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BDNF: A missing link between sympathetic dysfunction and inflammatory disease?

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Abstract

Nerve growth factor (NGF) plays a role in sympathetic neuron integrity and survival. Brain-derived neurotrophic factor (BDNF) also has trophic effects on sympathetic neurons. We report here the serendipitous finding that co-treatment of hippocampus with BDNF and the NGF antagonist TrkA-Fc leads to perivascular inflammation and marked vasoconstriction. This effect is not observed with either reagent alone or in combination with other control proteins. Because NGF supports sympathetic neuron health, we tested the hypothesis that BDNF combined with sympathetic compromise caused this effect. Superior cervical ganglia were removed bilaterally with concurrent BDNF infusion into hippocampus. Perivascular inflammation was observed at 3 days, but not 12 days post treatment, when sympathetic terminals had receded, suggesting that the presence of these terminals was necessary for inflammation. Since sympathetic dysfunction may lead to compensatory overactivity of norepinephrine (NE) signaling, we co-infused BDNF with NE in the hippocampus and observed perivascular inflammation. In humans, sympathetic overactivity has been reported in a variety of vascular diseases. Some of these diseases, e.g. primary Raynaud's, are not accompanied by serious inflammatory disease whereas others, such as scleroderma and systemic lupus, are. We speculate that BDNF may contribute to the transformation of sympathetic dysfunction to inflammatory disease.

Keywords: Neurotrophins; Sympathetic nervous system; Norepinephrine; Autoimmune; Inflammation; Systemic sclerosis; Raynaud's phenomenon; Scleroderma

1. Introduction

The sympathetic nervous system (SNS) is one branch of the autonomic nervous system. Norepinephrine (NE), the major neurotransmitter in the SNS, is released onto target organs, including lymphoid organs and blood vessels, by the sympathetic nerve fibers (Elenkov et al., 2000; Flavahan et al., 2000). The release of NE onto blood vessels leads to vasoconstriction and increased vascular tone.

The development and maintenance of sympathetic neurons is dependent, in part, on the neurotrophin family of growth factors, consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3, and NT-4/5 (Lewin and Barde, 1996; Fritzsch et al., 1997). These growth factors are expressed by a wide variety of cells, including neurons and astrocytes, and bind to one or more of a family of cell surface receptor tyrosine protein kinases (Trk) expressed widely in the periphery (Sheard et al., 2002; Mu et al., 1993; Goettl et al., 2004; Hikawa et al., 2002) as well as in the central nervous system (for review see Barbacid, 1994; Lessman et al., 2003). NGF binds TrkA, BDNF and NT-4/5 bind TrkB, and NT-3 preferentially binds TrkC. All four neurotrophins also bind to the low affinity

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NGF receptor p75 (for review see Teng and Hempstead, 2004).

Antagonists to neurotrophins lead to decreased investment of sympathetic terminals on target organs such as vasculature (Rush et al., 1997) while the addition of sympathetic agonists, such as NE, to certain cell types leads to decreases in neurotrophin levels including NGF (Peeraully et al., 2004). Additionally, alterations in sympathetic tone have been shown to result in increased blood pressure (Rossoni et al., 2003). It is clear that the interaction between the sympathetic nervous system and neurotrophins plays an important role in maintaining normal vascular tone.

Dysregulation of vascular tone can lead to abnormal dilation and constriction of vasculature. These vasospasms have been shown to contribute to local tissue damage including deposition of proteins and extravasation of white blood cells (WBCs) into local tissue (van Laar and Tyndall, 2003; Kahaleh, 1990). Abnormal inflammatory activity is hallmark in certain connective tissue diseases including Scleroderma (SSc) and Lupus. Patients with SSc present with vascular abnormalities, abnormally high sympathetic tonus (Zakrzewska-Pniewska et al., 1999), and altered blood levels of the circulating neurotrophin NGF (Matucci-Cerinic et al., 2001). BDNF is elevated in the synovium of patients with the autoimmune diseases rheumatoid arthritis (Weidler et al., 2005) and spondylarthritis (Rihl et al. 2005).

