

Purinergic signaling in acupuncture

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The proposed role of purinergic signaling in the physiological basis of acupuncture was first presented in 2009. Data showing that ATP is released from keratinocytes and other skin cells during acupuncture treatments lends weight to this hypothesis. ATP in turn activates P2X3 receptors on the sensory nerves in the skin, which then transmit those messages to motor neurons in the brain stem that control autonomic functions and modulate nociceptive activities. Here, we review and describe the recent evidence for purinergic signaling underlying acupuncture effects and propose ways to further test this hypothesis.

Introduction

It has been well established that adenosine 5'-triphosphate (ATP) is an intracellular energy source in cellular biochemistry. In 1970, Burnstock et al. suggested that ATP acted as a nonadrenergic, noncholinergic neurotransmitter in the gut (1), and in 1972 he named the extracellular actions of ATP, "purinergic signaling" (since ATP is a purine nucleotide), and formulated the purinergic signaling hypothesis (2).

In 2009, Burnstock proposed that purinergic signaling could be involved in the physiological mechanisms mediating acupuncture effects. This hypothesis suggested that mechanical deformation of the skin by needles or application of heat or electrical current leads to the release of large amounts of ATP from keratinocytes, fibroblasts, and other cell types in skin (Figure 1). The released ATP then activates P2X3 ion channel receptors on sensory nerves within the skin and tongue that transmit messages via sensory ganglia and the spinal cord to the brain stem and hypothalamus. These brain regions contain motor neurons that control autonomic functions, including cardiovascular, gastrointestinal, respiratory, and urinogenital activities—common targets of acupuncture treatments. These sensory neuron messages also modulate the pathways that lead to centers in the cortex responsible for conscious awareness of pain and other central nervous system activities, including sleep regulation (3). A number of subsequent studies have been published that also implicate purinergic signaling in various aspects of acupuncture, detailed below.

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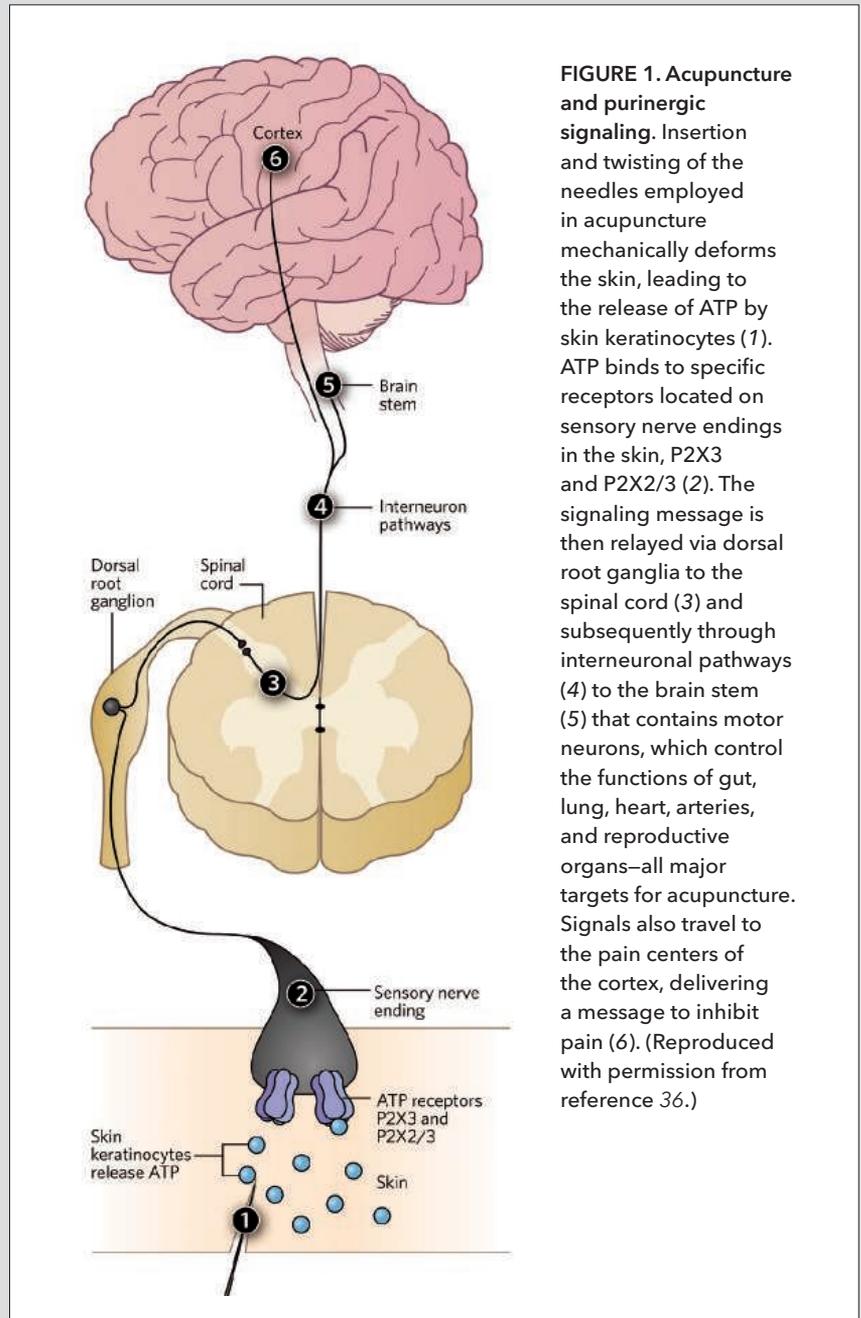


