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PACAP27 prevents Parkinson-like neuronal loss and motor deficits but not microglia activation induced by prostaglandin J2

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ABSTRACT

Neuroinflammation is a major risk factor in Parkinson's disease (PD). Alternative approaches are needed to treat inflammation, as anti-inflammatory drugs such as NSAIDs that inhibit cyclooxygenase-2 (COX-2) can produce devastating side effects, including heart attack and stroke. New therapeutic strategies that target factors downstream of COX-2, such as prostaglandin J2 (PGJ2), hold tremendous promise because they will not alter the homeostatic balance offered by COX-2 derived prostanoids. In the current studies, we report that repeated microinfusion of PGJ2 into the *substantia nigra* of non-transgenic mice, induces three stages of pathology that mimic the slow-onset cellular and behavioral pathology of PD: mild (one injection) when only motor deficits are detectable, intermediate (two injections) when neuronal and motor deficits as well as microglia activation are detectable, and severe (four injections) when dopaminergic neuronal loss is massive accompanied by microglia activation and motor deficits. Microglia activation was evaluated in vivo by positron emission tomography (PET) with [^{11}C](R)PK11195 to provide a regional estimation of brain inflammation. PACAP27 reduced dopaminergic neuronal loss and motor deficits induced by PGJ2, without preventing microglia activation. The latter could be problematic in that persistent microglia activation can exert long-term deleterious effects on neurons and behavior. In conclusion, this PGJ2-induced mouse model that mimics in part chronic inflammation, exhibits slow-onset PD-like pathology and is optimal for testing diagnostic tools such as PET, as well as therapies designed to target the integrated signaling across neurons and microglia, to fully benefit patients with PD.

1. Introduction

Neuroinflammation, particularly microglia and astrocytes, play a critical role in the pathogenesis of Parkinson's disease (PD) [19,22,55,84]. A critical factor of neuroinflammation is cyclooxygenase-2 (COX-2), which is highly induced in PD and negatively impacts neuronal function [7,25,40,85]. COX-2 is a key enzyme in the biosynthesis of prostaglandins, some of which are neuroprotective while others are neurotoxic [36]. The role of prostaglandins in PD pathology is poorly defined. In the previous studies we compared in vitro the neurotoxic effects of four different prostaglandins, i.e. A1, D2, E2, and J2, and established that prostaglandin J2 (PGJ2) was the most neurotoxic at the concentrations tested [47].

PGJ2 is derived from PGD2 [87], the principle cyclooxygenase product synthesized in the mammalian CNS [1,14,30]. From all prostaglandins, PGD2 levels change the most under pathological conditions [26,48]. PGD2 readily undergoes non-enzymatic dehydration to generate PGJ2, which is unique among prostaglandins as it is highly reactive and forms covalent Michael adducts with cellular proteins [87].

The levels of PGJ2 in the brain are highly elevated in rodent models of stroke [50,51] and traumatic brain injury (TBI) [31,45], reaching the 100 nM range. These levels represent average brain concentrations, but it is predicted that local cellular and intracellular concentrations of PGJ2 could be much higher [52]. Importantly, stroke and TBI increase the long-term risk for PD [8,35,73,86]. Moreover, PGJ2 impairs the ubiquitin/proteasome pathway (UPP) [41,47,76,90] and mitochondrial function [42,43,54], and potentiates dopamine toxicity [64]. Based on all of these findings we propose that PGJ2 plays an important role in PD pathogenesis. In effect, we showed that microinfusing PGJ2 alone

Abbreviations: COX-2, cyclooxygenase-2; DA, dopamine; DMSO, dimethyl sulfoxide; MPTP, 1-Methyl 4-phenyl 1,2,3,6-tetrahydropyridine; 6-OHDA, 6-Hydroxydopamine; PD, Parkinson's disease; PBS, phosphate buffered saline; PACAP, pituitary adenylate cyclase-activating polypeptide; PGD2 and J2, prostaglandin D2 and J2: respectively; PD, Parkinson's disease; PET, positron emission tomography; SEM, standard error of the mean; SNpc and SNpr, *Substantia nigra pars compacta and pars reticulata*: respectively; TBI, traumatic brain injury; TH, tyrosine hydroxylase; Ub-protein, ubiquitinated protein; UPP, ubiquitin/proteasome pathway; VTA, ventral tegumental area

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into the *substantia nigra/striatum* of mice is sufficient to induce molecular, cellular and behavioral deficits similar to those in PD [69].

