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Neurotrophins and acupuncture

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ABSTRACT

The aim of this review is to report recent findings and ongoing studies on the effects of acupuncture on endogenous biological mediators, in particular on neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Acupuncture is a therapeutic technique and is a part of Traditional Chinese Medicine (TCM). 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. The established research on the neuro-physiological correlates of acupuncture has pointed towards endogenous opioids as the principal biological mediators of the therapeutic actions of this ancient technique. More recently, several classes of molecules, such as neurotransmitters, cytokines and growth factors, have also been identified as possible mediators for specific acupuncture effects. This review will focus on the links between acupuncture and a class of growth factors known as neurotrophins (NTs), which are the main mediators of neural activity, plasticity and repair following neurodegeneration and/or traumatic injury. A special emphasis will be placed on the work of our laboratory investigating the role of nerve growth factor (NGF), the prototypical member of the neurotrophin family, as a mediator of acupuncture effects in the central nervous system (CNS) and as a modulator of sensory and autonomic activity.

1. Acupuncture from a western perspective

Acupuncture, which is part of Traditional Chinese Medicine, is a therapeutic technique and a system with empirical basis; it has been used in the treatment and prevention of disease for centuries in China and more recently in Western countries. Traditionally, acupuncture has been used to treat many different symptoms and diseases, such as acute infection and inflammation, dysfunction of autonomic nervous system, pain, metabolic disease, and peripheral and central nervous system injury and/or degeneration.

Acupuncture is a potent form of sensory stimulation. Most scientific studies of acupuncture have focused on sensory stimulation as the main mechanism (Andersson and Lundeberg, 1995; Zhao, 2008). 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 proposed that the effects of acupuncture are correlated to activation of type A and C sensory nerve fibers (Zhao, 2008). Particular significance has been given to a group of receptors in skeletal muscles, that both have low and high thresholds for mechanical stimulation and are innervated by A-delta and possibly C-fibers. Physiologically, these receptors are activated by strong muscle contractions, and their stimulation by acupuncture results in the activation of physiological processes similar to those resulting from physical exercise (Andersson and Lundeberg, 1995; Kaufman et al., 1984; Kniffki et al., 1981). Another group of receptors, named polymodal receptors (PMRs) have also been proposed as possible mediators for the effects of acupuncture and moxibustion (Kawakita, 1991; Kawakita and Gotoh, 1996; Kawakita et al., 2006). PMRs are free nerve endings receptors characterized by responsiveness to both mechanical and thermal stimuli (not necessarily noxious) (Kawakita and Gotoh, 1996; Kawakita et al., 2006). The activation of PMRs could explain the overlap in effects of techniques based on mechanical (acupuncture) or thermal (moxibustion) stimulation applied at the same anatomical point on the body surface.

Both acupuncture and physical exercise release endogenous opioids, which seem to be essential in the induction of acupuncture-mediated functional changes of different organ systems (Andersson and Lundeberg, 1995). Particular interest has been dedicated to β-endorphin, an endogenous opioid that influences a variety of hypothalamic and autonomic functions and is important in the regulation of pain perception, stress response, mood and immune functions (Holden et al., 2005; Vassilakopoulos et al., 2005). Indeed, the identification of β-endorphin as the factor mediating the pain-relieving effects of acupuncture represents a milestone in the history of acupuncture

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research (Kaptchuk, 2002). However, other systems may be involved in the acupuncture modulation of stress, pain, autonomic activity and immune systems. Research carried out in the last 20 years has shown that through acupuncture it is possible to affect synthesis, release and action of several neurotransmitters (catecholamine, glutamate, acetylcholine, GABA, and serotonin) and neuropeptides (oxytocin, NPY, CCK, VIP, SP, CGRP, and PACAP, among others) in both the central and peripheral nervous systems (CNS and PNS, respectively) (Carlsson, 2002; Hole and Berge, 1981; Kaptchuk, 2002; Ulett et al., 1998; Zhao, 2008; Zijlstra et al., 2003).

2. Neurotrophins

The neurotrophin (NT) family of proteins is responsible for the growth and survival of neurons during development and for the maintenance and function of adult neurons (Ernfors, 2001; McAllister, 2001; Poo, 2001; Rush et al., 1997). NTs also promote regeneration of damaged axons after various peripheral and central nervous system injuries (Chao et al., 2006; Connor and Dragunow, 1998; Olson, 1993b; Terenghi, 1999).

