HHS Public Access

Author manuscript

Chin J Integr Med. Author manuscript; available in PMC 2018 January 10.

Published in final edited form as:

Chin J Integr Med. 2017 November ; 23(11): 812–815. doi:10.1007/s11655-017-2789-x.

Nitric Oxide Signaling Molecules in Acupuncture Points: Toward Mechanisms of Acupuncture*

MA Sheng-xing

Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center and Department of Obstetrics and Gynecology, David Geffen School of Medicine, University of California at Los Angeles and Harbor-UCLA Medical Center, Torrance, CA 90502, United States

Abstract

Recent clinical trial studies have demonstrated that the effects of acupuncture on pain improvement are small and no difference between acupuncture points (acupoints) and non-points. Whether acupuncture needles must be inserted in specific points depends on whether acupoint specificity exists that is still not resolved, and is now urgent. Previous anatomical studies have demonstrated that acupoints exist higher number of nerve fibers/trunks, blood vessels, hair follicles, and sweat glands as well as density of the gap junction. Recent evidence shows that nitric oxide (NO) level is elevated in the acupoints/meridians and is associated with an enhanced expression of NO synthase endowed with transient receptor potential vanilloid type-1. There is growing evidence from international groups showing that acupuncture induces NO-mediated vasodilatation, which increases local blood flow and allows for a flush of algesic or sensitizing substances, leading to pain relief. Previous studies, using a novel biocapture system, have demonstrated that NOx^- (total nitrite and nitrate) and cGMP concentrations are consistently increased over skin acupuncture points (acupoints) compared to non-meridian control regions (NMCR) in humans. Dermal microdialysis in humans showed that NO-cGMP releases in the subcutaneous tissue of acupoint are higher than those in NMCR and increased by electroacupuncture (EA). Recent studies have demonstrated that low-frequency electrical stimulation and manual acupuncture with low stimulating force and rate produce an elevation of NO release predominantly over acupoints. In contrast, NO levels over the areas of the skin regions are moderately reduced by high-frequency EA stimulation. The reinforcement methods induce an elevation of vasodilator (NO) release over skin, which contribute to local warmness and beneficial effects of EA including pain relief. Although the specificity of acupoints and monitoring the quantitative response to acupuncture require further investigation, the results from anatomical and biochemical studies consistently show that acupoints exist higher levels of NO signaling molecules, and stimulus-evoked NO release is also with a higher level at acupoints. Results suggest that NO signaling molecules contribute to the specificity of acupoints, and selecting well-trained acupuncturetists for using correct acupoints and appropriate parameters should improve acupuncture clinical trial studies.

*Supported by NIH Grant (No. AT002478, AT004620, and AT004504) from the National Center for Complementary and Integrative Medicine (to SXM)

Correspondence to: Dr. MA Sheng-Xing, Tel: 1-310-974-9573, sxma@labiomed.org.

Keywords

acupuncture points; nitric oxide; electroacupuncture; manual acupuncture; reinforcement methods; biocapture; clinical trials

It has long been accepted that acupuncture, puncturing, and scraping needles at certain points on the body has effects of analgesia, anesthesia, and treatment of various diseases. This therapy has drawn the attention of many investigators and become a research subject of international interest around the world.[1-3] Over the last few decades there has been a number of studies addressing anatomical structures, chemicals, and physiological activities of acupuncture points (acupoints), and acupoint specificity was regarded as one of the core scientific issues with respect to acupuncture practice.[4-6] Recent years, a large meta-analysis of randomized clinical trials (RCT) with data from 17,922 patients with chronic pain and several groups of acupuncture clinical trials have demonstrated that the effects of verum acupuncture on pain improvement have significant, but small, differences compared to sham acupuncture procedures and no difference between acupoints and non-points.[7-9] In addition, dry needling, which simply replaces acupuncture as dry needling and acupoints as trigger points, has been utilized in the United States and other Western countries.[10,11] On the other hand, acupuncture education and practices are based on the classical principles of Chinese medicine(CM). According the description of CM, acupoints are located along meridian lines on the body surface, and acupuncture stimulates acupoints through regulation of the environmental balance of circulating the vital energy and blood through meridian system.[3-5,12] The acupoint specificity involved a fundamental question that is still not resolved is whether acupuncture needles must be inserted in specific points to have their greatest effects. In this paper, the evidence and research progresses for understanding of the anatomical, biophysical, and biochemical specificity of acupoints have been summarized with an emphasis on recent development of nitric oxide (NO) related signaling molecules in acupoints at physiological levels and following acupuncture stimulations.

