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Electroacupuncture and nerve growth factor: potential clinical applications

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ABSTRACT

The nerve growth factor (NGF) is a neurotrophin regulating the survival and function of peripheral sensory and sympathetic neurons and of forebrain cholinergic neurons. Both peripheral neuropathies and brain cholinergic dysfunctions could benefit from NGF-based therapies, but the clinical use of NGF has been so far hampered by the development of important side effects, like hyperalgesia and autonomic dysfunctions. Acupuncture is a therapeutic technique and is a part of traditional Chinese medicine. Western descriptions of the clinical efficacy of acupuncture on pain, inflammation, motor dysfunction, mood disorders, and seizures are based on the stimulation of several classes of sensory afferent fibers and the consequent activation of physiological processes similar to those resulting from physical exercise or deep massage. Recently, it has been shown that peripheral sensory stimulation by electro-acupuncture (EA) could improve brain NGF availability and utilization, at the same time counteracting the major side-effects induced by NGF administration. This review focuses on the emerging links between EA and NGF with special emphasis on the work carried out in the last decade in our laboratory, investigating the role of NGF as a mediator of EA effects in the central nervous system and as a modulator of sensory and autonomic activity.

Key words

Nerve growth factor • Electroacupuncture • Forebrain cholinergic neurons • Peripheral neuropathies • Reproduction • Immune system

Introduction

Nerve Growth Factor

Nerve growth factor (NGF) is the first discovered target-derived neurotrophic factor, essential for the development and maintenance of neurons in the peripheral nervous system (PNS) and for the functional integrity of cholinergic neurons in the central nervous system (CNS) (Aloe et al., 1997). NGF can also affect non-neuronal cells and the maintenance of a balanced interplay between the nervous, endocrine and immune systems. NGF concentrations increase during stress and have a modulatory role in the hypothalamic-pituitary-adrenal (HPA) axis (Aloe et al., 2002).

The NGF exerts its biological action activating 2 different transmembrane receptors: the high-affinity, NGF-selective tyrosine kinase A (TrkA) and the low-affinity, non-selective p75 neurotrophin receptor ($p75^{NTR}$). The major cytosolic/endosomal pathways activated by the TrkA receptors are Ras-mitogen activated protein kinase, extracellular signal-regulated kinase, phosphatidylinositol 3-kinase-Akt, and phospholipase C-γ (Klesse et al., 1999; Chao et al., 2006; Reichardt, 2006). $p75^{NTR}$ is a glycoprotein that regulates signaling through TrkA (Friedman et al., 1999; Schor, 2005; Reichardt, 2006); binding of NGF to $p75^{NTR}$ activates additional signaling pathways (NF-κB or the ceramide or the MEKKs/JNK pathways) that, in the absence of coexpressed TrkA,

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may signal a cell to die via apoptosis (Friedman et al., 1999; Miller et al., 2001; Schor, 2005).

Interest in the possible therapeutic benefits of NGF has increased during the last two decades (Olson, 1993; Seiger et al., 1993; Apfel et al., 1995; Apfel et al., 1998; Eriksdotter Jonhagen et al., 1998; Lambiase et al., 2000; McArthur et al., 2000; Tuveri et al., 2000; Aloe, 2004; Chiaretti et al., 2005; Tuszynski et al., 2005; Allen et al., 2006). Preclinical and clinical trials have been conducted to investigate the potential role of NGF in the treatment of central and peripheral neurodegenerative diseases and more recently corneal, diabetic and pressure ulcers, and vasculopathies (Koliatsos et al., 1991; Seiger et al., 1993; Eriksdotter Jonhagen et al., 1998; Apfel et al., 2000; Bonini et al., 2000; McArthur et al., 2000; Lambiase et al., 2003; Allen et al., 2006). Clinical blockade of NGF could be useful for the treatment of neuropathic pain, as well as for some kind of dysautonomias (Hefti et al., 2006). Purified NGF has been administered intranasally (Chen et al., 1998; Yasuno et al., 2000; Capsoni et al., 2009) and intracerebroventricularly in subjects affected by Alzheimer's disease (Seiger et al., 1993; Eriksdotter Jonhagen et al., 1998), intravenously in patients with peripheral neuropathies (Apfel et al., 2000), and topically in subjects with corneal ulcers (Aloe et al., 2008), pressure ulcers (Aloe, 2004; Aloe et al., 2008) and ulcers induced by autoimmune disorders (Tuveri et al., 2000; Aloe, 2004; Generini et al., 2004). Despite promising early results, patients in most investigations experienced side effects such as weight loss and hyperalgesia, especially following systemic delivery of the neurotrophin (Apfel, 2002). Thus, the rationale exists for pursuing alternative therapeutic strategies aimed at endogenous modulation of NGF and/or at counteracting the NGF-associated side effects, without interfering with its therapeutic actions.