The first NT to be discovered as able to promote neuronal survival and neurite outgrowth was nerve growth factor (NGF). NGF is 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). For over 35 years, NGF has been considered to be a very powerful and selective growth factor for sympathetic and sensory neurons and for cells derived from the neuronal crest (Alleva et al., 1993; Cowan, 2001; Rush et al., 1997). In the CNS, the greatest quantities of NGF are produced in the cortex, the hippocampus and in the pituitary gland; although significant quantities of this NT are also produced in other areas, including the basal ganglia, thalamus, spinal cord and in the retina (McAllister, 2001).

Trophic effects are exerted by NGF on NGF-responsive neurons both during development and in adulthood (Alleva et al., 1993; Aloe et al., 2002; Aloe et al., 1994; Cowan, 2001; Maestripieri et al., 1990; McAllister, 2001; Rush et al., 1997; Taglialatela et al., 1991). The spectrum of NGF action was once considered limited to differentiation functions related to specific central and peripheral neuronal classes; more recent studies have revealed that NGF actions also extended to other kinds of non-neuronal cells. Indeed, NGF is implicated in the functioning of the immune-hematopoietic system (Aloe, 2001; Aloe et al., 1997; Bonini et al., 1999) and in the neuro-endocrine system (Aloe et al., 1997). NGF can affect the maintenance of a balanced interplay between the nervous, endocrine and immune systems (Alleva et al., 1993; Aloe et al., 2002; Aloe et al., 1999).

The second NT to be characterized after NGF was brain-derived neurotrophic factor (BDNF). This factor is found in a range of tissue and cell types, such as the retina, CNS, motor neurons, PNS and peripheral tissues (Lindsay, 1996; Mufson et al., 1999; Skup, 1994). Activity of BDNF in the hippocampus, cortex, and basal forebrain plays a crucial role in the process of learning and memory (Lee and Son, 2009; Lu and Gottschalk, 2000; Yamada et al., 2002; Yamada and Nabeshima, 2003). Mice with BDNF knocked out suffer developmental defects in the brain and the sensory nervous system, and usually die soon after birth, which suggests an important role of BDNF in normal neural development (Bartoletti et al., 2002; Vitalis et al., 2002). Several studies have established links between low levels of BDNF and conditions such as depression, schizophrenia, Alzheimer's disease, Huntington's disease, Rett syndrome, and dementia (Pezet and Malcangio, 2004; Schulte-Herbruggen et al., 2007). Moreover, stress and the stress hormone corticosterone have been shown to reduce brain BDNF levels in rats, leading to hippocampus atrophy (Duman and Monteggia, 2006; Schaaf et al., 2000), a condition described in humans suffering from chronic depression.

The neurotrophin-3 (NT-3) is closely related to both NGF and BDNF. It may be involved in the maintenance of the adult nervous system, and may affect embryonic neuronal development. The first NT to be expressed in the PNS through embryogenesis, NT-3 can sustain either mitogenesis or the exit of neuronal progenitors from the cell cycle. During perinatal development, NT-3 supports the survival and differentiation of sensory neurons, many of which become dependent on NGF during later stages of development (Lewin and Barde, 1996). In the CNS, expression levels of NT-3 are highest during perinatal development, where the most prominent expression is in the hippocampus, the neocortex, and the cerebellum (Zhou and Rush, 1994). In the adult, expression levels of NT-3 and BDNF are comparable in most areas of the brain (Ernfors et al., 1990; Maisonpierre et al., 1990). NT-3 is also an important factor for the development and maintenance of enteric nervous system (Chalazonitis, 2004). NT-3 has been shown to be elevated in the cerebrospinal fluid of children with hydrocephalus (Hochhaus et al., 2001) and in the skin of diabetic patients (Kennedy et al., 1998). Preclinical and clinical trials suggest a possible pharmacological use for NT-3 in the treatment of Charcot-Marie-Tooth disease type 1A (Sahenk et al., 2005) and in functional constipation (Parkman et al., 2003).