Anatomical Studies of Acupoints

Several groups of anatomical studies have identified that the number of nerve fibers/trunks, blood vessels, hair follicles, and sweat glands are enhanced over acupoints compared to their adjacent control areas.[13-15] Early anatomical analysis of 309 acupoints in humans have shown that 152 are located at, and 157 are in the vicinity of a nerve trunk; 24 are situated at, and 262 are adjacent to a major artery and/or vein. They concluded that most acupoints are intimately related to the distribution of nerve trunks and blood vessels. At the light microscopic level, the numbers of nerve bundles, nerve fibers, and nerve endings were higher in the skin under the low impedance acupoints than those in their adjacent control areas in both patients and rats.[14,15] It has been suggested that low impedance acupoints on the skin may reflect the variation in anatomical concentration of nerve fibers beneath the skin and represent areas of potentially high neuronal activity.[14]

Over the last three decades, acupoints have been discovered to have the high density of the gap junction, which may be related to the high electrical conductance in the areas.[16,17] NO is a potent modulator of gap junctional coupling in endothelial cells[18] and that the NO

donors reduce the inhibitory junction potentials, thus increasing conductance.[19] NO-induced vasodilation through both cGMP-dependent and independent pathways relies on gap junctional communication.[20] Biochemical studies have shown that NO content is higher in the skin and subcutaneous tissue of acupoints/meridians lines compared to non-meridian areas in rats, and elevated NO level in the acupoints/meridians is associated with an enhanced expression of NO synthase protein levels.[21] Double immunostaining of transient receptor potential vanilloid type-1 (TRPV1) receptor and neuronal nitric oxide synthase (nNOS) revealed co-localization of TRPV1 and nNOS in both subepidermal nerve fibers and in dermal connective tissue cells.[22] A high expression of TRPV1 endowed with nNOS in subepidermal nerve fibers exist in the acupoints and the expression is increased by electroacupuncture (EA). The results suggest that the higher expression of TRPV1 in the subepidermal nerve fibers and its upregulation after EA stimulation may play a key role in mediating the transduction of EA signals to the CNS, and its expression in the subepidermal connective tissue cells may play a role in conducting the local effect of the EA. These findings are also consistent with results which indicate that NO and nNOS levels are enhanced in the acupoints, and suggest that NO may enhance gap junctional conductance and TRPV1 mediated communication which involve in meridian functions.[21,22]

Nitric Oxide Level over and in Acupoints at Physiological Status

The skin represents a unique non-systemic site for the cutaneous measurement of NO metabolites and other biomolecules.[23-25] Weighing 10-12 kg on average, the skin is the largest reservoir for NO derivatives and donors, comprised of N-nitroso compounds, nitrite, and nitrate.[26] Nitrite is 25 times higher in skin, compared to the plasma of healthy volunteers.[24,26,27] In human epithelial cells, NO can be produced enzymatically by three NO synthases (eNOS, nNOS, iNOS),[24,26] or non-enzymatically through the nitrate-nitrite-NO pathway.[25,27]

NO, with a half-life of a few seconds, rapidly oxidizes into nitrite and nitrate, and measurements of these stable metabolites adequately indicate changes in NO activity and production in tissues.[28-30] Investigators have developed an NO-scavenging compound, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO), for use in biological systems.[31,32] A painless, non-invasive biocapture device that use PTIO to scavenge and quntify NO-related biomolecules over specific skin regions has developed in our laboratories.[33] The standard device (a hollow, semi-cylindrical tube 5.0 cm × 0.3 cm) is adhered to the skin surface using a custom double-sided adhesive over the skin region along the acupoints, meridian lines without acupoint (MWOP), and non-meridian control region (NMCR) under the participant's arm or leg. Then, aqueous PTIO solution will be placed inside the tubing contacting the surface of the skin for 20 min in order to absorb NO.[33,34] The liquid was drained from the tube. Concentrations of NOx^- (total nitrite and nitrate, the stable metabolites of NO), and nitrotyrosine and cGMP in the samples were quantified by using chemiluminescence and an enzyme-linked immunoSorbent assay in a blinded fashion, respectively.[33-36]

