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Neural mechanism of electroacupuncture's hypotensive effects

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ABSTRACT

EA at P 5–6 and S 36–37 using low current and low frequency may be able to reduce elevated blood pressure in a subset of patients (~70%) with mild to moderate hypertension. The effect is slow in onset but is long-lasting. Experimental studies have shown that EA inhibition of cardiovascular sympathetic neurons that have been activated through visceral reflex stimulation is through activation of neurons in the arcuate nucleus of the hypothalamus, vlPAG in the midbrain and NRP in the medulla, which, in turn, inhibit the activity of premotor sympathetic neurons in the rVLM. The arcuate also provides direct projections to the rVLM that contain endorphins. Glutamate, acetylcholine, opioids, GABA, nociceptin, serotonin and endocannabinoids all appear to participate in the EA hypotensive response although their importance varies between nuclei. Thus, a number of mechanisms underlying the long-lasting effect of EA on cardiovascular function have been identified but clearly further investigation is warranted.

Hypertension affects approximately 1 billion individuals worldwide (Hajjar and Kotchen, 2003). Although a number of treatment strategies have been developed for this disease, therapy has not been perfected and often is associated with adverse side effects. Acupuncture is a potential alternative to pharmacological therapy (WHO, 2003). Drug therapy indiscriminately blocks many receptors that can lead to a multiplicity of side effects. Acupuncture, on the other hand, is associated with a very low (0.13%) incidence of side effects (MacPherson et al., 2001; Mayer, 2000) and is relatively inexpensive. As such, there has been increasing interest worldwide in exploring alternative medical therapies like acupuncture for treating chronic conditions like hypertension (Eisenberg et al., 1993, 1998).

Chinese and Southeast Asian medical professionals have long utilized acupuncture, and its potent and more standardized alternative, electroacupuncture (EA), to treat disease. Electroacupuncture incorporates a small electrical current administered through needles from a battery driven device. Both clinical and basic science reports suggest that acupuncture's usefulness includes management of ischemic cardiac chest pain and hypertension (Ballegaard et al., 1986, 1990, 1991). There are a number of clinical reports suggesting that acupuncture can reduce blood pressure (BP) (Zhang, 1956; Tam and Yiu, 1975; Alshelevich et al., 1985; Akhmedov et al., 1993; Dan, 1998). However, there are weaknesses in prior acupuncture research. For example, power tests to determine proper sample size, randomization in the application of acupuncture in control and test subjects, and appropriate control groups and/or control acupoints are often missing. Furthermore, the period time for follow-up after acupuncture treatment generally has been inadequate. Many studies have not used ambulatory 24 hour blood pressure (BP) monitoring to provide comprehensive assessment of blood pressure and few studies have utilized EA, which, as noted above, can be easily standardized. In some studies patients have been on antihypertensive medications and many have used a large number of acupoints overlying neural pathways, whose efficacy has not been validated. Furthermore, for the most part, patients have not been divided into effective (~70%) and non-effective (~30%) groups, an important point to consider in acupuncture trials since it is well recognized that acupuncture only works in a subset of patients (Cao et al., 1983; Han, 1987; Li et al., 2004; Li and Longhurst, 2007). Thus, to evaluate definitively the effectiveness of acupuncture in the management of hypertension, rigorously designed and conducted randomized clinical trials still are needed to overcome many methodological limitations of previous investigations.

1. Clinical studies

In 2004 we reported that EA typically does not change BP significantly in healthy subjects at rest (Li et al., 2004). In contrast, ~70% of the healthy human during exercise demonstrate inhibition of the stress-induced increase in systolic and mean blood pressures (SBP and MBP) as well as the double product (SBP × heart rate, reflecting myocardial oxygen consumption) (Longhurst et al., 1980) following 30 min EA at P 5–6 acupoints (Li et al., 2004). Conversely, EA at control acupoints G 37–39 does not alter the exercise-related increase in blood pressure or the double product. This study confirmed the efficacy of the P 5–6 and LI 4 acupoints in lowering elevated blood pressure in humans and reinforced the role of G 37–39 as suitable control acupoints for evaluating non-specific placebo responses. The

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strong effect at P5–6 on exercise-induced elevations in BP and the absence of an influence of acupuncture at G 37–39 further reinforces the existence of point specific effects of acupuncture on cardiovascular function that result from stimulation of some but not other acupoints (Fig. 1).