Electroacupuncture

Acupuncture is a potent form of sensory stimulation. This is the key perspective point from which Western scientific community has studied the mechanisms of acupuncture treatment, first on pain relief and then in a wide spectrum of diseases, including infections, inflammations, dysfunctions of the autonomic, peripheral and central nervous system, and metabolic disorders (Andersson et al., 1995; Ulett et al., 1998; Kaptchuk, 2002). Needle insertion into the skin and deeper tissues results in particular patterns of afferent activity in peripheral nerves. The inserted needles are stimulated by manual rotation or through the application of electrical stimulation, generally referred to as electro-acupuncture (EA). It has been reported that manual acupuncture and EA effects can be correlated to activation of all kinds of type A (alpha, beta, gamma and delta) and C sensory nerve fibers. However, particular significance has been given to a group of receptors in the skeletal muscles, which have both low- and high-threshold for mechanical stimulation, and are innervated by A-delta fibers and possibly C-fibers. Physiologically they are activated by strong muscle contractions and have been denoted ergoreceptors. Based on this, it has been suggested that EA with repetitive muscle contraction results in the activation of physiological processes similar to those resulting from physical exercise (Andersson et al., 1995).

Both acupuncture and physical exercise release endogenous opioids, which seems to be essential in the induction of acupuncture-mediated functional changes of different organ systems. However, other systems may be involved in the acupuncture modulation of stress, pain, autonomic activity and immune systems. Indeed, research carried out in the last 20 years shown that through acupuncture it is possible to affect synthesis, release and action of several neurotransmitters (catecholamines, glutamate, acetylcholine, GABA, serotonin) and neuropeptides (among others: oxytocin, NPY, CCK, VIP, SP, CGRP, PACAP) in both CNS and PNS. It has recently been proposed that at least some of the effects attributed to acupuncture are mediated by neurotrophins, particularly NGF (Manni et al., 2010a).

NGF and EA: potential clinical application

Reproduction

Data on the correlation between NGF and EA first came from our work on the estradiol-valerate (EV)-induced polycystic ovary (PCO) in rats (Stener-Victorin et al., 2000; Stener-Victorin et al., 2003; Manni et al., 2005c) and then on CNS physiology and pathology. EA treatments in EV-treated rats

counteract the increase of ovarian NGF levels and lower them towards control levels (Stener-Victorin et al., 2000). The results suggested that EA inhibits hyperactivity in the sympathetic nervous system (Cao et al., 1983; Chao et al., 1999), a major pathogenetic cause of PCO (Barria et al., 1993; Lara et al., 1993; Lara et al., 2000; Lara et al., 2002). Supporting this hypothesis, in a following study (Stener-Victorin et al., 2003) we demonstrated the effect of EA in reducing ovarian NGF along with ovarian and hypothalamic sympathetic activation markers. We also demonstrated that an interplay among the NGF/NGF receptor system and adrenergic responsiveness characterizes ovarian pathology in this rat model (Manni et al., 2005a; Manni et al., 2005b; Manni et al., 2005c; Manni et al., 2006), as EV injection dysregulated the ovarian expression of both TrkA and $p75^{NTR}$, as well as α_1- and β_2-adrenergic receptors. Neutralization of endogenous NGF reversed most of these abnormalities (Manni et al., 2006). Almost identical results were obtained by EA treatment (Manni et al., 2005c), demonstrating that the results were probably achieved through the actions of EA on the NGF system. In recently published studies on a dihydrotestosterone (DHT)-induced PCO syndrome (PCOS) model, it has been further demonstrated that both EA and physical training effectively improve PCO-related metabolic disturbances (Manneras et al., 2008), alter sympathetic markers and ovarian morphology (Manneras et al., 2009) and normalize the DHT-induced increase of $mRNA^{NGF}$ (Manneras et al., 2009). Overall, the data on EA/NGF interaction in PCOS models indicated that the therapeutic potential of EA is exerted via its ability to modulate the activity of the autonomic nervous system by inducing a long-lasting depression of the sympathetic nervous system, with a concomitant down-regulation of NGF in peripheral organs.