The latest NT to be discovered is NT-4/5 (Hallbook et al., 1991; Ip et al., 1992). Expression of NT-4/5 is prominent in the postnatal hippocampus, neocortex, cerebellum and in the thalamic nuclei, and continues until adulthood (Friedman et al., 1998). Trophic effects of NT-4/5 have been described in cultures of noradrenergic neurons from locus coeruleus (Friedman et al., 1993) suggesting neurotrophic influences of NT-4/5 in this brain region. NT-4/5 is also produced in rat skeletal muscle in an activity-dependent manner (Funakoshi et al., 1995), with a role in the growth and remodeling of adult motor neuron innervation. While knockout of any other NTs has proven lethal during early postnatal development, mice lacking NT-4/5 have only shown unimportant deficits and develop normally to adulthood (Ibanez, 1996).

The biological actions of neurotrophins are exerted via specific receptors: tropomyosin kinase receptors (Trk) A, B and C, which are typical tyrosine kinase receptors (Huang and Reichardt, 2003). Different neurotrophins selectively bind to the subtypes of Trk receptors: NGF activates TrkA, BDNF and NT-4/5 bind to TrkB, and NT-3 binds to TrkC. The major cytosolic/endosomal pathways activated by the Trk receptors are Ras-mitogen activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K)-Akt, and PLC-γ (Chao et al., 2006; Klesse and Parada, 1999; Reichardt, 2006). All of the neurotrophins also bind to, with approximately equal affinity, and activate the low-affinity, non-selective p75 neurotrophin receptor ($p75^{NTR}$). This receptor is a transmembrane glycoprotein that regulates signaling through TrkA (Friedman and Greene, 1999; Reichardt, 2006; Schor, 2005); in addition, binding of NGF to $p75^{NTR}$ activates additional signaling pathways that, in the absence of co-expressed TrkA, may signal a cell to die via apoptosis (Friedman and Greene, 1999; Miller and Kaplan, 2001; Schor, 2005).

3. The link between acupuncture and NGF: established observations

Interest in the possible therapeutic benefits of NGF has increased during the last two decades (Allen and Dawbarn, 2006; Aloe, 2004; Apfel and Kessler, 1995; Apfel et al., 1998; Chiaretti et al., 2005; Eriksdotter Jonhagen et al., 1998; Lambiase et al., 2000; McArthur et al., 2000; Olson, 1993a; Seiger et al., 1993; Tuszynski et al., 2005; Tuveri et al., 2000). Preclinical and clinical trials have been conducted to investigate the potential role of NGF in the treatment of central and peripheral neurodegenerative diseases, peripheral neuropathies, and more recently epithelial derangements with neurotrophic origin, such as those acting in corneal, diabetic and pressure ulcers, and in

vasculopathies (Allen and Dawbarn, 2006; Apfel et al., 2000; Bonini et al., 2000; Eriksdotter Jonhagen et al., 1998; Koliatsos et al., 1991; Lambiase et al., 2003; McArthur et al., 2000; Seiger et al., 1993). Nevertheless, 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 nasally (Capsoni et al., 2009; Chen et al., 1998; Yasuno et al., 2000) and intracerebroventricularly (i.c.v.) in subjects affected by Alzheimer's Disease (AD) (Eriksdotter Jonhagen et al., 1998; Seiger et al., 1993), 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 (Aloe, 2004; Generini et al., 2004; Tuveri et al., 2000). Despite promising early results, patients in most investigations experienced side effects such as weight loss, pain 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.

Early data on the correlation between NGF and acupuncture, came from the work on the estradiol-valerate (EV)-induced polycystic ovary (PCO) in rat (Bai et al., 2004; Manni et al., 2005c; Stener-Victorin et al., 2003; Stener-Victorin et al., 2000) and then on CNS physiology and pathology. It was first demonstrated that 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 also provided support for the theory that EA inhibits hyperactivity in the sympathetic nervous system (Cao et al., 1983; Chao et al., 1999), which has been indicated as a major pathogenetic cause of PCO (Barria et al., 1993; Lara et al., 2000; Lara et al., 2002; Lara et al., 1993). Supporting this hypothesis, in a following study (Stener-Victorin et al., 2003) the efficacy of EA was demonstrated in reducing ovarian NGF along with ovarian and hypothalamic sympathetic activation markers. However, further studies on the effects of EA on brain and ovarian NGF content in EV-induced PCO models (Bai et al., 2004) indicated that the efficacy of EA treatments was confined to peripheral field, since it reduced the ovarian but not the hypothalamic NGF over-expression induced by EV.

