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RESVERATROL PROTECTS NEURONS FROM CANNULAE IMPLANTATION INJURY: IMPLICATIONS FOR DEEP BRAIN STIMULATION

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Abstract—Brain-implantable electrodes such as those used in deep brain stimulation (DBS) have a promising future in end-stage Parkinson's disease therapy. However, there is considerable injury when electrodes penetrate brain tissue. For instance, broken blood vessels and glial scar formation may impede continual DBS or electrical recording from specific neurons. To begin addressing this key safety issue, we tested the therapeutic potential of resveratrol in reducing brain trauma caused by DBS-like surgery. Microinfusion of resveratrol (10 μM) directly applied to the sub-thalamic nucleus (STN) of the rat brain significantly minimized the formation of astrocytic gliosis in response to a 27-G precision-glide cannula implant. The therapeutic effects of resveratrol extended to the ''kill zone'', a boundary zone of about 100 μm comprising the cannula implant and surrounding neurons. We also found that resveratrol not only provided almost complete protection from mechanical injury to the brain, but that it also prevented undesirable motor deficits often seen in animals with lesions to the STN. Lastly, continuous infusion of resveratrol over a 4-week period led to the inhibition of pro-apoptotic, neurodegenerative and cell division cycle genes that may be associated with a reduction in astrocytic gliosis and glial scar formation within the STN. Taken together, these data suggest that application of resveratrol to the brain is an effective adjunct surgical procedure for minimizing acute neuronal injury when electrodes are implanted directly into the STN.

Key words: astrocytic gliosis, cell division cycle genes, Parkinson's disease, motor deficits, sub-thalamic nucleus, rat.

INTRODUCTION

Resveratrol (trans-3,5,4′-trihydroxystilbene) is a small polyphenol molecule (M.W. = 228.24) isolated from the roots of *Polygonum cuspidatum* (Japanese knotweed) and found to be a pharmacologically active constituent of red wine. In recent years, compelling evidence from both *in vivo* and *in vitro* studies indicates that resveratrol may prevent major causes of morbidity and mortality such as cancer, cardiovascular disease, diabetes and neurodegenerative disorders (Baur and Sinclair, 2006). Although the precise mechanisms by which resveratrol exerts its wide range of beneficial effects on disease are not yet clear, there is convincing evidence that resveratrol activates silent information regulator 1 (SIRT1) signaling pathways that also considerably expand the life span of several experimental animals (Della-Morte et al., 2009; Baur, 2010). SIRT1 is a mammalian nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase localized mostly to the nucleus of brain neurons (Michán and Sinclair, 2007; Zakhary et al., 2010) whose activity is particularly overexpressed following periods of caloric restriction (Michán and Sinclair, 2007). Because resveratrol activates metabolic pathways that slow aging, this polyphenol molecule may therefore provide a broad strategy to prevent age-related degenerative changes and age-related pathologies such as Parkinson's disease (PD).

PD is a neurodegenerative disease whose pathogenesis is well understood: The progressive loss of dopamine neurons in the substantia nigra, a nucleus of the midbrain (Rosenthal, 1998). This cell loss causes a spectrum of movement disorders, including the clinical triad of resting tremor, rigidity and bradykinesia (Maetzler et al., 2009; Philippens et al., 2010). Although powerful drugs that attenuate the underlying causes of the disease are available, long-term treatment outcomes in many cases remain unsatisfactory. Furthermore, it has come as an acute disappointment that newly FDA-approved drugs only convey minor benefits, if any, compared to older drugs (e.g., levodopa, L-DOPA). Thus, a non-pharmacological initiative is underway in the neurosciences to overcome the long stagnation in drug therapy for PD. For instance, deep brain stimulation (DBS), optogenetics analysis and human pluripotent stem cells could potentially be used as therapies, disease models, or in drug screening assays. Of all of the aforementioned therapies, DBS in particular, has become an alternative treatment for patients who no longer respond to L-DOPA administration (Pizzolato and Mandat, 2012).

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Abbreviations: d_2H_2O, double-distilled water; DBS, deep brain stimulation; DMSO, dimethyl sulfoxide; GFAP-ir, glial fibrillary acidic protein-immunoreactivity; KPBS, potassium phosphate- buffered saline; LH, luteinizing hormone; PD, Parkinson's disease; PF, paraformaldehyde; QPCR, quantitative polymerase chain reaction; SIRT1, silent information regulator 1; STN, sub-thalamic nucleus.

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With DBS, patients have electrodes surgically inserted in the sub-thalamic nucleus (STN), a discrete diencephalic nucleus that controls movement initiation (Deniau et al., 2010). However, inserting electrodes through the skull and into the brain can cause astrocytic gliosis, inflammation and cell dystrophy. This scarring process, in turn, is a major source of failure in chronically implantable electrodes (Pancrazio, 2008). Thus, the specific hypothesis for the current experiments was to test the untapped therapeutic potential of resveratrol in reducing brain trauma caused by DBS-like surgery. To accomplish this goal, we inserted bilateral cannulae into the rat STN, thus mimicking the surgical procedure of DBS, and then μM doses of resveratrol were directly administered through the cannulae. In addition to testing the efficacy of resveratrol in protecting nerve tissue from mechanical injury, we also measured changes in behavioral activity in rats treated peripherally with resveratrol. Finally, we analyzed gene expression patterns from STN material following peripheral resveratrol treatment.

