Editorial Commentary

Purinergic mechanisms mediate acupuncture-induced analgesia

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Introduction

Pain is one of the conditions which verifiably benefits from acupuncture (AP) treatment in clinics. In spite of convincing meta-analyses of randomized, sham controlled clinical investigations or evaluated Cochrane reviews, there is still some disbelief about the efficiency of this therapeutic maneuver (1,2). In fact, it is hard to understand why AP strongly relieves some types of pain (low back pain, neck pain, osteoarthritis), while leaving other types of probably similar etiology only slightly or not at all affected (dental pain, coloscopy and intraoperative pain). Moreover, the strongest evidence for a positive outcome of AP treatment is in the case of postoperative nausea and vomiting, conditions not related to pain.

Convincing arguments for the efficiency of AP, especially of electroacupuncture (EAP) in case of various painful conditions, were supplied by experiments on laboratory rodents (3,4). In this Editorial Commentary we will reflect to ideas presented in these overviews. The authors refer to numerous original publications supporting the participation of endogenous opioid peptides in AP-induced analgesia both in animals and human subjects (4). Thereafter they emphasize that in addition to peripheral and central opioids, purinergic signaling also contributes to analgesia.

Purinergic signaling by the appropriate receptors

Adenosine 5'-triphophate (ATP) is not only a means for the intracellular storage of energy, but also an extracellular transmitter/signaling molecule subserving intercellular communication (5). It has been shown that ATP is released together with classic neurotransmitters (noradrenaline, acetylcholine) as a (co)transmitter on the one hand, but on the other hand it also supports the communication between almost all non-excitable cell types of the animal/human body through activation of a whole range of plasma membrane receptors (6). These receptors belong to two classes: P2X receptors (seven subtypes: P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, P2X7) mediate rapid responses in the millisecond range and P2Y receptors (eight mammalian subtypes: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, P2Y14) mediate slow, protracted responses in the range of seconds or even hours or days. Subsequently it was reported that ATP can be enzymatically degraded by ecto-ATPases by successive dephosphorylation of the mother molecule, to adenosine 5'-diphosphate (ADP) and eventually to adenosine. ATP is an agonist for all P2X receptor-types, while ADP is an agonist for certain P2Y receptors. Adenosine activates its own receptor-types (A1, A2A, A2B, A3) and thereby causes sometimes even opposite effects to those induced by ATP/ADP itself.

Purinergic signaling as a mechanism of AP-induced analgesia

It has been suggested that AP may release ATP by mechanical stimulation of mast cells located in subcutaneous/muscular tissue in the neighborhood of thin-diameter sensory nerve fibers which respond otherwise to painful stimuli. ATP/ADP or adenosine thereafter activates their receptors located at the very terminals of these peripheral nerves and either locally, or via indirect effects at spinal cord/brain centers, causes analgesia (7).

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As mentioned above, the reviews of Tang *et al.* (3,4) discuss the literature evidence collected in the past years to support the purinergic hypothesis of AP analgesia. They righteously mention that it is extremely important to work on laboratory animals because of the following reasons: (I) in contrast to many clinical studies it is easy to establish well controlled experimental conditions; (II) the results obtained can be evaluated by strict and validated statistical methods using the appropriate number of animals in each group; and (III) it is possible to utilize inbred populations as well as genetically modified knockout, knock-in and transgenic animals and their wild-type controls.

AP-induced analgesia is mediated by A1 adenosine receptors

It has been reported in a seminal paper that gentle manual rotation of an AP needle inserted into the right Zusanli acupoint of mice released ATP (rapidly degraded to adenosine) into the tibialis anterior muscle/subcutaneous tissue, as measured by microdialysis and high pressure liquid chromatography (8). This adenosine appears to stimulate an inhibitory adenosine receptor, localized at the respective nerve terminals and thereby eliminates the mechanical and thermal allodynia induced by the previous injection of Complete Freund's Adjuvant (CFA) into the right hind paw. CFA is known to induce inflammatory pain. Spared injury to the sciatic nerve generates neuropathic pain in the right hind paw which could also be releaved by manual AP delivered to the right Zusanli acupoint. From these results the authors concluded that ATP released into the interstitial tissue, is enzymatically decomposed to adenosine and this adenosine acts locally to abrogate both inflammatory and neuropathic pain. The authors convincingly demonstrated the involvement of a specific adenosine receptor of the so-called A1 subtype by using selective pharmacological antagonists and A1 receptor deficient mice.