More recently, in a preliminary study, we evaluated the influence of EA in patients with mild to moderate hypertension (BP 140–180/90–110 mm Hg) (Li and Longhurst, 2007). In this investigation, a group of 18 hypertensive patients without medication were assessed with 24 hour ambulatory BP monitoring and low frequency, low intensity EA at P 5–6 and St 36–37 once weekly for 30 min for eight weeks of therapy. Over a period of four to eight weeks of treatment their peak SBP and average SBP/24 h were reduced by approximately 18 and 10 mm Hg, respectively. Similar to our observations during exercise, diastolic BP and heart rate were unchanged by EA. Following termination of EA treatment the peak and average SBP remained low for four additional weeks, but over the next four weeks the BP of most patients returned to near their pretreatment level. The blood pressures of six other patients receiving EA for 30 min at control acupoints LI 6–7 and GB 37–39 once weekly for eight weeks were unaltered. These early data suggest that EA at select acupoints known to have a strong cardiovascular action, performed once weekly for 4–8 weeks, significantly reduces BP. This beneficial effect is slow in onset but persists for a prolonged period of time.

2. Mechanistic studies

The mechanism of the inhibitory effect of EA on cardiovascular function was first studied in the 1980s. Li and Yao (Yao et al., 1982a,b; Li, 1984) evaluating several experimental hypertensive animal models, including conscious dogs, spontaneously hypertensive rats and stress-induced hypertension in Sprague Dawley (SD) rats, demonstrated that EA reduces BP through activation of opioid receptors in the brain (Li and Yao, 1992; Xie et al., 1997). Chiu et al. (1997) reported that acupuncture treatment decreases BP in hypertensive patients in association with a decrease in plasma renin