Data have also been produced by our group in the context of male reproduction (Manni et al., 2010b), using a mouse model of psychosocial stress (isolation), that is known to play a detrimental role on male fertility, deregulating spermatogenesis and leading to poor sperm quality (Sasagawa et al., 2001). It was found that testis NGF content was increased by EA in control and decreased in isolated mice; EA augmented the phosphorylation of TrkA and increased testicular content of the $p75^{NTR}$ in isolated mice; the catecholamine rate-limiting enzyme tyrosine hydroxylase was increased by social isolation and diminished by EA in the testis of isolated mice, suggesting normalization of catecholaminergic activity. Concomitant to the NGF system modulation, EA counteracted the isolation-induced testicular down-regulation of the anti-apoptotic protein Bcl-2, suggesting an action on spermatogenesis. Our data indicate that EA, most probably affecting the neuroendocrine axis activity and/or the sympathetic tone, could affect the testicular homeostasis, counteracting the effect of stress on male fertility.

Peripheral neuropathies

NGF system has been indicated as a major therapeutic target in peripheral neuropathies, secondary to diabetes, viral infections, chemotherapy treatments or injuries (Apfel et al., 1995; Riaz et al., 1996; Apfel, 2002). However, this specific and promising pharmacologic use of NGF has been hampered by the development of side effects (Apfel, 2002). Exploring the possibility to overcome such undesired effects, we investigated the possibility to administer EA as a supportive therapy in NGF-treated animals. The aim was to improve NGF use by the responsive tissues, thus possibly reducing the pharmacological doses and avoiding NGF-associated side effects, especially when the neurotrophin is administered intravenously. We demonstrated that the action of acupuncture at the sensory neuron level could allow integration between NGF administration and EA. Indeed, after chronic NGF administration, EA overcomes the development of the NGF-associated hyperalgesic response, a result that facilitates the pharmacological use of the neurotrophin. The effect of EA is probably related to the cellular mobilization of pain mediators such as the neuropeptide substance P (SP) and the transient receptor-potential vanilloid receptor 1 (TRPV-1). It is worth noting that the effects of combined treatment with NGF and EA have been so far subject of few investigations. Indeed, the effects of such a therapy with intranasal NGF and EA have been recently investigated only by another group in a model of focal brain ischemia (Cheng et al., 2009). The authors demonstrated that intranasal administration of NGF and EA may have a synergistic effect in preventing ischemic injury and enhancing functional recovery after focal cerebral ischemia, which may be attributed to enhanced cell proliferation and survival.

The link between EA and NGF in the treatment of peripheral neuropathies has been preliminary investigated by our group using the streptozotocin (STZ)-induced rat model of type 1 diabetes (Manni et al., 2011). Treating adult rats with EA early after diabetes induction, we found that the thermal hyperalgesia was corrected by EA and that the parallel increase of NGF and TrkA in the spinal cord was also counteracted by EA. STZ also induced variations of skin/spinal TrkA phosphorylation, increase of skin SP and spinal TRPV-1 ion channel and decrease of spinal GABA that were counteracted by EA. Our results indicated that EA efficacy in the treatment of diabetic neuropathies might be based upon its actions on spinal/peripheral NGF synthesis/utilization and on normalization of the levels of several sensory neuromodulators.