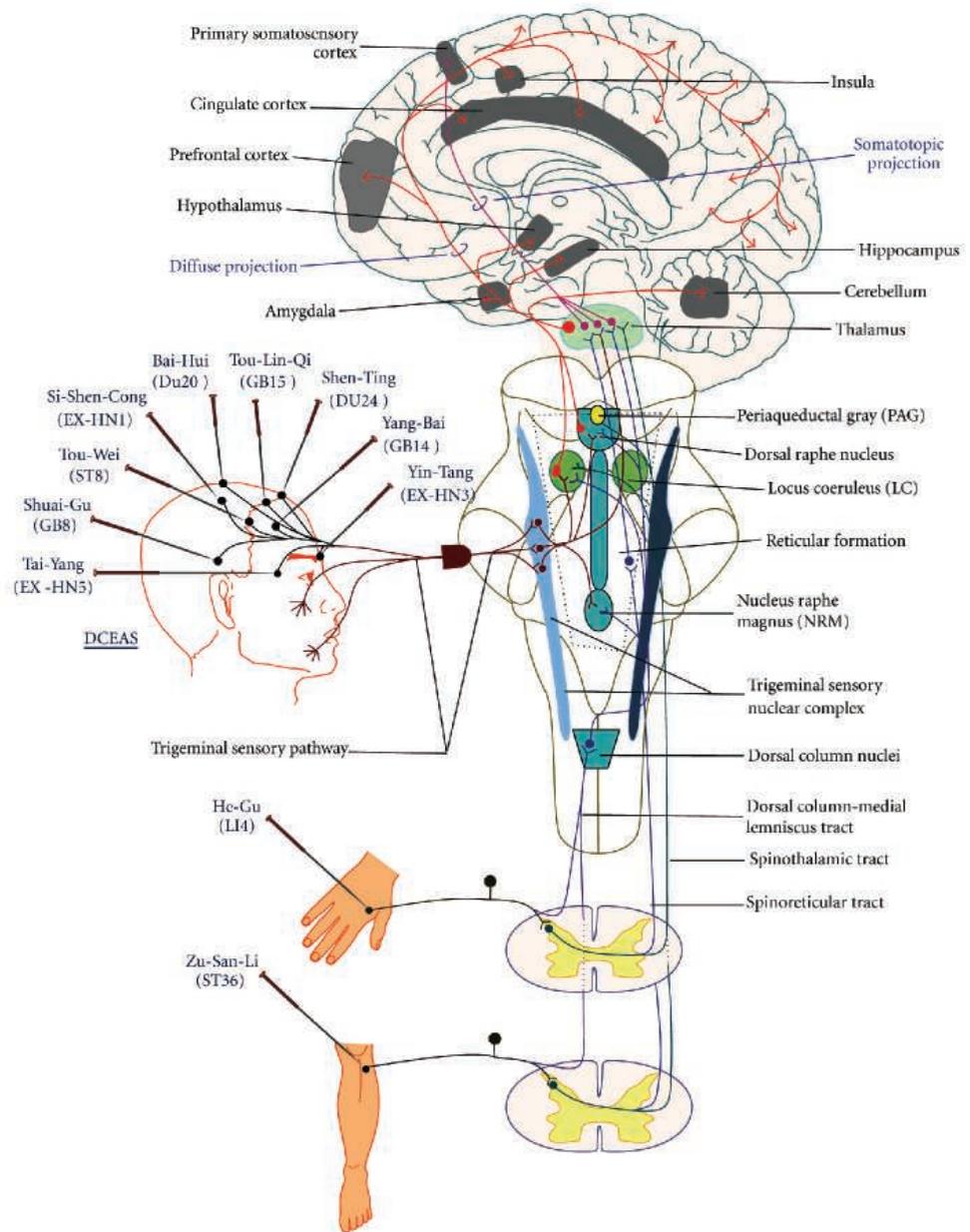
FIGURE 1. Acupuncture and purinergic signaling. Insertion and twisting of the needles employed in acupuncture mechanically deforms the skin, leading to the release of ATP by skin keratinocytes (1). ATP binds to specific receptors located on sensory nerve endings in the skin, P2X3 and P2X2/3 (2). The signaling message is then relayed via dorsal root ganglia to the spinal cord (3) and subsequently through interneuronal pathways (4) to the brain stem (5) that contains motor neurons, which control the functions of gut, lung, heart, arteries, and reproductive organs—all major targets for acupuncture. Signals also travel to the pain centers of the cortex, delivering a message to inhibit pain (6). (Reproduced with permission from reference 36.)