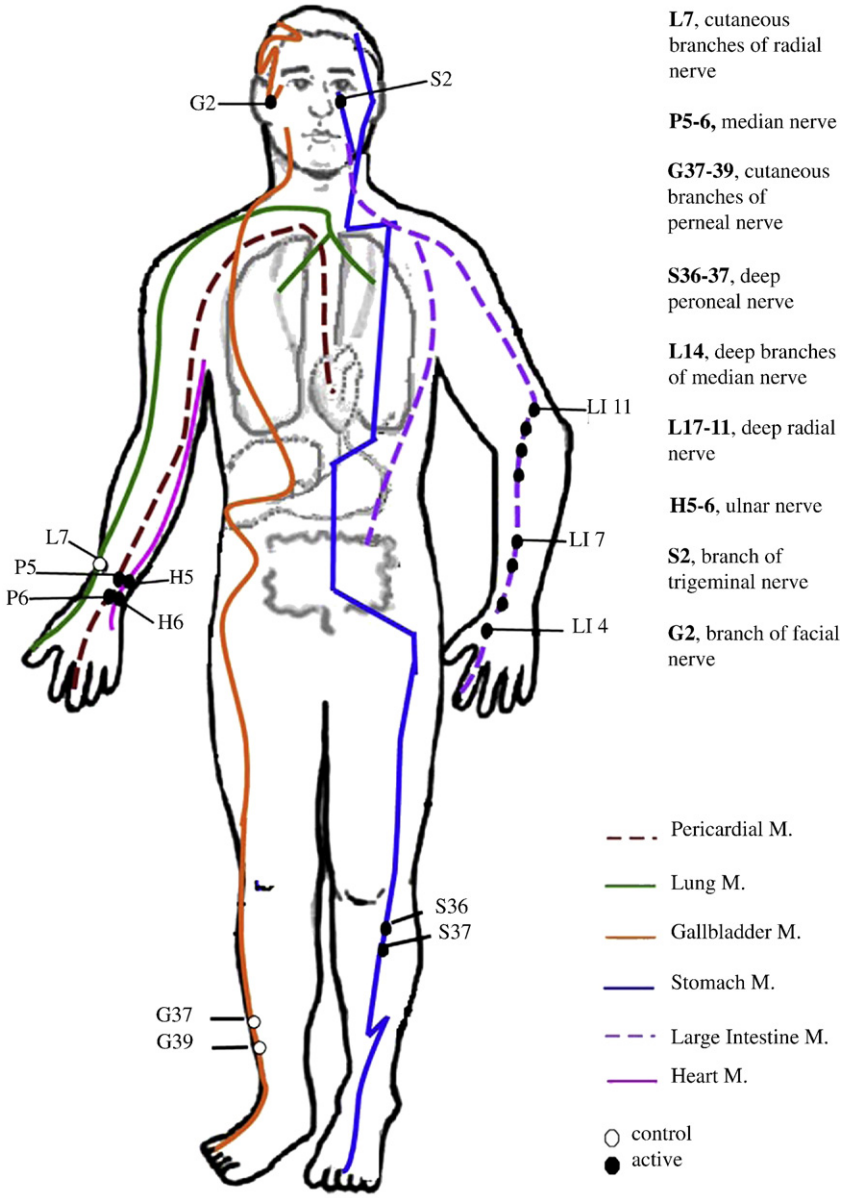

Fig. 1. Effective and ineffective acupoints in EA inhibition of reflex cardiovascular sympathoexcitation. ● effective acupoints — P 5, P 6, LI 4, S 36, S 37; ○ ineffective acupoints — L 7, G 37, G 39. Abbreviations: L — lung meridian (green line), P — pericardial meridian (brown line), LI — large intestine meridian (purple line), S — stomach meridian (blue line), G — gallbladder meridian (orange line).

activity. In the late 1990s we (Li and Longhurst) began our collaboration, resulting in a series of experimental studies on the mechanisms involved in low frequency EA regulation of cardiovascular function.

2.1. Acupoint specificity, afferent fibers and stimulation parameters

2.1.1. Point specificity

We have evaluated the effectiveness of activating different acupoints on reflex-induced increases in BP, caused by the gallbladder or splanchnic nerve (SN) stimulation in cats. This visceral reflex leads to stimulation of the sympathetic nervous system through activation of cardiovascular premotor sympathetic neurons in the rVLM. We observed that EA at P 5–6 (pericardial meridian, overlying the median nerve) and LI 10–11 (large intestine meridian, overlying the deep radial nerve) is most effective in its influence on cardiovascular excitatory reflexes (Fig. 1). EA at LI 4–L7 (large intestine and lung meridians, overlying branches of median and the superficial radial nerve) and S 36–37 (stomach meridian overlying the deep peroneal nerve) is less effective, while EA at LI 6–7 and K1-B67 does not influence BP. Furthermore, direct stimulation of the deep or superficial nerves underneath the acupoints produces similar results (Tjen-A-Looi et al., 2004; Li et al., 1998). Similar observations have been made in a rat model employing gastric distension (Zhou et al., 2005a,b).

2.1.2. Stimulation parameters and afferent fibers

Previous studies using compound action potential recording techniques suggest that finely myelinated fibers are the predominate pathway stimulated during low current, low frequency EA (Li and Yao, 1992; Cheung et al., 2001). In our feline model, we have demonstrated that both myelinated and unmyelinated nerve fibers are activated by stimulation of the median nerve (P 5–6 acupoints) by using a very small current (0.1 to 3.0 mA) to determine the motor threshold as a guide to achieve a similar stimulus intensity between animals. Of the 62 fibers recorded, 37% were C-fibers (<2.5 m/s) and 63% Aδ fibers (>2.5 m/s) (Li et al., 1998). Thus, both myelinated and unmyelinated fibers appear to participate in the acupuncture-cardiovascular effect. Recently, we observed that neonatal capsaicin-treated rats depleted of substance P from primary afferents are insensitive to the inhibitory EA effect during gastric distention. Thus, the inhibitory effect of EA at P 5–6 on cardiovascular excitatory reflexes involves unmyelinated group IV fibers (Tjen-A-Looi et al., 2005).