EXPERIMENTAL PROCEDURES

Animals

All animals used for this study were adult, male Long–Evans rats (200–300 g) purchased from Harlan Laboratories Inc. (Indianapolis, IN, USA). Upon arrival, rats were individually housed in microenvironment chambers in the *vivarium* at Hope College. Animals were given a standard rodent diet and water *ad libitum* and kept on a 12:12-h light–dark cycle with lights on at 0700 h. All experiments were performed during the lights-on period and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and with approval from the Hope College Animal Care and Use Committee (HCACUC). Body weights were recorded daily to monitor the animals' general health and recovery from surgery. All efforts were made to minimize animal stress and to reduce the number of rats used for the experiments described below.

Microinjections into the STN: Time course

Rats were deeply anesthetized (0.1 ml/100 g body weight; IP) with a ketamine cocktail (ketamine, xylazine, and acepromazine in a 5.0:2.5:1.0 ratio, respectively) and ketofen (5 mg/kg, IM) for analgesia. Once anesthetized, the rats were placed in a Kopf stereotactic frame and a vertical incision along the top of the skull was made under sterile conditions. Hamilton cannula syringes (27-gauge; Brain Infusion Kit, Alzet Inc., Cupertino, CA, USA) were directed toward the right and left STN. Stereotaxic coordinates relative from Bregma: AP = −3.72 mm, DV = 8 mm and ML = 2 mm, according to the Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 1998). Resveratrol was delivered at a dose of 10 μM dissolved in 50 nl of dimethyl sulfoxide (DMSO) and slowly injected over a 5-min period to either the right or left STN. This particular dose (i.e., 10 μM) of resveratrol was determined from *in vitro* studies using human cells (Torres et al., 2011). DMSO (the vehicle solution) was delivered at the same rate and at a similar volume to that of resveratrol to the STN of the non-resveratrol treated hemisphere. Thus, this is a within-subject design experiment with the right or left STN randomized for microinjections of either resveratrol or DMSO. Five min after the initial drug delivery, cannulae were raised 1 mm and a second 50 nl of resveratrol or DMSO was injected immediately before the cannulae were removed from the brain parenchyma. This procedure allowed resveratrol or DMSO to flow through the needle tract during the withdrawal phase of the surgery. We arbitrarily selected four different survival times following mechanical injury: 48 h, 1 week, 2 weeks and 4 weeks (n = 4–6 rats/time period). Rat brains were then collected and analyzed for trauma with the following screening assays: Histology (Nissl stain), immunocytochemistry (glial fibrillary acidic protein [GFAP]-immunoreactivity [ir]) and histochemistry (Fluoro-Jade B stain).

Nissl stain histology

Rat brains were mounted on a sliding microtome stage and cut into 40-μm coronal sections. A set of these sections were directly mounted onto gelatin–chrome-alum-coated slides in anatomical order from the rostral (Bregma −3.30 mm; subincertal nucleus) to the caudal (Bregma −4.52 mm; substantia nigra, reticular) domains of the lesion. The slides were allowed to dry overnight and then processed for Nissl stain to assess neuronal injury and other chromatolysis changes of the lesion.

GFAP immunocytochemistry and Fluoro-Jade histochemistry

Immunocytochemistry for GFAP was performed on a second set of rat brain sections (40 μm) using a standard immune-fluorescent reaction procedure as previously described by our group (Saldanha et al., 2010). In brief, rat brain sections were fixed *in situ* with 4% paraformaldehyde (PF) in 0.1 M potassium phosphate-buffered saline (KPBS). After 4 days of PF incubation, brain sections were transferred to 0.1 M KPBS with 10% sucrose for 48 h for stabilization purposes and then stored in a cryoprotective solution (0.9% NaCl, 30% sucrose, 1% polyvinyl-pyrrolidine MW 40,000, 30% ethylene glycol in 0.05 M KPBS) at −20 °C until processed for immunocytochemistry. Coronal brain sections were incubated for 72 h at 4 °C with an anti-α GFAP polyclonal rabbit antibody (Dako Inc., Carpinteria, CA, USA) at a dilution factor of 1:1000. Thereafter, the brain sections were incubated with Texas Red anti-(rabbit anti-mouse IgG (H + L); Vector Laboratories Inc., Burlingame, CA, USA) for 3 h at room temperature at a dilution factor of 1:500. After several rinses of KPBS washes, brain sections were mounted onto Superfrost Plus slides (VWR Scientific, West Chester, PA, USA), dried on a slide warmer for 20 min, and then air dried for 24 h. Dried slides were then dipped into double-distilled water (d_2H_2O) for 5 s and then transferred to a Fluoro-Jade solution (made by adding 1 mL of stock Fluoro-Jade solution into 99 mL of 0.1% acetic acid in distilled water) for 30 min. The 0.01% stock solution was prepared by dissolving 10 mg of Fluoro-Jade into 100 mL of distilled water. Background staining was reduced by using a low concentration (0.0001%) of the Fluoro-Jade stock solution. After staining, the slides were once again placed in d_2H_2O after which coverslips were applied using Hard-mount (Vector Labs, Burlingame, CA, USA). In some experiments, alternate sets of slides were processed for GFAP-ir as described above, but with Ni–DAB as the primary chromogen (Vector Laboratories Inc., Burlingame, CA, USA).

Analysis of GFAP and Fluoro-Jade

Qualitative analysis of GFAP and Fluoro-Jade double-labeled cells was performed on coronal brain sections tested positive for GFAP-ir. The image processing system consisted of a Leica DMC480 digital camera mounted on a Leica DM5000b microscope. The camera was attached to a Scion VG-5 Frame Grabber (Scion Corp., Frederic, MD, USA) mounted onto a Macintosh G5 computer (Apple Computer, Cupertino, CA,