Exploring the mechanisms underlying the EA effect on ovarian NGF expression and activity in EV-induced PCO, we demonstrated that an interplay among the NGF/NGF receptor system and adrenergic responsiveness characterizes the development of ovarian pathology in this rat model (Manni et al., 2005a; Manni et al., 2006; Manni et al., 2005b; Manni et al., 2005c). Indeed, EV injection alters the ovarian content of both TrkA and $p75^{NTR}$, at the same time deregulating α_1- and β_2-adrenergic receptors (ARs). Neutralization of endogenous NGF reversed most of the abnormalities in ovarian ARs and NGF receptors and expression of the sympathetic marker tyrosine hydroxylase (TH) (Manni et al., 2006). Almost identical results were obtained through EA treatment (Manni et al., 2005c), demonstrating the sympathetic tone-lowering capacity of the technique, and showing that the results were probably achieved through the actions of EA on the NGF system. Interestingly, similar results were also found when EV-injected rats experienced voluntary physical exercise for five weeks (Manni et al., 2005a), further confirming that the effects of acupuncture are based on physiological and anatomical substrates that are common with physical exercise. In recently published studies on a dihydrotestosterone (DHT)-induced PCO syndrome (PCOS) model, fulfilling the criteria of human PCOS, the group of Elisabet Stener-Victorin at Gothenburg University (Sweden) 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 NGF mRNA (Manneras et al., 2009). Overall, the above-reviewed results support a general consideration: at least some of 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 branch, which is associated with a peripheral down-regulation of NGF in organs.

The modulatory action exerted by EA on NGF in the peripheral field seems to take place not only in target organs but also in PNS structures. Indeed, it has been reported that both NGF mRNA and the number of NGF-expressing neurons are increased by EA in the spinal cord of cat after dorsal rhizotomy (Wang et al., 2007), indicating an involvement of the neurotrophin in the EA-induced spinal plasticity after injury. EA also increases both NGF mRNA and protein levels in spared L6 dorsal root ganglion (DRG) neurons after removal of adjacent ganglia (Chen et al., 2007), further supporting a neurotrophin-based plasticity phenomenon induced by EA. In a recent study, we demonstrated that the action of acupuncture at the sensory neuron level could allow integration between NGF administration and EA (Aloe and Manni, 2009). 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). One report also addressed the anti-inflammatory properties of acupuncture and associated such therapeutic results with the acupuncture-induced variations of peripheral NGF content (Chae et al., 2007).

While evidence for an acupuncture-specific CNS response is still emerging from histochemical, biochemical, molecular and neuroimaging studies (Campbell, 2006; Guo et al., 1996; Hole and Berge, 1981; Hui et al., 2000; Kwon et al., 2000; Wu et al., 1999; Zhang et al., 2004), few investigations have addressed the link between acupuncture effects and NGF modulation in the brain and other CNS structures. 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 (VEGF) (Pagani et al., 2006). Indirect evidence pointing to an NGF involvement in the EA-induced CNS response comes from receptor-activation studies. Applied to 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). Using protein kinase inhibitors of specific intracellular signaling pathways, the authors 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 demonstrated in further papers that EA stimulation can reverse the ischemia-induced increase of TRPM7, an ion channel implicated directly as central components of neuronal death pathways (Aarts and Tymianski, 2005), by enhancing TrkA activity, which triggers the downstream PI3K pathway (Zhao et al., 2005; Zhao et al., 2007).

4. The actions of acupuncture could be mediated by all NTs

Beside NGF, all members of the neurotrophin family seem to be potential candidates for mediating the biological actions of acupuncture (see Table 1). Indeed, the possibility of using EA-induced NT modulation in animal models of neurological and neurodegenerative disease has been investigated in the last few years. EA stimulation at acupoint ST-36 has been proven effective in restoring hippocampal BDNF mRNA that was declined by immobilization stress, suggesting that EA may relieve the neuropathological effects of stress by modulating brain NTs (Yun et al., 2002). The effect of EA on brain BDNF has been also studied in a mouse model of Parkinson's Disease (PD) (Liang et al., 2002). It was found that high-frequency EA is effective in reducing the degeneration of dopaminergic neurons in the ventral midbrain and that this could be

Table 1
Some of the effects of acupuncture might be mediated by NTs. Below the results obtained in experimental studies are summarized.