This is a most impressive study, containing probably only a single issue not sufficiently clarified. It is stated that ATP is released by AP from keratinocytes, although in the meantime a number of publications strongly suggest that mast cells are the sources of ATP (9). Mast cells are immune cells which play a role in anti-inflammatory responses, wound-healing, angiogenesis, immune tolerance and defense against pathogens. Three pieces of evidence support the notion that mast cells release ATP on AP-induced stimulation: firstly, mast cell-deficient rats exhibit less mechanical analgesia than their wild-type counterparts; secondly, mechanical stimuli lead to a rise in intracellular Ca^{2+} in mast cells and release ATP in a Ca^{2+}-dependent manner, and; thirdly, non-specific P2 receptor antagonists or selective antagonists of the P2X7 receptor-subtype attenuate the release of ATP from mast cells. Another open question relates to the molecular mechanism that senses mechanical forces at the mast cell membrane and transduces it to trigger ATP release. Recently a mechanosensitive ion channel called "Piezo1" was discovered; it may be the immediate sensor of AP stimulation of the mast cell membrane (10).

AP-induced analgesia is mediated by P2X3, P2X4 and P2X7 receptors

Data related to the effect of ATP itself, released locally by AP, are much less conspicuous than those related to adenosine effects. In most experiments, EAP was used as a stimulus, which was reported to relieve experimental inflammatory, neuropathic and visceral pain in a multiplicity of studies. Of the P2X receptor types, P2X3 (11), P2X4 (12) and P2X7 (13,14) were proposed to mediate the AP-induced analgesia (3,4). In contrast to the evidence presented on the participation of adenosine, the conclusiveness of these data suffer from the contradiction that ATP is known to cause pain rather than analgesia, by activating the above mentioned P2X receptor-types. Therefore, the use of pharmacological antagonists of P2X3, P2X4 or P2X7 receptors will abolish not only the purinergically-induced AP analgesia but also the purinergically-induced pain. The same is true for experiments in mice, the P2X receptor-types of which were genetically deleted. Thus, we have at our disposal only indirect methodological approaches to confirm the participation of P2X receptor-types in AP-induced analgesia, instead of the direct pharmacological and genetic tools.

In fact, it was found that painful stimuli caused an up-regulation of the respective receptor mRNAs and proteins along the pain conducting pathways, starting with the peripheral dorsal root ganglia (DRGs) and the central spinal cord dorsal horn segments. Subsequently, both AP and the use of selective pharmacological antagonists reversed/prevented the up-regulation of these receptors at the mentioned sites, suggesting their involvement in the AP-induced pain-relief.

Hence, the generally accepted idea is that the stimulation of P2X3, P2X4 and/or P2X7 receptors located at the terminals of sensory nerve fibers (peripheral endings of the

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DRGs) causes *dequi*, a sensation of soreness, numbness, distension, aching or heaviness, which is thought to be essential for the therapeutic outcome. A couple of scenarios may explain the development of analgesia: (I) at least the P2X3 receptor is known to rapidly desensitize; the depolarization of the neuronal membrane caused by its activation declines to its resting value in spite of the continuous presence of the agonist ATP. In consequence, the conduction of action potentials via the central terminals of the DRGs to the spinal cord dorsal horn is interrupted and thereby there is no perception of pain. (II) Alternatively, P2X4/P2X7 receptors which are located at peripheral immune cells, such as monocytes/macrophages could release various bioactive molecules themselves blocking action potential generation at the terminals of DRG neurons. (III) A still simpler explanation for the inverse analgesic effect mediated by P2X receptors would be that the AP-induced impulses evoked in sensory fibers of the skin connect with central interneurons to inhibit the pathways directed to the higher pain centers of the brain.

It is important to note that P2X7 receptors are located at the resident macrophages of the CNS, the so called microglial cells, which release pro-inflammatory cytokines, chemokines, proteases, reactive oxygen/nitrogen species and even the excitotoxic glutamate/ATP to cause pain. Microglial P2X4 receptors release brain-derived neurotrophic factor (BDNF) onto spinal cord sensory neurons and also induce pain. Thereby, the preponderance of the algesic and analgesic outflow of bioactive cell products may decide whether ATP causes pain or analgesia.