Central nervous system

A possible NGF-based therapeutic effect of EA on CNS pathologies has also been investigated in the last decade. Recently we demonstrated that low-frequency EA applied to a rat model of human retinitis pigmentosa, induced a partial recovery of the normal morphological features of the retina, an increases of NGF synthesis and an improved retinal vascularization parallel to a local increase of the vascular endothelial growth factor (Pagani et al., 2006). Moreover, given in a rat model of cerebral ischemia-reperfusion EA reverses the high levels of ischemia-induced expression of the NR1 subunit of the NMDA glutamate receptor, and up-regulates levels of TrkA (Sun et al., 2005). In the same work it has been found that the neuroprotective effects of EA appear to be mediated by stimulation of the PI3-K pathway (Sun et al., 2005). Using the same experimental stroke model, the authors also demonstrated that EA stimulation can reverse the ischemia-induced increase of ion channels having a key role in the neuronal death pathways (Aarts et al., 2005), by enhancing TrkA activity, which triggers the downstream PI3-K pathway (Zhao et al., 2005; Zhao et al., 2007). Clues are being also emerging about a possible NGF-mediated EA therapeutic value in neurodegenerative disease affecting the forebrain cholinergic (FBC) system. These neurons projects to cortex and hippocampus, receiving NGF from their innervation targets as trophic support (Dreyfus, 1989). Using the STZ-induced rat model of type 1 diabetes, we have recently demonstrated that diabetic rats have a decreased brain NGF and NGF receptor expression (Sposato et al., 2007), suggesting the presence of NGF-dependent cholinergic dysfunction. We have applied EA treatment to STZ-injected rats (Manni et al., unpublished), demonstrating that it was effective in protecting forebrain cholinergic neurons from the STZ-induced loss of TrkA and choline acetyl-transferase, at the same time counteracting the STZ-induced NGF protein decrease in the cortex and hippocampus. Indirect evidence of a protective effect of EA on the cholinergic brain also comes from our study on a psychosocial stress in mice (Manni et al., 2009). We demonstrated that EA may modulate cognition as well as brain neurotrophins content in mice subjected to social isolation.

Immunological system

The effect of EA on immune cells and/or immunological markers have also been investigated in recent years. EA can influence the synthesis and expression of pro- and anti-inflammatory molecules (Huang et al., 2005) as well as mast cell (MC) activity, possibly modulating allergic response (Calcutt, 2002, Yoo et al., 2004). We used an experimental model of pharmacological induced anaphylactic reaction in adult rats, through injection of the MC activating compound 48/80, and investigated the effects of low-frequency EA on mast cell activation and release of NGF (Manni et al., 2010a). Both MC and NGF have been shown to play an important role during the allergic reaction. We hypothesized that the interplay between MC and NGF could be modulated by the treatment with EA. We found that repeated EA stimulation differentially affects peritoneal, lung and dermal mast cells. In Fig. 1 the effect of 48/80 compound and EA on peritoneal mast cell NGF and degranulation is depicted. We found that EA treatment counteracted the 48/80-induced increase of NGF in peritoneal cells. Moreover it was also found that EA counteracted MC degranulation in the lungs, suggesting its effectiveness in the treatment of allergic asthma. Since MC are critically involved in allergic responses (Song et al., 2009), these observations suggest that EA, through the modulation of MC activity, can influence the release of their preformed mediators, including NGF, in allergic reactions.