Experimental model	Animal	Stimulation	Summary of results	NT involved	Reference
EV-induced PCO	Rat	Low-frequency EA	— EA reverses EV-induced ovarian NGF up-regulation. — Normalization of ovarian TrkA, $p75^{NTR}$, adrenoceptors and tyrosine hydroxylase.	NGF	(Bai et al., 2004; Manni et al., 2005c; Manni et al., 2005c; Stener-Victorin et al., 2003; Stener-Victorin et al., 2000)
DHT-induced PCOS	Rat	Low-frequency EA	— Improvement of ovarian morphology and function in DHT-treated rats. — EA reverses DHT-induced NGF-, ADRB3- and NPY-mRNA up-regulation in mesenteric adipose tissue. — Decrease of DHT-induced NPY-mRNA up-regulation in mesenteric adipose tissue.	NGF	(Manneras et al., 2009)
Spinal rhizotomy	Cat	High-frequency EA	— Increase of NTs expression in spared L6 DRG.	NGF, BDNF, NT-3, NT-4	(Chen et al., 2007; Liu et al., 2009; Wang et al., 2007)
Spinal transection	Rat	Mixed frequency EA	— Promotion of grafted stem cells survival and migration toward lesioned site. — Increase of spinal NT-3 expression in lesioned/transplanted rats.	NT-3	(Chen et al., 2008)
Spinal transection	Rat	Mixed frequency EA	— Improvement of grafted bone marrow mesenchimal stem cells survival and differentiation. — Increase of spinal NT-3 expression in lesioned/transplanted rats. — Functional recovery of lesioned/transplanted rats.	NT-3	Ding et al. (2009)
MFB transection	Rat	Different frequency EA	— 100 Hz stimulation prevents degeneration of dopaminergic neurons. — 100 Hz stimulation induces BDNF mRNA in ventral midbrain.	BDNF	Liang et al. (2002)
Middle cerebral artery occlusion	Rat	Mixed frequency EA	— Down-regulation of NMDA-NR1subunit. — Up-regulation of TrkA. — Activation of the PI3-K pathway.	NGF (TrkA)	Sun et al. (2005)
Neuropathic pain	Rat	Low-frequency EA	— Improvement of NGF-induced thermal hyperalgesia. — Normalization of NGF-induced variation of TRPV-1 content in DRG and paw skin. — Modulation of SP contents in DRG and paw skin.	NGF	Aloe et al. (2008a)
Retinitis Pigmentosa	Rat	Low-frequency EA	— Increase of NGF and BDNF in the retina of RCS rats. — Increase of TrkA and TrkB receptors in the retina of RCS rats. — Increase of VEGF expression and improvement of retina vascularization in RCS rats.	NGF, BDNF	Pagani et al. (2006)
Carrageenan-induced inflammation	Rat	Manual acupuncture	— Inhibition of inflammatory response. — Decrease of tissue NGF.	NGF	Chae et al. (2007)
Immobilization stress.	Rat	Low-frequency EA	— BDNF mRNA expression was decreased by stress and normalized by EA, in rat hippocampus.	BDNF	Yun et al. (2002)

supported by the EA-induced up-regulation of BDNF in the same area (Liang et al., 2002). The link between acupuncture and BDNF has also been investigated in a rat model of stroke with loss of motor function (Kim et al., 2009). The authors demonstrated that, at least in the early recovery stage, no additional effects of low-frequency EA on motor recovery, brain BDNF or TrkB levels were evident. However, a prolonged treatment period could elicit different results (Kim et al., 2009).