To overcome the neurotoxic effects of PGJ2 we tested the impact of the pituitary adenylate cyclase-activating polypeptide (PACAP). PACAP is a potent neuroprotective lipophilic peptide in different models of neuronal injury such as stroke, Parkinson's disease, Huntington's disease, traumatic nerve injury, retinal degeneration, and others, where it exhibits anti-apoptotic, anti-inflammatory and anti-oxidant effects [4,18,53,66,72]. In addition, we previously established that PACAP prevents neuronal death, proteasome deficits and the decrease in cAMP levels induced by PGJ2 in vitro [57]. PACAP signals survival via the activation of the G-protein coupled receptor PAC1R (pituitary adenylate cyclase 1 receptor), which is expressed in the substantia nigra pars compacta (SNpc), in the ventral tegmental area (VTA), and other brain areas [38,81]. Nanomolar concentrations of the two forms of PACAP, PACAP38 and the truncated form PACAP27 (the latter used in our experiments), activate adenylate cyclase and elevate intracellular cAMP [60]. PACAP was shown to mitigate 6-OHDA and MPTP-induced loss of dopaminergic neurons in animal models of PD [17,71,72,89]. Furthermore, PACAP deficiency sensitizes dopaminergic neurons to paraquat-induced neuroinflammation in vivo [92]. Some studies support the view that PACAP27 is a more potent neuropeptide than PACAP38 and VIP (vasoactive intestinal peptide) in, for example, voltage clamped preparations of rat jejunum [15]. Together, these findings support our investigation on the efficacy of PACAP27 against the neurodegenerative effects of PGJ2 with our in vivo model of PD.

Our current study extends our previous one [69], in that we establish progressive stages of pathology induced by four consecutive PGJ2 microinfusions one week apart. These three stages mimic the slow-onset cellular and behavioral deficits in PD: mild (upon one injection) when only slight motor deficits are evident, intermediate (upon two injections) when neuronal and motor deficits as well as microglia activation are significantly detectable, and severe (upon four injections) when dopaminergic neuronal loss is massive accompanied by microglia activation and motor deficits. We also show that PET imaging of the brain with [^{11}C](R)PK11195 qualitatively detects microglia activation induced by PGJ2. Finally, we establish that in the intermediate stage, PACAP27 protects against neuronal loss and motor deficits, but not in the severe stage, which most likely coincides with a point of no return. PACAP27 failed to prevent microglia activation in this rodent model of PD-like pathology. It is clear that brain injury, initiated in our model by a neurotoxic product of inflammation, involves integrated signaling across neurons and microglia. Ideally, therapeutic interventions for PD should target all cells involved regardless of cell type, i.e. neurons and glia.

2. Materials and methods

2.1. Materials

Drugs: PGJ2 (cat. # 18500, Cayman Chemical) in DMSO, and PACAP27 (pituitary adenylate cyclase-activating polypeptide, cat. # H-1172, Bachem Bioscience) in sterile water. The final DMSO concentration in PBS was 17% for all microinfusions. The solutions were freshly prepared and stored for a maximum of 2 h at 4 °C and in the dark. *Primary antibodies*: dopaminergic neurons [tyrosine hydroxylase (TH), 1:1000, cat.# MAB318 (mouse) or AB152 (rabbit), Millipore]; GABAergic neurons [GAD67, 1:1,000, cat.# MAB5406 (mouse), Millipore]; all neurons [NeuN, 1:50, cat.# MAB377 (mouse), Millipore]; microglia and macrophages [Iba1, 1:500, cat.# 019-19741 (rabbit), Wako Chemicals]; and ubiquitinated proteins [1:200, cat.# Z0458 (rabbit), Dako Cytomation]. *Secondary antibodies*: Alexa Fluor 568 (1:100, cat.# A11036, rabbit) and Alexa Fluor 488 (1:100, cat.# A11029, mouse) both from Invitrogen. Vectashield Hard Set™ mounting medium with DAPI (cat.# H-1500, Vector Laboratories).