A serious studies performed in more than 200 human subjects have demonstrated that PTIO, a NO-scavenging compound, can be used to capture and quantify NOx^-, cGMP,

nitrotyrosine, and other small biomolecules in our device. [33-36] Repeated local application of PTIO solutions does not cause any adverse symptoms such as skin irritation. The biocapture device is defined as a nonsignificant risk device that has been evaluated and approved by our institutional Investigational Review Board (IRB) and has also maintained IRB approval throughout the investigation based on the Food and Drug Administration (FDA) regulation. Our studies demonstrated, using this biocapture device, that concentrations of NOx^- and cGMP can be captured over the forearm along the Pericardium Meridian (PC) and the leg along the Bladder Meridian regions by using this device in humans.[33,34] NOx^- and cGMP levels are consistently increased over skin acupoints compared to non-meridian control regions. The results are consistent with data from dermal microdialysis in healthy humans reporting that NO-cGMP releases in the subcutaneous tissue of acupoint are higher than those in NMCR and increased by acupuncture stimulation.[37] The investigation of NO bioavailability over the skin surface is confirmed by other studies,[38] and is a potential physiological indicator for therapeutic manipulation of the skin microvasculature and in pathophysiology.[23,29,34] These results suggest that NO is physiologically released/generated from the skin surface with a high level at acupoints.

Stimulus-Evoked Nitric Oxide Release over Skin Regions

Kimura, et al.[39] reported that acupuncture induces cutaneous vasodilatation in the forearms of humans, which is attenuated by application of NO synthesis inhibitor. It is postulated that acupuncture stimulation improves local circulation and allows for a flush of algesic or sensitizing substances, leading to pain relief.[40-42] We have demonstrated that dialysate NO-cGMP releases in the subcutaneous tissue of the forearm skin along the PC acupoints are increased by EA stimulation.[37] The results are consistent with the results reported that low-frequency (6 Hz) transcutaneous electrical nerve stimulation induces an elevation of NO and cGMP release biocaptured over PC acupoints in humans.[36]

Recently, the effects of reinforcing method using manual acupuncture (MA) *vs.* reductive EA on local NO release have been examined using the novel biocapture device over skin regions in humans.[43] Results show that NO levels biocaptured over the skin regions are increased following MA by twisting/rotating the needle with gentle amplitude and moderate speed. In contrast, NO levels over the areas of the skin regions are moderately reduced by high-frequency EA (30 Hz), a reduction method. Consistently, NO levels biocaptured over the skin regions are elevated by either electrical heat and transcutaneous electrical nerve stimulation with low-frequency (6 Hz).[36,43] The results suggest that heating and MA/EA/transcutaneous electrical nerve stimulation (TENS) with low stimulating force and rate, which are reinforcement methods, produce an elevation of NO release predominantly over acupoints, but EA with a higher stimulating force and rate, which are reduction methods, cause an inhibition of NO generation.

These results suggest that local NO-cGMP release biocaptured over skin acupoints are consistently induced by low stimulating frequency/force of MA, EA, and TENS but not by high stimulation rate/force. It is well-documented that NO improved local circulation and allowed for a flush of analgesic or sensitizing substances for pain relief.[40-42] Acupuncture induces an elevation of vasodilator (NO) release over skin regions, and elevated NO

improves local circulation, which contribute to local warmness and the beneficial effects of acupuncture such as pain relief, improvements of sweating, and inflammation. These results suggest that local NO release is critically dependent on the stimulating force/intensity and speed/frequency of MA/EA, and utilizing the appropriate parameters for an investigated technique carries significant clinical relevance to conduct meaningful acupuncture research. Our results suggest that utilizing the appropriate parameters for an investigated technique and monitoring the quantitative response to acupuncture are worth considering in acupuncture clinical trials. Moreover, the present data shows that NO level is higher over acupoints at physiological level, and stimulus-evoked NO release is also with a higher level at acupoints. Whether the effect of acupoint stimulation has better effect than non-acupoint stimulation or how to select stimulating force/speed for specific diseases/symptoms by monitoring the quantitative response to acupuncture require further investigation. The results from both anatomical and biochemical studies consistently suggest that NO signaling molecules involve in specificity of acupoints, and patients with acupuncture treatment and clinical research should select well-trained acupuncturetists for using correct methods and acupoints.