Modulation of the neuroprotective effects of EA by NTs has also been demonstrated in an animal model of spinal neuroplasticity. A significantly higher number of NGF-, BDNF- and NT-3-positive neurons were observed in the cat spinal cord after partial dorsal rhizotomy and high-frequency EA, indicating that the spinal plasticity promoted by EA could be induced by increased levels of NTs (Wang et al., 2007). Using the same experimental model, it has also been demonstrated that high-frequency EA up-regulates both mRNA and levels of NGF, BDNF and NT-3 in spared L6 dorsal root ganglion (DRG) neurons, extending to primary sensory neurons the hypothesis of a neurotrophin-mediated effect of EA on plasticity in damaged neurons (Chen et al., 2007). The same observations have also been extended to NT-4, suggesting an involvement of the entire NT family in mediating EA-induced spinal cord plasticity after peripheral nerve lesion (Liu et al., 2009).

Recently, the effects of EA on the survival and migration of transplanted stem cells in the injured spinal cord have been correlated to spinal NT-3 content in rats (Chen et al., 2008). The results showed that an increased number of surviving stem cells, as well as their migration length toward caudal tissue, were associated with increased spinal NT-3 levels after EA treatment in spinally transected rats (Chen et al., 2008). A further paper demonstrated that mixed-frequency (alternating strings of dense and sparse, 2 and 60 Hz frequencies) EA treatment promotes grafted stem cell survival and differentiation, as well as increased spinal NT-3, parallel to functional recovery in spinal transected rats (Ding et al., 2009).

5. The research on acupuncture and NTs: future directions

Therapeutic strategies for human diseases based on the biological mechanisms underlying the link between acupuncture and NTs, should be based on: the ability of sensory stimulation through acupuncture-based techniques to modulate central and peripheral expression and activity of NTs, according to the disease state and the general homeostatic properties of acupuncture; and the possibility of integrating the pharmacological use of NTs with acupuncture treatment.

The ability of acupuncture and EA to modulate NT content in both CNS and peripheral tissues and exert therapeutic effects on animal models of pathologies has been extensively described in the last

decade and reviewed above. Results of the different studies show that tissue NT content may be differentially influenced by acupuncture, and may mainly depend on the experimental conditions (see Table 1). Apparently controversial results indicate that different mechanisms and biological mediators are probably responsible for the variation of NTs induced by EA. As for peripheral organ NT content, based on the results from EV-induced rat PCO, it is possible to hypothesize that the effects of EA on sympathetic tone drive the tissue expression and content of NGF (Manni et al., 2005a; Manni et al., 2006; Manni et al., 2005c; Stener-Victorin et al., 2003; Stener-Victorin et al., 2000). Indeed, NGF gene expression could be down-regulated by the EA-mediated reduction of sympathetic activity and ARs challenged by norepinephrine (NE). This is supported by findings upon the regulation of NGF by catecholamine and by the activation of β_2-AR (Colangelo et al., 2004; Culmsee et al., 1999; Samina Riaz and Tomlinson, 2000; Semkova et al., 1996). Thus, mechanisms based upon the EA modulation of neurotransmitters and neuromodulators could be responsible for the change in NTs in peripheral organs and nervous tissues. It is conceivable that NTs could be also regulated by neurotransmitters such as serotonin (Angelucci et al., 2000; Krzan et al., 2001) and/or by neuropeptides such as colecystokinin-8 (CCK-8) (Manni et al., 2001; Manni et al., 2000; Manni et al., 2002; Tirassa et al., 1999; Tirassa et al., 1998) which are both affected by EA (Bian et al., 1993; Han et al., 1986; He et al., 1992; Takagi and Yonehara, 1998; Xing et al., 2007). Confirming the latter hypothesis, our preliminary data suggest that CCK-8 antagonists partially block the EA-induced regulation of tissue NGF content (Manni et al., unpublished).