Interesting, recently reported findings, were not yet reported in the review of Tang *et al.* (3), Stephan *et al.* (15) and Zhang *et al.* (16) presented evidence for the existence of a new 'cognate' ASIC3/P2X3 receptor and its involvement in pain sensation. Acid-sensing ion channel 3 (ASIC3) exhibits a high structural similarity to P2X3 receptors; although the amino acid composition is different, the trimeric structure and the ion conductive pathways are similar. In addition, low threshold acute acidic pain mediated by the activation of ASIC3 channels was prevented by EAP, as compared with transient receptor potential vanilloid channel 1 (TRPV1) mediated high threshold acidic pain. ASIC3 and P2X3 receptors appeared to interact with each other in response to both protons and ATP, by forming the above mentioned ASIC3/P2X3 'cognate' receptor.

AP-induced analgesia is mediated by P2Y1 receptors

A definite weakness of the available scientific literature is that there exist only few investigations on the involvement of the ATP/ADP-sensitive P2Y receptors in AP-induced analgesia. At least for P2Y1 receptors some data support an analgesic effect by the blockade of the N-type voltage-sensitive Ca^{2+} current in DRG neurons (17). This may lead to a decreased release of the nociceptive transmitter glutamate from layer 1 sensory neurons in the spinal cord dorsal horn and thereby interrupt the synaptic contact to higher areas of the brain. Alternatively, a negative interaction between P2Y1 receptors and the pain-causing TRPV1 channels may also result in analgesia (18).

However, still more findings support the notion that a number of P2Y receptor-types (P2Y1, P2Y6, P2Y11, P2Y12) boost painful sensation, and their blockade by selective antagonists has an analgesic effect (4). While the reason for this apparent controversy is unknown, consonant with the existence of pain-mediating P2Y receptors, EAP was reported to inhibit visceral hypersensitivity caused by the intracolonic injection of acetic acid as a model of irritable bowel syndrome (19). In these experiments, both a selective antagonist of P2Y1 receptors and an antagonist of the mitogen activated protein kinase/extracellular regulated kinase 1 (MAPK/ERK) decreased the intensity of pain. The analgesia induced by EAP simultaneously with the down-regulation of the astrocytic marker glial fibrillary acidic protein (GFAP)- and P2Y1 receptor-immunoreactivities led to the conclusion that EAP depresses visceral hypersensitivity by inhibiting P2Y1 receptors and their downstream signaling via the MAPK/ERK pathway in astrocytes. A strong argument for the participation of astrocytes and their P2Y1 receptors in AP-induced analgesia was supplied by experiments which documented the blockade of this analgesic reaction by the intrathecal infusion of the selective astrocytic neurotoxin, fluorocitrate.

In this case, not the EAP-induced local release of ATP/ADP was supposed to be involved, but that of a bioactive molecule down-regulating P2Y1 receptor-activity. Astrocytes on their behalf may cause neuronal effects indirectly by modifications in K^+ uptake and redistribution, Cl^- and water fluxes, Na^+/Ca^{2+}, or Na^+/HCO_3^- exchange, and neurotransmitter uptake, etc. (20). Furthermore, astrocytes may release gliotransmitters by vesicular Ca^{2+}-dependent mechanisms, as well as by connexin/pannexin

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(hemi)channels, and other non-exocytotic pathways, modifying neuronal functions.

Translation of data relating to AP from animals to humans

A shortcoming of the available experimental studies is that they usually deal with EAP rather than manual AP; EAP represents a much stronger stimulus than manual AP, and may therefore not perfectly model the standard clinical situation in humans. It is most praiseworthy that in the important work of Goldman and coworkers (8) the authors twist the AP needle inserted into the Zusanli acupoint every 5 min for 30 min in total, similar to the human applicability of AP. However, comparable studies favoring the participation of P2 receptors in AP-induced analgesia are hitherto missing.

Conclusions

In conclusion, in spite of some ambiguous explanations in the discussed reviews or Tang *et al.* (3,4), there is still strong evidence for the involvement of purinergic mechanisms in the analgesic effect of AP. Apparently, a whole range of purinergic signaling molecules (ATP/ADP, adenosine) and receptors (P2X3, P2X4, P2X7, ASIC3/P2X3, P2Y1) may function probably even simultaneously. Purinergic mechanisms may complement the previously reported effects of AP due to the release of opioid peptides in peripheral tissues and the CNS.

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