The current paper reports the serendipitous finding that application of brain-derived neurotrophic factor (BDNF) to hippocampal tissue with abnormal sympathetic tonus results in the development of an inflammatory process which neither abnormal sympathetic tonus nor BDNF alone can induce. These data suggest an etiological role of BDNF in perivascular inflammation via a neuroimmune mechanism.

2. Methods

2.1. Stereotaxic surgeries

We anesthetized adult Sprague–Dawley rats (275–375 g) using the pre-anesthetic chlorpromazine (3 mg/kg) followed by ketamine (210 mg/kg). Animals were then shaved and treated with povidone–iodine solution. Incisions were made in the scalp and, after placing two anchor screws into the skull, a 4 mm indwelling cannula (Plastics One, Roanoke, Virginia) was placed − 2.6 mm ML and − 3.7 mm AP from bregma (Paxinos and Watson, 1986) into the left hippocampus. The cannulae were attached to heat-sealed polyvinyl catheters filled with sterile phosphate-buffered saline (PBS). We then attached the cannula to the skull using dental acrylic to secure the cannula to the anchor screws, sutured the incision with 3-0 nylon suture (Henry Schein, Melville, NY), and applied topical antibiotic cream. We recovered the rats under heat lamps until fully awake.

2.2. Pump surgeries

We re-anesthetized rats 7 days after placing cannulae into the hippocampus as shown in the experimental timeline (Fig. 1). We placed an incision across the nape of the neck and withdrew the tubing attached to the cannulae. We next attached a 14 day, 0.5 μL/h osmotic mini-pump (Alza Corporation, Mountain View, California) to the catheter end. Rats received one of the following infusions: BDNF (12 μg/day)+TrkA-Fc (30 μg/day), BDNF+TrkB-Fc (30 μg/day), BDNF+TrkC-Fc (30 μg/day), BDNF+NE (250 pg/day), BDNF+hFc (30 μg/day), BDNF alone, TrkA-Fc alone, PBS alone, NE alone, and hFc. The concentration for BDNF was selected based on previous BDNF infusion studies (see Croll et al.,1998). The TrkA-Fc dose was selected to ensure at least a 10-fold molar excess of TrkA-Fc over endogenous and exogenous neurotrophin levels, as in vitro data for receptor-body traps show that a 10M excess solution is necessary to block neurotrophin activity (Croll et al., 1998). Receptor body traps, such as TrkA-Fc, bind to endogenous protein ligands, thereby preventing them from binding to their endogenous receptors. The NE dose was selected based on amounts used in the literature and on estimates of pathophysiological NE (see Globus et al., 1989). All solutions were dissolved in sterile PBS, and not all treatments were used in all experiments. We then placed the pump subcutaneously along the back, closed the incision with 3-0 nylon suture, and applied topical antimicrobial cream to the wound.

2.3. Sympathectomies

At the same time as pump surgery, we removed the superior cervical ganglion (SCG) bilaterally from some rats receiving BDNF only or PBS only (see Fig. 1 for timeline). Rats in the same experiment received a sham surgery. Briefly, we made an incision across the neck exposing the muscles and thyroid gland. We separated the thyroid gland from underlying tissues and wrapped it in sterile, saline-soaked gauze above the incision site. We next dissected down to the right superior cervical ganglion, isolated it from surrounding fascia and blood vessels, and used micro-scissors to remove it completely. We then repeated this procedure for the left superior cervical ganglion. After

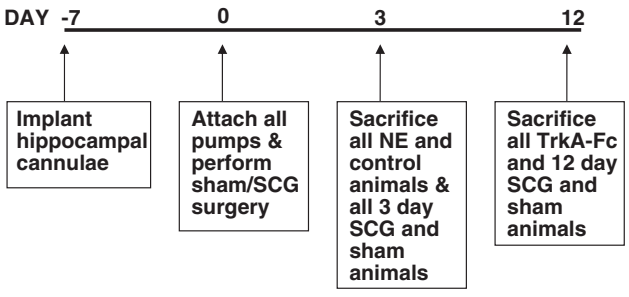

Fig. 1. Schematic of experimental timelines.