In our rat model of reflex hypertension, sham acupuncture involving needle insertion without manipulation at P 5–6 or LI 6–7 acupoints did not attenuate the gastric distention-induced reflex increase of BP, thus demonstrating that this procedure can serve as a control for EA. However, EA at P 5–6, H 6–7 (overlying the ulnar nerve) or S 36–37 with low current (2 mA) and low frequency (2 Hz) for 30 min inhibited the reflex increase in BP for 30–40 min. Increasing the stimulation frequency to 40 or 100 Hz did not inhibit the elevated BP. In this regard, we observed a reciprocal relationship between the frequency of stimulation and the afferent response. Thus, it appears that low frequency, low current EA in a point specific manner optimally influences reflex-induced hypertension (Zhou et al., 2005a,b).

2.2. EA inhibition of neural activity in the rostral ventrolateral medulla

Over the last ten years, our laboratory has demonstrated experimentally that EA at select acupoints, such as Neiguan–Jianshi along the pericardial meridian (P 5–6) inhibits sympathoexcitatory related increases in BP and has a beneficial effect on myocardial ischemia through a reduction of myocardial oxygen demand (Li et al., 1998). EA-induced inhibition of elevated blood pressure as well as premotor sympathetic neural firing in the rVLM cardiovascular center is long-lasting, since it is maintained for at least an hour in most studies. We have found that administration of naloxone to non-specifically block opioid receptors, or gabazine to block γ-aminobutyric acid type A receptors in the rVLM abolishes the prolonged EA inhibition (Tjen-A-Looi et al., 2003, 2007).

We also have examined the central neural mechanisms that underlie point specific cardiovascular responses. As noted above, we observed that the rVLM is an important brain stem region that processes somatic inputs during acupuncture stimulation. Electrophysiological studies of neuronal activity in the rVLM have shown that, as compared to cardiovascularly inactive points (LI 6–7, G 37–39), P 5–6 and certain acupoints along the large intestine meridian (LI 4–11), located over deep somatic neural pathways, provide more convergent afferent input to cardiovascular premotor sympathetic neurons in the rVLM (Tjen-A-Looi et al., 2004). Similarly, as noted below, stimulation of cardiovascularly active points (as compared to less active or inactive points) provides more input to nuclei that constitute a long-loop pathway (see below), which appears to be critical to the sympathoinhibition occurring during electroacupuncture (Li et al., 2006, 2009).

2.3. Supramedullary nuclei in EA-cardiovascular response (the long-loop pathway)

Studies in the 1980s by Li and his colleagues (see reviews in Li and Yao, 1992 and Cheung et al., 2001) demonstrated in rabbits that the inhibitory effect of EA and direct stimulation of the deep peroneal nerve during blood pressure elevations elicited by the defense reaction were abolished by destruction of the arcuate either electrolytically or following decerebration caudal to the arcuate nucleus. These experiments do not unequivocally prove that the arcuate plays a role in the EA response since these techniques may have destroyed cell bodies and fiber in passage. However, the cardiovascular response elicited by the defense reaction also can be inhibited by stimulation of the arcuate with dl-homocysteic acid (DLH) (Huangfu and Li, 1987, 1988). These data in aggregate thus suggest that the arcuate, which contains a sizeable population of β-endorphin neurons, contributes to the inhibitory effect of EA (Huangfu and Li, 1987), at least with regard to its influence of the cardiovascular defense reaction.