Different stimulation modalities could also account for the results obtained by different investigators. Indeed the high-frequency EA seems to elicit a general increase of NTs, at least in neuronal tissues (Chen et al., 2007; Liang et al., 2002; Liu et al., 2009; Wang et al., 2007), while low-frequency EA induces both a decrease and an increase of tissue NT content, depending on the experimental conditions and models used (Bai et al., 2004; Manneras et al., 2009; Manni et al., 2005b; Manni et al., 2005c; Pagani et al., 2006; Stener-Victorin et al., 2003; Stener-Victorin et al., 2000; Yun et al., 2002). Stimulus modality represents an intriguing future research possibility that could adequately elucidate the mechanism(s) involved in NT modulation by acupuncture. Preliminary data from our laboratory indicate that 4 treatment with

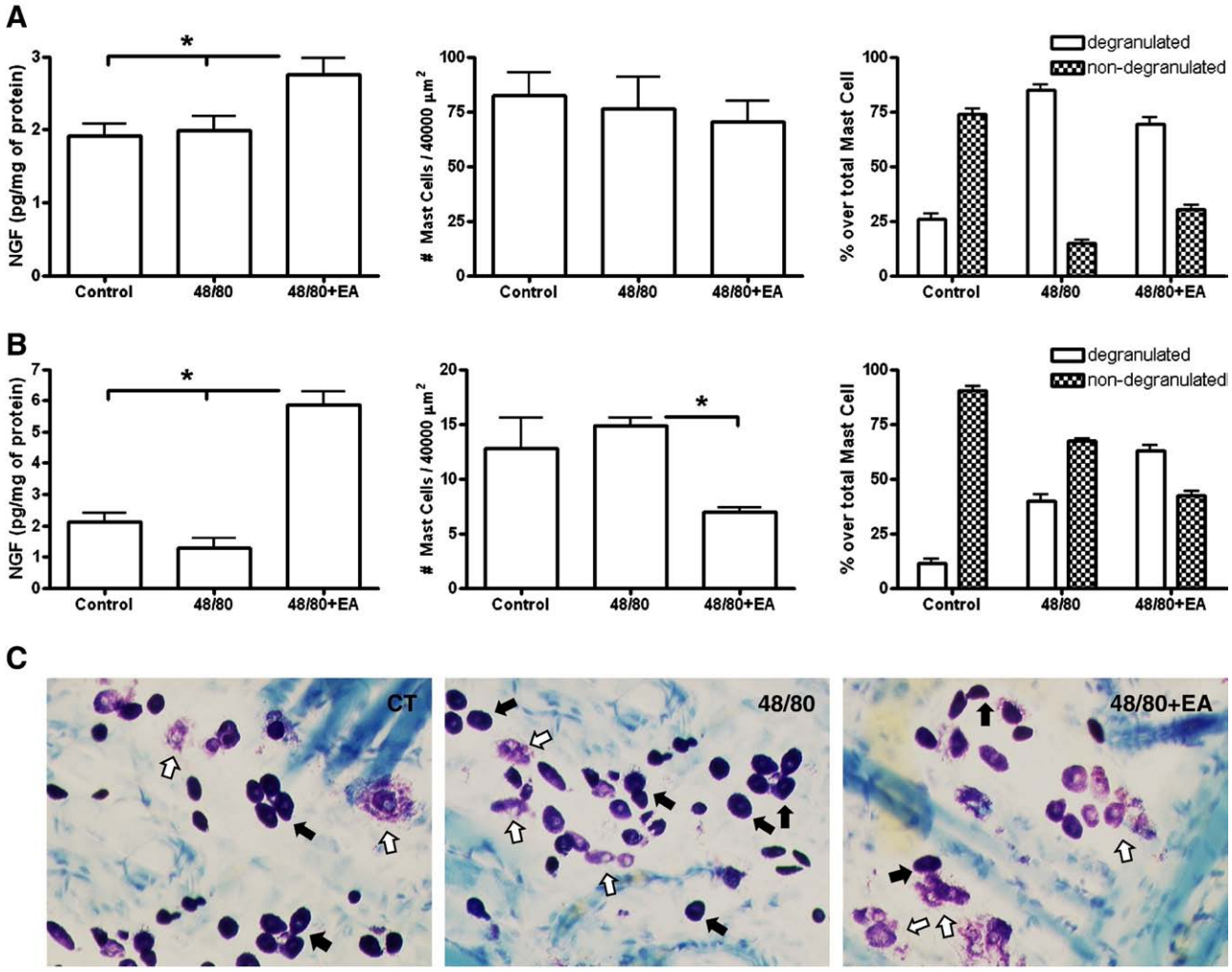

Fig. 1. Both mast cells (MCs) and NGF have shown to play an important role during the allergic reaction. We hypothesized that the interplay between MCs and NGF could be modulated by the treatment with EA. We used an experimental model of pharmacological induced anaphylactic reaction in adult rats, through injection of the MC activating compound 48/80. Rats were divided in 3 experimental groups: CT: non-treated controls; 48/80: treated once a week for four weeks with compound 48/80; 48/80 + EA: treated with 48/80 + 2 weekly sessions of low-frequency EA for four weeks. Panel A: EA induced an increase of NGF in the lung of 48/80-treated rats. Total MCs number was not affected by any of the treatments, while the percentage of intact or activated (degranulated) MCs was quite different among the experimental groups, pointing at EA as a possible complementary therapy in the treatment of allergic asthma. Panel B: EA induce a significant increase of NGF and a reduction of MCs number in the tongue of 48/80-treated rats. The fraction of activated MCs is increased by 48/80 and further increased by EA. Thus, despite the EA-induced decrease of MC number, this data advise against the use of EA in allergic disease manifestation such as the urticaria–angioedema. Panel C: Section of tongue tissue were stained with toluidine blue, to evidence the presence of MC. Examples of resting MCs are pointed by black arrows. Activated MCs are pointed by white arrows. Magnification ×300.

high-frequency, but not low-frequency, EA given at acupoint ST36 in unconscious healthy mice effectively enhances NGF synthesis in selected brain areas (hippocampus, hypothalamus, and striatum), with a parallel modulation of cholinergic and adrenergic synthesis enzymes, choline acetyl-transferase (ChAT) and tyrosine hydroxylase (TH) (Manni et al., unpublished).