Supporting evidence for the hypothesis

Studies that have established the components involved in the purinergic signaling pathway include: (i) release of ATP (in response to mechanical or chemical stimulation)

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FIGURE 2. Schematic illustration of the central neural pathways that carry afferent (sensory) neural impulses following acupuncture treatment from various parts of the body. Brain areas that commonly respond in neuroimaging studies to acupuncture stimulation are indicated with gray shadow. DCEAS: dense cranial electroacupuncture stimulation. (Reproduced from 28, with permission from Hindawi Publishing Corporation.)



from keratinocytes (4-6) and possibly from Merkel cells, which contain high levels of ATP (7, 8); ATP has also been shown to be released from keratinocytes upon heating (9); (ii) immunohistochemical data demonstrating the presence of P2X3 receptors on sensory nerve fibers in the skin (10-12) and tongue (13); (iii) in an isolated tongue/lingual nerve preparation, mechanical activation of the tongue with De Frey hairs was shown to result in a discharge in the lingual sensory nerve fibers that was mimicked by ATP activation and blocked by P2X3 receptor antagonists (14); and (iv) both presynaptic inhibition via adenosine A_1 and P2Y receptors, and enhancement via P2X and A_{2A} receptors at synapses in

the central nervous system have been reported (15).

Subsequent papers have built upon and extended evidence in support of purinergic signaling underlying acupuncture effects. Several studies have associated the skin cells affected by acupuncture techniques with purinergic signaling. For example, ATP has been shown to be released from human keratinocytes in response to mechanical stimulation by hypo-osmotic shock (16), as well as from keratinocytes in response to heat (17). Additionally, mast cells, which accumulate around the acupuncture needles, also release ATP in response to mechanical stimulation (18). Another skin cell type, human subcutaneous fibroblasts, can

also release ATP in response to bradykinin and histamine (19, 20). Tsutsumi et al. demonstrated that mechanical stimulation can evoke the propagation of calcium waves between human keratinocytes, induced by ATP and activation of P2Y₂ receptors (21, 22), which is consistent with the earlier results from Koizumi et al. (5). Tuina (traditional therapeutic massage) and moxibustion (a traditional Chinese medicine therapy using a moxa, often made from dried mugwort, either used as a fluff or processed into a cigar-shaped stick; it can be used indirectly, with acupuncture needles, or burned on to the patient's skin) may also act via the purinergic signaling pathway (23). Papers describing the release of ATP from human epidermal keratinocytes via connexin hemichannels and vesicles involving vesicular nucleotide transporter have recently been published (24–26). A 2010 study has claimed that adenosine, following breakdown of released ATP during acupuncture, can act as a prejunctional inhibitor of neurotransmission via A₁ receptors, resulting in anti-nociceptive actions (27). Valuable reviews are available describing the neural pathways from different skin regions to structures in the brain stem and higher brain centers. These pathways are important because different acupuncture sites may activate different neural pathways impinging on specific nuclei in the brain stem that control autonomic functions potentially modulated by acupuncture (Figure 2) (28, 29).

Purinergic signaling and electroacupuncture

Electroacupuncture is a form of acupuncture where a small electric current is passed between pairs of acupuncture needles. This is thought to augment traditional acupuncture and is believed to be particularly helpful in treating pain.

The supraspinal antinociception effect of electroacupuncture has been associated with P2X3 receptor activation in the midbrain periaqueductal gray region (30). Moreover, the analgesic effect of electroacupuncture on chronic neuropathic pain has been shown to be mediated by P2X3 receptors in rat dorsal root ganglion neurons (31). Following these studies, electroacupuncture was shown to result in a reduced expression of P2X3 and P2X2 receptors in the dorsal root ganglion of rats with chronic neuropathic pain (32) and visceral hypersensitivity (33). Electroacupuncture at *He-Mu* points can also reduce P2X4 receptor expression in colon and spinal cord in visceral hypersensitivity (34). Moreover, in a review by Lin et al., the neuroprotective effects of acupuncture were reported to act via increasing brain derived neurotrophic factor (BDNF) expression via stimulation of ATP (35).