2.3.1. Arcuate and vlPAG nuclei

We also have evaluated the role of the hypothalamic arcuate nucleus and its interaction with the midbrain ventrolateral periaqueductal gray (vlPAG) and rVLM in the EA-cardiovascular sympathoexcitatory responses (Li and Yao, 1992; Li et al., 2006, 2009; Tjen-A-Looi et al., 2006). Electrophysiological recordings show that spontaneous activity of neurons in the arcuate and the vlPAG is low (5 ± 1 and 4 ± 1 imp/s, respectively) and that they respond to both visceral splanchnic nerve (SN) and somatic acupoint stimulation. As noted above, we also have observed a gradation of responses to stimulation of different acupoints. In this regard, the evoked responses in both regions are greater during stimulation of the P 5–6, LI 4–11, H 5–6 and S 2–G 2 acupoints (named for the pericardial, large intestine, heart, small intestine and gallbladder meridians, located over the median, deep radial, ulnar and cranial nerves) as compared to responses resulting from stimulation of LI 6–7 (large intestine, over superficial radial nerve) or G 37–39 (gallbladder meridian, over superficial peroneal nerve (Li et al., 2006, 2009)). Thus, we have observed a similar gradation of response in the central nuclei responsible for the EA-cardiovascular response as we observed during our acupoint specificity studies (see above) during stimulation of the acupoints along several meridians that overlie different mixed somatic nerves.

Microinjection of the excitatory amino acid DLH, into the arcuate nucleus augments the responses of vlPAG neurons, while microinjection of small concentrations of 50 nl of 1 mM kainic acid (KA) causes reversible depolarization blockade that transiently deactivates arcuate neurons and decreases the vlPAG responses to SN stimulation (Li et al., 2006). Additionally, 30 min of EA at P 5–6 increases the SN-

evoked discharge of vlPAG neurons, a response that can be blocked by microinjection of KA into the arcuate nucleus. We also have found that a single microinjection of DLH into the arcuate nucleus, like EA, inhibits the reflex increase in BP induced by application of bradykinin (BK) to gallbladder for approximately 30 min. Finally, microinjection of KA into the arcuate blocks the inhibitory influence of EA at P 5–6 on the BK-induced blood pressure response. In aggregate, these results suggest that the arcuate nucleus receives variable input from a number of somatic nerves, with greater input from deep and lesser input from superficial (cutaneous) pathways. Furthermore, excitatory projections from the arcuate nucleus to the vlPAG appear to be essential to the inhibitory influence of EA on the reflex increase in BP induced by SN and gallbladder afferent stimulation.

2.3.2. Direct and indirect vlPAG-rVLM projections

The vlPAG provides inhibitory input to premotor sympathetic neurons in the rVLM that ultimately reduces sympathetic outflow and reflex elevations in blood pressure (Tjen-A-Looi et al., 2006). Direct projections from the vlPAG to the medulla have been documented in tract tracing studies (Loewy, 1990). However, a vlPAG projection to the raphe, in particular the nucleus raphe obscurus (NRO) also exists and might form an indirect pathway from the vlPAG to the rVLM that is operative in the EA-cardiovascular response (Li and Yao, 1992; Cheung et al., 2001). Our more recent studies have suggested that the nucleus raphe pallidus (NRP), located more ventrally than the NRO or the nucleus raphe magnus, contains more cells activated during stimulation of the median nerve with EA at the P 5–6 acupoints, as judged by the concentration of c-Fos labeling (Guo et al., 2008). Our recent preliminary data suggest that brief chemical blockade of the NRP with KA may transiently reverse activation of neurons in the rVLM during stimulation of the vlPAG as well as EA modulation of visceral excitatory reflexes (unpublished data). Furthermore, the NRP can inhibit rVLM activity, including activity of bulbospinal premotor sympathetic neurons. Serotonin projections from the raphe likely play a role in modulation of cardiovascular activity (Dean and Woyach, 2004; Dean, 2005; Tjen-A-Looi et al., 2007; Moazzami et al., 2007; Guo et al., 2008). Although these studies are preliminary, they suggest that an indirect connection from the vlPAG to the rVLM that involves a serotonergic connection from the NRP plays an important role in the long-loop modulation of cardiovascular sympathetic outflow during reflex visceral stimulation. These studies do not eliminate the possibility that direct projections between the vlPAG and the rVLM also serve a functional role in EA-cardiovascular modulation. The direct or indirect projections from the vlPAG to the rVLM complete the long-loop pathway and provide an important source for the inhibitory influence of EA on rVLM premotor neurons and ultimately sympathoexcitatory cardiovascular responses (Li et al., 2006; Tjen-A-Looi et al., 2006).