References

1. Acupuncture. NIH Consensus Statement Online. 1997; 15:1–34.
2. Yin C, Buchheit TE, Park JJ. Acupuncture for chronic pain: an update and critical overview. Curr Opin Anaesthesiol. 2017; 30:583–592. [PubMed: 28719458]
3. Ma SX. Neurobiology of acupuncture: Toward CAM. Evid Based Complement Alternat Med. 2004; 1:41–47. [PubMed: 15257325]
4. Chan SH. What is being stimulated in acupuncture: evaluation of the existence of a specific substrate? Neurosci Biobehav Rev. 1984; 8:25–33. [PubMed: 6328387]
5. Xing JJ, Zeng BY, Li J, Zhuang Y, Liang FR. Acupuncture point specificity. Int Rev Neurobiol. 2013; 111:49–65. [PubMed: 24215917]
6. Campbell A. Point specificity of acupuncture in the light of recent clinical and imaging studies. Acupunct Med. 2006; 24:118–122. [PubMed: 17013358]
7. MacPherson H, Maschino AC, Lewith G, Foster NE, Witt CM, Vickers AJ, et al. Characteristics of acupuncture treatment associated with outcome: an individual patient meta-analysis of 17,922 patients with chronic pain in randomised controlled trials. PLoS One. 2013; 8:e77438. [PubMed: 24146995]
8. Manheimer E, White A, Berman B, Forys K, Ernst E. Meta-analysis: Acupuncture for low back pain. Ann Intern Med. 2005; 142:651–663. [PubMed: 15838072]
9. Vickers AJ, Cronin AM, Maschino AC, Lewith G, MacPherson H, Foster NE, et al. Acupuncture for chronic pain: individual patient data meta-analysis. Arch Intern Med. 2012; 172:1444–1453. [PubMed: 22965186]
10. Dunning J, Butts R, Mourad F, Young I, Flannagan S, Perreault T. Dry needling: a literature review with implications for clinical practice guidelines. Phys Ther Rev. 2014; 19:252–265. [PubMed: 25143704]
11. Fan AY, Xu J, Li YM. Evidence and expert opinions. Dry needling versus acupuncture (II): The American Alliance for Professional Acupuncture Safety (AAPAS) White Paper 2016. Chin J Integr Med. 2017; 23:83–90. [PubMed: 28265852]
12. Beijing College of Traditional Chinese Medicine, Shanghai College of Traditional Chinese Medicine, Nanjing College of Traditional Chinese Medicine. , editor. Essentials of Chinese acupuncture. Beijing: Foreign Languages Press; 1980. p. 301-310.

13. Chan WW, Weissensteiner H, Rausch WD, Chen KY, Wu LS, Lin JH. Comparison of substance P concentration in acupuncture points in different tissues in dogs. Am J Chinese Med. 1998; XXVI: 13–18.
14. Luciani RJ. Direct observation and photography of electroconductive points on human skin. Am J Acup. 1978; 6:311–317.
15. Wang ZT, Wu SL, Cao YC, Zhu ZX, Xu RM. Morphological study on the low impedance line along channel. Acup Res. 1987; 1:82–85.
16. Fan JY, Xi SY, Liu Z, Wei ZM. The role of gap junctions in determing skin conductance and their possible relationship to acupuncture points and meridians. Am J Acup. 1990; 18:163–170.
17. Zheng JY, Fan JY, Zhang YJ, Guo Y, Xu TP. Further evidence for the role of gap junctions in acupoint information transfer. Am J Acup. 1996; 24:291–296.
18. Hoffmann A, Gloe T, Pohl U, Zahler S. Nitric oxide enhances *de novo* formation of endothelial gap junctions. Cardiovasc Res. 2003; 60:421–430. [PubMed: 14613872]
19. Cayabyab FS, Daniel EE. K^+ channel opening mediates hyperpolarizations by nitric oxide donors and IJPs in opossum esophagus. Am J Physiol. 1995; 268(5 Pt 1):G831–G842. [PubMed: 7762667]
20. Javid PJ, Watts SW, Webb RC. Inhibition of nitric oxide-induced vasodilation by gap junction inhibitors: a potential role for a cGMP-independent nitric oxide pathway. J Vasc Res. 1996; 33:395–404. [PubMed: 8862145]
21. Ma SX. Enhanced nitric oxide concentrations and expression of nitric oxide synthase in acupuncture points/meridians. J Alter Comp Med. 2003; 9:207–215.
22. Ibrahim TS, Chen ML, Ma SX. TRPV1 expression in acupuncture points: Response to electroacupuncture stimulation. J Chem Neuroanat. 2011; 41:129–136. [PubMed: 21256210]
23. Raiszadeh MM, Ross MM, Russo PS, Schaepper MA, Zhou W, Deng J, et al. Proteomic analysis of eccrine sweat: implications for the discovery of schizophrenia biomarker proteins. J Proteome Res. 2012; 11:2127–2139. [PubMed: 22256890]
24. Paunel AN, Dejam A, Thelen S, Kirsch M, Horstjann M, Gharini P, et al. Enzyme-independent nitric oxide formation during UVA challenge of human skin: characterization, molecular sources, and mechanisms. Free Radic Biol Med. 2005; 38:606–615. [PubMed: 15683717]
25. Weller R, Pattullo S, Smith L, Golden M, Ormerod A, Benjamin N. Nitric oxide is generated on the skin surface by reduction of sweat nitrate. J Invest Dermatol. 1996; 107:327–331. [PubMed: 8751965]
26. Suschek CV, Schewe T, Sies H, Kröncke KD. Nitrite, a naturally occurring precursor of nitric oxide that acts like a ‘prodrug’. Biol Chem. 2006; 387:499–506. [PubMed: 16740120]
27. Weitzberg E, Lundberg JO. Nonenzymatic nitric oxide production in humans. Nitric Oxide. 1998; 2:1–7. [PubMed: 9706737]
28. Ignarro LJ, Fukuto JM, Griscavage JM, Rogers NE, Byrns RE. Oxidation of nitric oxide in aqueous solution to nitrite but not nitrate: Comparison with enzymatically formed nitric oxide from L-arginine. Proc Natl Acad Sci. 1993; 90:8103–8107. [PubMed: 7690141]
29. Ignarro LJ. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol. 1999; 30:535–560.
30. Ma SX, Ignarro LJ, Byrns R, Li XY. Increased nitric oxide production in posterior hypothalamus and central sympathetic function on arterial pressure tolerance to nitroglycerin in rats. Nitric Oxide: Biol Chem. 1999; 3:153–161.
31. Akaike T, Yoshida M, Miyamoto Y, Sato K, Kohno M, Sasamoto K, et al. Antagonist action of imidazolineoxl N-oxides against endothelium-derived relaxing factor/ NO through a radical reaction. Biochemistry. 1993; 32:827–832. [PubMed: 8422387]
32. Yoshida M, Akaiki T, Wada Y, Sato K, Ikeda K, Ueda S, et al. Therapeutic effects of Imidazolineoxyl N-oxide against endotoxin shock through its direct nitric oxide-scavenging activity. Biochem Biophys Res Commun. 1994; 202:923–930. [PubMed: 8048966]
33. Ma SX, Li XY, Sakurai T, Pandjaitan M. Evidence of enhanced non-enzymatic nitric oxide generation on the skin surface of acupuncture points: An innovative approach in humans. Nitric Oxide: Biol Chem. 2007; 17:60–68.