Conclusions

Evidence in support of the hypothesis of purinergic signaling mediating the physiological mechanisms underlying acupuncture effects has been accumulating over recent years. To help further test this hypothesis, I propose that experienced acupuncturists focus on acupuncture sites that induce effects that can be quantified, such as an

increase or decrease in heart rate or blood pressure, and identify specific neurons that are activated in the brain using noninvasive scanning techniques. If acupuncture-induced effects can be identified and quantified, researchers could then test whether ATP mimicks the responses and if P2X3 receptor antagonists block the effects. Moreover, we suggest that researchers conduct experiments recording responses from sensory neurons in the skin and tongue in animal models and distinguish between low-threshold fibers involved in acupuncture and high-threshold fibers that mediate nociception, as well as recordings from the motor nerves in the brainstem responsible for autonomic functions.

References

1. G. Burnstock, G. Campbell, D. Satchell, A. Smythe, *Br. J. Pharmacol.* **40**, 668 (1970).
2. G. Burnstock, *Pharmacol. Rev.* **24**, 509 (1972).
3. G. Burnstock, *Med. Hypotheses* **73**, 470 (2009).
4. N. Mizumoto, M. E. Mummert, D. Shalhevet, A. Takashima, *J. Invest. Dermatol.* **121**, 1066 (2003).
5. S. Koizumi et al., *Biochem. J.* **380**, 329 (2004).
6. H. E. Burrell et al., *J. Biol. Chem.* **280**, 29667 (2005).
7. R. Crowe, M. Whitear, *Cell Tissue Res.* **190**, 273 (1978).
8. M. Silberstein, *Med. Hypotheses* **75**, 272 (2010).
9. S. Mandadi et al., *Pflugers Arch.* **458**, 1093 (2009).
10. E. J. Bradbury, G. Burnstock, S. B. McMahon, *Mol. Cell. Neurosci.* **12**, 256 (1998).
11. G. Burnstock, *Br. J. Anaesth.* **84**, 476 (2000).
12. M. Taylor, J. C. Peleshok, A. Ribeiro-da-Silva, *J. Comp. Neurol.* **514**, 555 (2009).
13. X. Bo et al., *Neuroreport* **10**, 1107 (1999).
14. W. Rong, G. Burnstock, K. M. Spyer, *J. Physiol.* **524**, 891 (2000).
15. G. Burnstock, *Physiol. Rev.* **87**, 659 (2007).
16. N. Azorin et al., *Exp. Dermatol.* **20**, 401 (2011).
17. J. R. Gifford, C. Heal, J. Bridges, S. Goldthorpe, G. W. Mack, *J. Physiol.* **590**, 6403 (2012).
18. L. Wang et al., *Evid. Based Complement. Alternat. Med.* **2013**, 350949 (2013).
19. R. Pinheiro et al., *Cell Commun. Signal.* **11**, 70 (2013).
20. R. Pinheiro et al., *J. Biol. Chem.* **288**, 27571 (2013).
21. M. Tsutsumi et al., *Cell Tissue Res.* **338**, 99 (2009).
22. M. Tsutsumi et al., *Skin Res. Technol.* **16**, 146 (2010).
23. L. Fan, L. M. Yin, *J. Acupunct. Tuina Sci.* **12**, 125 (2014).
24. T. P. Barr et al., *PLOS ONE* **8**, e56744 (2013).
25. T. Takahashi et al., *J. Invest. Dermatol.* **133**, 2407 (2013).
26. K. Inoue et al., *J. Invest. Dermatol.* **134**, 1465 (2014).
27. N. Goldman et al., *Nat. Neurosci.* **13**, 883 (2010).
28. Z. J. Zhang, X. M. Wang, G. M. McAlonan, *Evid. Based Complement. Alternat. Med.* **2012**, 429412 (2012).
29. Q. Q. Li et al., *Evid. Based Complement. Alternat. Med.* **2013**, 267959 (2013).
30. Z. Xiao et al., *Brain Res.* **1330**, 31 (2010).
31. W. Z. Tu et al., *Neurochem. Int.* **60**, 379 (2012).
32. R. D. Cheng et al., *Chin. J. Integr. Med.* **19**, 374 (2013).
33. Z. Wang et al., *Neural Regen. Res.* **8**, 802 (2013).
34. X. Guo et al., *Neural Regen. Res.* **8**, 2069 (2013).
35. D. Lin et al., *Int. J. Mol. Sci.* **15**, 3234 (2014).
36. G. Burnstock, *The Scientist* **25**, 24 (2011).