To complete our studies of interactions between nuclei concerned with the long-loop pathway, we have activated rVLM neurons by stimulating the SN to investigate whether the arcuate directly or indirectly inhibits rVLM activity (Tjen-A-Looi et al., 2003, 2004, 2006, 2007; Li et al., 2006, 2009). Microinjection of KA into the arcuate significantly blocks EA-inhibition of SN-induced reflex increases in rVLM neuronal activity. Furthermore, microinjection of DLH into the arcuate, like EA, inhibits reflex increases of rVLM neuronal discharge (Li et al., 2009). Thus, the arcuate regulates not only PAG but also rVLM activity.

2.3.3. Direct and indirect arcuate-rVLM projections

As noted previously, neurons in the vlPAG receive convergent input from a number of somatic nerves stimulated during EA, as well as from the arcuate nucleus. Bilateral microinjection of KA into the rostral vlPAG partially reverses rVLM neuronal responses and cardiovascular inhibition during DLH stimulation of the arcuate. Conversely, depolarization blockade of the caudal vlPAG completely reverses arcuate evoked rVLM responses (Li et al., 2009). In parallel studies, we have observed that arcuate neurons can be antidromically activated from the rVLM and that arcuate perikarya are labeled with a retrograde tracer microinjected into the rVLM (Li et al., 2009). Many neurons from the arcuate that project to the rVLM are activated by EA stimulation (c-Fos positive) and they frequently contain opioid peptides (Guo et al., 2004). As such, the vlPAG, particularly the caudal vlPAG, appears to be required for inhibition of rVLM neuronal activation by the ARC and subsequent EA-related cardiovascular activation. However, direct projections from the arcuate nucleus to the rVLM, likely serve as an important source of β-endorphin since this projection contains this opioid peptide (Li et al., 2009). This latter observation is consistent with our earlier anatomical study showing that cells in the rVLM contain enkephalin but not β-endorphin (Guo et al., 2004). Hence, EA-cardiovascular responses that result from the action of β-endorphin on μ-opioid receptors located on rVLM sympathoexcitatory premotor neurons (Li et al., 2001) depend on this hypothalamic-medullary projection.

2.4. Neurotransmitters responsible for EA-modulation of BP

2.4.1. Opioids, GABA and serotonin in rVLM

In the 1980s and 1990s preliminary studies (Huangfu and Li, 1988; Li and Yao, 1992; Zhang et al., 1992) suggested that EA inhibition of pressor responses in any of several models of hypertension might be related to the action of opioids, GABA and serotonin (5-HT) in the rVLM.

More recently (Li et al., 2001) we have demonstrated that the EA inhibition of reflex autonomic responses in cat is related to the activation of μ- and δ-, but not -opioid receptors in the rVLM, suggesting that endorphins, enkephalins and perhaps endomorphin, but not dynorphin are responsible for EA modulation of cardiovascular responses. We also have found that nociceptin is involved in the early but not the late influence of EA on cardiovascular excitatory reflexes (Crisostomo et al., 2005; Tjen-A-Looi et al., 2007).

As noted above, we (Guo et al., 2004, 2008; Guo and Longhurst, 2007) have demonstrated the presence of enkephalinergic neurons in the rVLM and endorphinergic neurons in the arcuate nucleus that project directly to the rVLM, and that both neurotransmitter systems are activated by EA. Additionally, preliminary electrophysiological studies (Li et al., 2008) have shown that reciprocal excitatory glutamatergic (NMDA and non-NMDA) projections exist between the arcuate nucleus and vlPAG that may participate in the long-term inhibition of cardiovascular function. Preliminary investigation suggests also that this reciprocal projection may include a cholinergic component in the arcuate nucleus but not in the vlPAG (Li et al., 2008).

Furthermore, EA, through presynaptic endocannabinoid CB1 receptor stimulation, reduces the vlPAG release of GABA but not glutamate during EA (Fu and Longhurst, 2009). Reduced GABA disinhibits vlPAG neurons, thus increasing their activity, which, in turn, inhibits rVLM cardiovascular sympathetic neurons and related sympathoexcitatory reflex responses (Tjen-A-Looi et al., 2009). It is clear therefore that a variety of neurotransmitter systems underlie the cardiovascular action of EA. The list includes both excitatory and inhibitory neurotransmitters and their importance varies from one nucleus to another.