2.2. Mice

Eleven-week old male FVB mice (N = 43; body weight: 25–32 g) were obtained from Charles River. Mice were singly housed on a 12-h light/dark cycle, maintained at 23 °C and 50–70% humidity, with food and water available ad libitum. Mice were allowed to acclimate for two weeks before surgery. All procedures were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at Hunter College, CUNY and at Weill Cornell Medical College.

2.3. Surgery and microinfusion

We followed the same procedures as described in our previous study [69], except that mice received unilateral injections of vehicle (DMSO) or drugs (PGJ2 and PACAP27) only into the SN. Briefly, at thirteen weeks of age, mice were anesthetized by isoflurane inhalation (induction 2–2.5%, maintenance 1.5–2%) administered in 100% oxygen and placed into a stereotaxic frame (Model 51730D, Stoelting Co., Wood Dale, IL) fitted with a gas anesthesia mask (Model 50264, Stoelting Co.). A burr hole was drilled in the skull at coordinates relative to bregma for the SNpc [67]: rostral–caudal (RC) = −3.25 mm; medial–lateral (ML) = 1.25 mm; and ventral = 4.13 mm. All injections were administered to the right SNpc, while the contralateral (left) side served as an internal control. A 2 μL microinjection Hamilton syringe (7002 KH) with a 25-gauge needle was slowly inserted into the brain and left in place for five minutes. Thereafter, 2 μL of solution was infused at an injection rate of 0.2 μL/min (Quintessential stereotaxic injector, Model 53311, Stoelting Co.). The needle was left in place an additional five minutes to ensure total diffusion of the solution. Following injection, the needle was slowly removed and the incision was closed with monofilament absorbable sutures (cat. # 033899; Butler Schein Animal Health, Dublin, OH). After surgery, mice were administered with a subcutaneous injection of 0.5 cc Lactated Ringer's solution, given wet palatable rodent chow, and kept in a warm place to recover. Subsequent injections to the SNpc were administered via the same drill hole established during the first surgical procedure.

2.4. Groups

Mice were randomly assigned to the treatment groups (Fig. 1). For behavioral and immunohistochemical analyses, mice in each group received either two (2×, n = 3) or four (4×, n = 6) injections of DMSO, or either one (1×, n = 7), two (2×, n = 6) or four (4×, n = 6) injections of PGJ2. Moreover, to assess the therapeutic efficacy of PACAP27, other groups of mice received two injections of PACAP27 (2×, n = 3), or two injections of PACAP27 + PGJ2 (2×, n = 3) and were compared to the mice receiving DMSO (2×) or PGJ2 (2×). In preliminary studies we established that PACAP27 (4×) was ineffective against four PGJ2 injections, thus we did not utilize a 4× PACAP treatment group in our current paradigm. Behavior was assessed four weeks after the last microinfusion. Following behavioral assessment, animals were perfused intracardially and the brains removed for immunohistochemical analyses. To obtain maximal signal for μPET imaging, one group of mice (4×, n = 5) received four unilateral DMSO injections and another group (4×, n = 5) received four unilateral PGJ2 injections. Mice underwent μPET scanning one week after the last PGJ2 or DMSO injection. The concentrations per unilateral injection were 16.7 μg/2 μL for PGJ2 and 50 ng/2 μL for PACAP27, administered individually or co-injected. All injections were administered one week apart.

2.5. Behavior

Mice were tested for Parkinsonian-like behavior four weeks after their last injection (Fig. 1). Scorers were blind to the experimental