The possibility of using acupuncture treatment as a support for the pharmacological use of NTs represents a further therapeutic value arising from the established link between acupuncture and NTs. This hypothesis has been tested in a study exploring the combined use of low-frequency EA and NGF(Aloe and Manni, 2009). 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. The results were highly encouraging: EA is effective in reducing NGF-induced hyperalgesia, and a clinical test of this hypothesis seems valuable. Furthermore, the supporting role of acupuncture and NTs for stem cell transplantation has been explored. (Chen et al., 2008; Ding et al., 2009), indicating a promising clinical research development.

A number of recent studies have also investigated the effect of EA on immune cells and/or immunological markers and found that EA can influence the synthesis and expression of pro- and anti-inflammatory molecules as well as allergic response (Cabioglu and Cetin, 2008). For example, EA can modulate the activation of mast cells that are known to be functionally implicated in allergic mechanisms (Wu et al., 2008; Zhang et al., 2008). Using laboratory animals, we recently investigated the effects of low-frequency EA on mast cell activation and release of NGF. We found that repeated EA stimulation in unconscious adult rats differentially affects peritoneal, lung and dermal mast cells (Manni et al., unpublished data), as shown in Fig. 1. Since mast cells are critically involved in allergic responses (Bonini et al., 1999), these observations suggest that EA, through the modulation of mast cell activity, can influence the release of preformed mast cell mediators of allergic reactions, including NGF, and participate in allergic responses and tissues homeostasis.

6. Conclusions

The insertion of needles in the body surface and their manipulation or stimulation by means of electrical currents, initiate a number of reactions at the spinal level and centrally in the brain. As a consequence of the acupuncture-induced variation in neural activity, the input and output of neural circuits, and throughout the CNS, 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.

The intersection of the huge basic NT research field with research on neuro-physiological correlates of acupuncture widens the comprehension of the biological mechanisms regulating the synthesis, secretion and utilization of NTs in both physiological and pathological conditions.

Based on the presently published studies, the relationship between the therapeutic efficacy of acupuncture-based techniques and NT expression and activity in both the CNS and in the peripheral field is undoubtedly emerging. Since major problems could be associated with the pharmacological approach of NT administration, such as the selection of the site and modality of delivery (Lambiase et al., 2007; Thorne and Frey, 2001; Tuszynski et al., 2005), and the unpleasant and sometimes dangerous side effects reported after pre-clinical and clinical trials (Day-Lollini et al., 1997; Eriksdotter Jonhagen et al., 1998; Winkler et al., 1997), the development of acupuncture-based treatment protocols aimed at modulating endogenous NTs could represent a suitable alternative to the direct delivery of NTs.

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