2.5. Mechanism of the long-lasting effect of EA

We have observed that EA inhibition of rVLM cardiovascular sympathetic premotor neuron activity lasts for about 1 h after cessation of EA (Tjen-A-Looi et al., 2003). EA inhibition of cardiovascular excitatory reflex responses in anesthetized rabbits, rats and cats lasts from 1 to 6 h (Guo et al., 1981; Lovick et al., 1995; Li et al., 1998, 2002). In conscious animals (dog and spontaneous hypertensive rats) EA modulation of blood pressure can last for 1 to 12 h (Lin and Li, 1981; Yao et al., 1982a,b). In a preliminary clinical study, we have shown that EA inhibition of BP in hypertensive patients can continue for at least four weeks after terminating treatment (Li and Longhurst, 2007). Thus, inhibition of cardiovascular function by acupuncture cannot be explained simply by the short

term neural occlusive response originating from somatic-visceral convergent interaction on common rVLM interneurons (Tjen-A-Looi et al., 1997). However, there are several mechanisms that potentially might be involved in the prolonged action of EA on cardiovascular function. For example, the inhibitory effect of EA on rVLM sympathetic premotor neuronal responses to excitatory visceral input lasts for 30–40 min after the cessation of EA as a result of opioid and GABA modulation (Tjen-A-Looi et al., 2007).

Recently, we found that besides opioid and GABA modulation in the rVLM, the long-loop pathway, including the arcuate nucleus and the vlPAG, likewise plays a role in the prolonged cardiovascular influence of acupuncture. In this regard, prolonged inhibition of rVLM neurons by EA requires an intact arcuate nucleus since microinjection of the glutamatergic antagonist, kynurenic acid into this hypothalamic region blocks EA inhibition of reflex-induced hypertension, including the prolonged action of EA in the hypertensive response (Tjen-A-Looi et al., 2007). Furthermore, preliminary studies suggest that reciprocal excitatory projections between the arcuate nucleus and the vlPAG may form a reinforcing circuit that can be activated for prolonged periods by EA, lasting as long as 30–60 min (Li et al., 2008).

Additionally, other mechanisms may be evoked by EA to induce the very prolonged inhibition of BP that can last for several weeks in patients with mild to moderate hypertension. In this regard, although limited in scope, studies have suggested that acupuncture increases mRNA expression of opioid precursors in the brain (He et al., 1995; Guo et al., 1996). Preliminary data from our laboratory using real time PCR suggest that preproenkephalin in the rVLM is increased after completion of a single 30 min application of EA at P 5–6 acupoints of rats (Li et al., 2010). The roles of opioid mRNA expression and other neurotransmitter precursors, as well as neurotransmitters released in the hypothalamus, midbrain and other regions of the medulla, particularly in studies involving repetitive EA over time, are worthy of further investigation.

3. Summary

EA at P 5–6 and S 36–37 using low current and low frequency may be able to reduce elevated blood pressure in a subset of patients (~70%) with mild to moderate hypertension. The effect is slow in onset but is long-lasting. Experimental studies have shown that EA inhibition of cardiovascular sympathetic neurons that have been activated through visceral reflex stimulation is through activation of cells in the arcuate nucleus of the hypothalamus, vlPAG in the midbrain and NRP in the medulla, which, in turn, inhibit the activity of premotor sympathetic neurons in the rVLM. The arcuate also provides direct projections to the rVLM that contain endorphins. Glutamate, acetylcholine, opioids, GABA, nociceptin, serotonin and endocannabinoids all appear to participate in