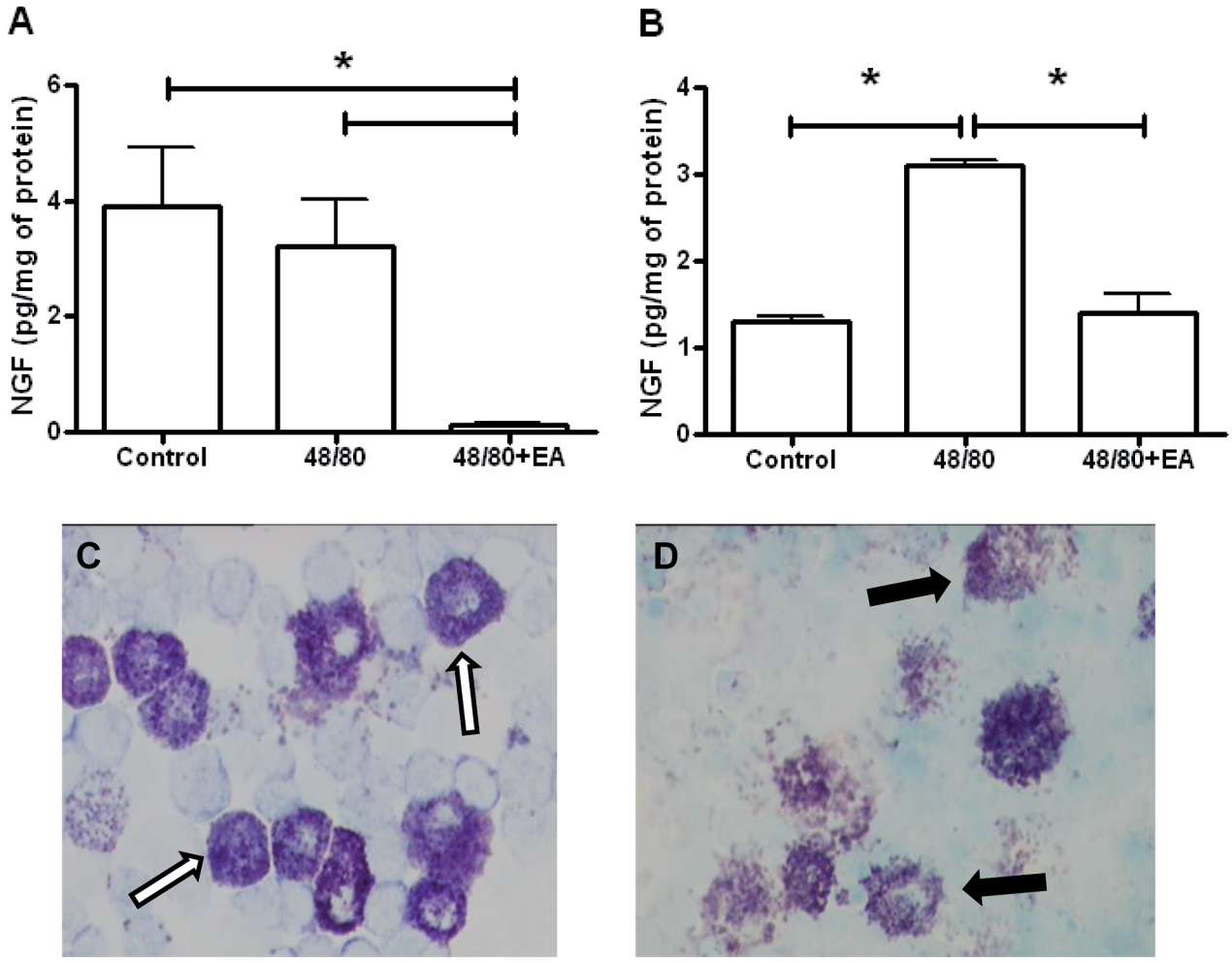

Fig. 1. - Effect of EA stimulation on peritoneal mast cells in a rat model of anaphylactic reaction induced by the compound 48/80. We used an experimental model of pharmacological induced anaphylactic reaction in adult rats, through injection of the mast cell (MC) activating compound 48/80. Rats were treated once a week for four weeks with compound 48/80. 48/80+EA rats were also treated with two weekly sessions of low frequency EA for four weeks.
Panel A: EA induced a decrease of NGF, measured by ELISA (R&D System, Minneapolis, USA) in peritoneal wash of 48/80-treated rats.
Panel B: Induction of anaphylactic reaction by treatment with compound 48/80 caused the rising of NGF content in the cellular component of peritoneal wash that was counteracted by EA.
Panel C-D: Cyto-centrifuge samples of peritoneal washes from Controls (C) and 48/80 (D) groups. Slides were stained with toluidine blue, to evidence the presence of MC. The increase of mast cells degranulation (black arrows) induced by compound 48/80 was not affected by EA. Examples of resting MCs are pointed by white arrows. Activated MCs are pointed by black arrows. Magnification x300.

Summary

The insertion of needles at the body surface and their manipulation or stimulation by means of electrical currents triggers a number of reactions at the site of insertion, the spinal level and centrally in the brain (see Fig. 2). EA stimulation induces variation in neural activity throughout the nervous system, and the synthesis and release of a number of neuromodulators is affected, with consequences that might have therapeutic value in a large number of disease states. Recently the link between EA and the neurotrophin NGF has been investigated, and their possible synergistic clinical use suggested. Both the treatment with EA and of its use as a supportive therapy together with NGF administration have been demonstrated in preclinical studies to affect reproductive organs behavior, regulating gameto-