34. Ma SX, Li XY, Smith BT, Lim N. Changes in nitric oxide, cGMP, and nitrotyrosine concentrations over skin along the meridians in obese subjects. Obesity. 2011; 19:1560–1567. [PubMed: 21151015]

35. Ma SX, Lee P, Li XY, Jiang I, Ma E, Hu J. Influence of age, gender, and race on nitric oxide release over acupuncture points-meridians. Sci Rep. 2015; 5:17547. [PubMed: 26621821]

36. Ma SX, Mayer E, Lee P, Li XY, Gao EZ. Transcutaneous electrical nerve stimulation increased nitric oxide-cyclic cGMP release biocaptured over skin surface of the pericardium meridian and acupuncture points in humans. Acup & Electro-Therapeutics Res INT J. 2015; 40:73–86.

37. Lim N, Ma SX. Responses of nitric oxide-cGMP releases in acupuncture point to electroacupuncture in human skin in vivo using dermal microdialysis. Microcirculation. 2009; 16:434–443. [PubMed: 19468961]

38. Ha Y, Kim M, Nah J, Suh M, Lee Y. Measurements of location-dependent nitric oxide levels on skin surface in relation to acupuncture point. Evid Based Complement Alternat Med. 2012; 2012:781460. [PubMed: 23049611]

39. Kimura K, Takeuchi H, Yuri K, Wakayama I. Effects of nitric oxide synthase inhibition on cutaneous vasodilation in response to acupuncture stimulation in humans. Acupunct Med. 2013; 31:74–80. [PubMed: 23076431]

40. Sandberg M, Lundeberg T, Lindberg LG, Gerdle B. Effects of acupuncture on skin and muscle blood flow in healthy subjects. Eur J Appl Physiol. 2003; 90:114–119. [PubMed: 12827364]

41. Sandberg M, Lindberg LG, Gerdle B. Peripheral effects of needle stimulation (acupuncture) on skin and muscle blood flow in fibromyalgia. Eur J Pain. 2004; 8:163–71. [PubMed: 14987626]

42. Sandberg M, Larsson B, Lindberg LG, Gerdle B. Different patterns of blood flow response in the trapezius muscle following needle stimulation (acupuncture) between healthy subjects and patients with fibromyalgia and work-related trapezius myalgia. Eur J Pain. 2005; 9:497–510. [PubMed: 16139178]

43. Ma SX, Lee P, Anderson TL, Li XY, Jiang I. Response of local nitric oxide release to manual acupuncture and electrical heat in humans: Effects of reinforcement methods. Evid Based Complement Alternat Med. 2017; 2017:1–8.