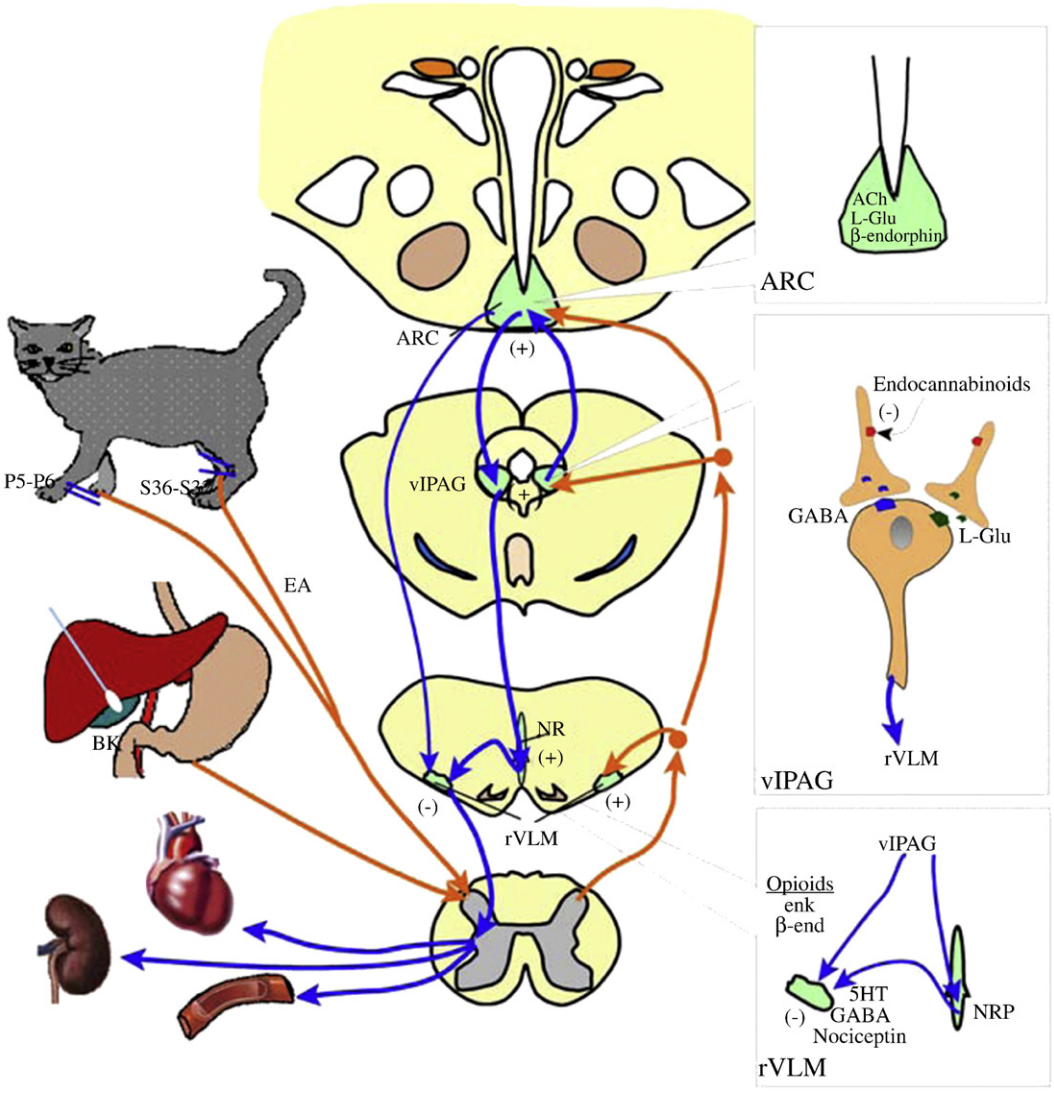

Fig. 2. Neural circuits of acupuncture's action on cardiovascular sympatheoexcitatory visceral reflex elevation of blood pressure. Abbreviations: ARC, arcuate nucleus; vlPAG, ventrolateral periaqueductal gray; NR, nuclei raphe; rVLM, rostral ventrolateral medulla.

the EA hypotensive response although their importance varies between nuclei (Fig. 2). Thus, a number of mechanisms underlying the long-lasting effect of EA on cardiovascular function have been identified but clearly further investigation is warranted.

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