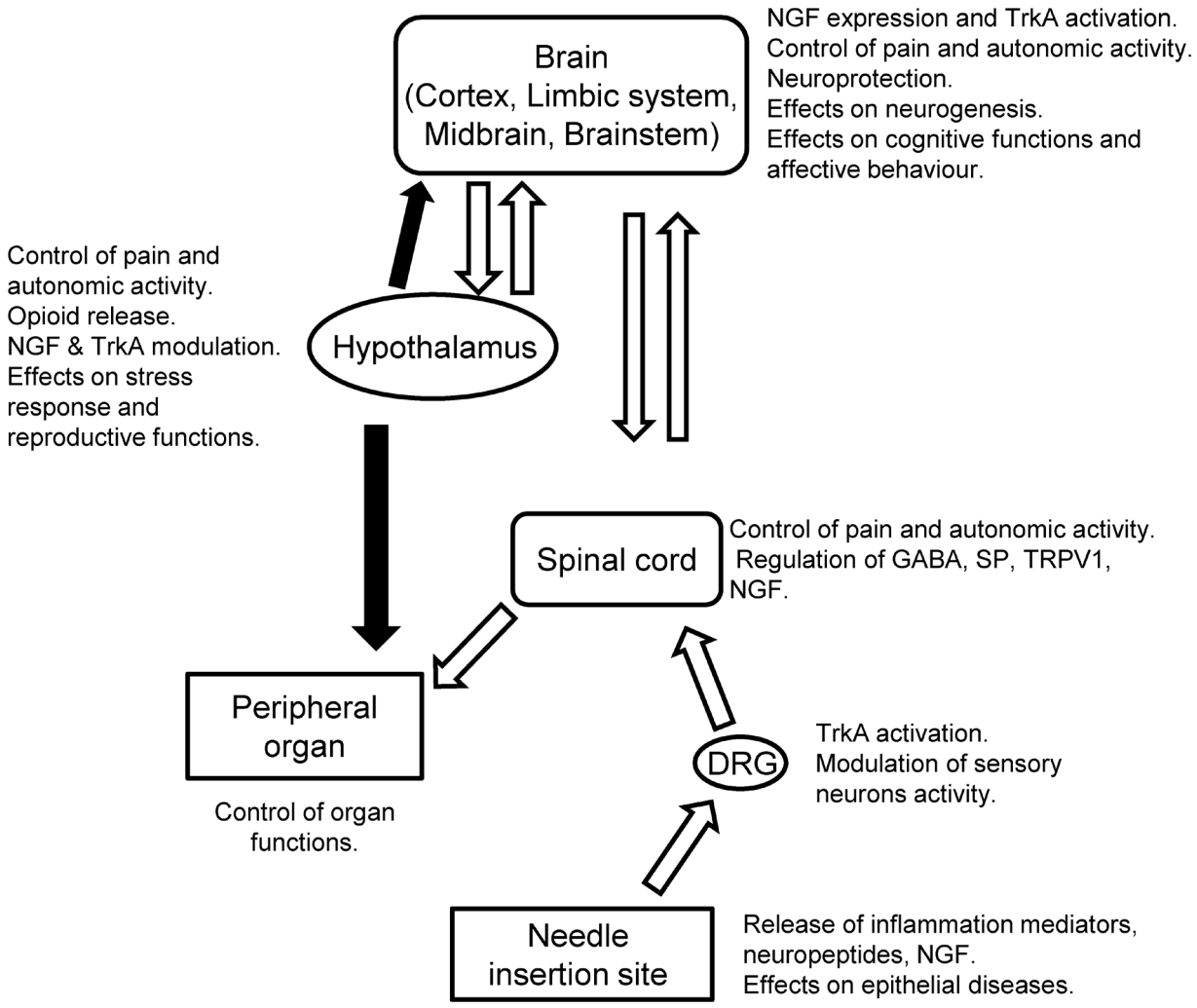

Fig. 2. - Schematic representation of neural pathways activated by EA treatments. The insertion of needles at peripheral sites and their stimulation by electrical currents induces the local release of tissue mediators of inflammation and activates the sensory spino-thalamic and spino-hypothalamic pathways. Neural (white arrows) or neuro-hormonal (black arrows) output is generated by stimulation in a series of relay stations along the afferent pathway. Beside every relay station, the relevant effects of EA stimulation linked to modulation of NGF system are indicated. Every NGF-related effect of EA could be considered as a possible therapeutic target for disease of the nervous, endocrine and immune systems. DRG: dorsal root ganglion; for other abbreviations, see the text.

genesis and fertility, autonomic nervous system and the stress response, the pain modulating system, the central cholinergic system and immunological functions regulating allergic response. The possibility to support NGF administration with EA in neurological, endocrine and immune diseases may represent a novel therapeutic approach, integrating Western pharmacology and physical therapy of traditional Chinese medicine.

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