

Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies

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Brain functions are regulated by chemical messengers that include neurotransmitters and neuropeptides. Recent studies have shown that acupuncture or electrical stimulation in specific frequencies applied to certain body sites can facilitate the release of specific neuropeptides in the CNS, eliciting profound physiological effects and even activating self-healing mechanisms. Investigation of the conditions controlling this neurobiological reaction could have theoretical and clinical implications

Neuropeptides play important roles in various aspects of brain function (e.g. opioid peptides in pain control [1] and neuropeptide Y (NPY) in appetite modulation [2], among others). This review discusses evidence that neuropeptides could be mobilized by peripheral electric stimulation to benefit human health.

It has been shown that physiological and pathological conditions can induce release of neuropeptides. Two well-known examples are a severe painful stimulus inducing the release of opioid peptides to ease pain, and sucking of the nipples promoting the secretion of milk. Oxytocinergic neurons fire at a very low rate, of ~1 Hz (0.1–2.6 Hz) in basal conditions, but prolonged sucking by ten or more pups can bring the firing rate up to 16–50 Hz, followed by strong milk ejection within 10–12 seconds [3]. This finding suggests that neuropeptide release could be modulated by external stimulation.

Clinically, intracranial [4] or intra-spinal [5] electrical stimulation has been used through neurosurgical procedures to provide relief for patients suffering from chronic pain, with a success rate of 50–80% after one year of treatment. This pain-relief effect could involve the release of neuropeptides [6], raising the attractive possibility that non-invasive methods might be used to modulate neuropeptide release for therapeutic intervention. The question is, would such an approach be effective and practical?

Frequency-dependent neuropeptide release *in vitro*

In isolated rat neurohypophyses, field electrical stimulation induces the release of arginine vasopressin (AVP) and oxytocin (OT) into the incubation medium. Stimulation at a frequency such as 15–30 Hz was much more effective than a lower frequency such as 2–3 Hz in

triggering peptide release [7], and burst stimulation was more effective than constant-frequency stimulation [8]. Furthermore, in superfused rat spinal cord slices, the release of the neuropeptide substance P (SP) per pulse of electrical stimulation was increased by frequencies in the range of 20–50 Hz, whereas release of the small-molecule neurotransmitter 5-hydroxytryptamine (5-HT) per pulse remained constant [9]. Hokfelt proposed that peptide release requires bursting or high-frequency activities, whereas individual action potentials firing at a low frequency will not induce the release of peptides [10,11]. The facilitation of peptide release by high-frequency stimulation was considered to be due to the lengthening of the action potential duration, together with the increase in frequency, leading to an increase in Ca^{2+} entry and in the amount of secretion per unit of action potential [12]. This concept has been supported by more recent reports [13]. However, frequency requirement can vary for different neuropeptides. In a similar experimental setting, thyrotropin-releasing hormone (TRH) could be released by electrical stimulation at a frequency as low as 0.5 and 3 Hz [14].

Frequency-dependent release of CNS opioid peptides by peripheral electrical stimulation

Peripheral electrical stimulation can be provided via electrodes placed on the skin (transcutaneous electrical nerve stimulation, TENS) or via a probe inserted through skin into the tissue (percutaneous electrical nerve stimulation, PENS). If the point of stimulation is selected according to traditional acupuncture therapy, the process is usually called electroacupuncture (EA). In fact, the difference between PENS and EA is more hypothetical than practical. One study compared the analgesic potency and the underlying neurobiological mechanisms of EA and TENS, with the acupuncture needles or the skin electrodes placed at the same 'acupoints', and concluded that they operate through very similar, if not identical, mechanisms [15]. Thus, the mechanisms of the aforementioned methods of peripheral stimulation are discussed under the same heading.

To facilitate the release of opioid peptides in the CNS, one can use manual acupuncture [16] or EA [17] stimulation. The parameters of the latter can be precisely characterized. It was interesting to note that analgesia

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induced by low-frequency (4 Hz) stimulation, but not that induced by high-frequency (200 Hz) stimulation, can be reversed by low doses of the opioid antagonist naloxone [17], suggesting that low-frequency stimulation can increase the release of opioid peptides in the CNS. By changing the dose of naloxone or using various opioid receptor subtype-specific antagonists, we were able to show that analgesia induced by either low- or high-frequency stimulation are both mediated by opioid peptides [18,19]. The difference was that the former was mediated by μ and/or δ opioid receptors, whereas the latter was mediated by κ opioid receptors [20]. These results suggest that different kinds of opioid peptides are released under these different conditions.

Direct evidence comes from our study using radio-immunoassay of spinal perfusates from the rat [21], showing that 2 Hz peripheral stimulation produces a significant increase in the content of enkephalin-like immunoreactivity (IR) but not in that of dynorphin IR, whereas 100 Hz increases dynorphin IR but not enkephalin IR. In a follow-up double-blind study, in collaboration with Lars Terenius of the Karolinska Institute (Stockholm, Sweden), the results obtained in rats were fully confirmed in humans [22]. These studies suggest that (1) the principle proposed by Hokfelt in 1991 [11] might have to be revised, and (2) to support our hypothesis, more evidence, obtained using different approaches, is needed.

To test whether analgesia induced by stimulation at 2 and 100 Hz are mediated differentially in the spinal cord by enkephalin and dynorphin, respectively, we performed an antibody microinjection study. Our idea was that binding of an opioid peptide molecule to its antibody to form a large protein complex would hinder its approach to the receptor, resulting in a loss of its biological function. Indeed, intrathecal injection of enkephalin antiserum resulted in a dramatic decrease in the efficacy of 2 Hz EA analgesia. This effect of antiserum diminished as the EA frequency was increased to 128 Hz. By contrast, dynorphin antiserum produced an equally dramatic decrease in the analgesic effect produced by 128 Hz EA, but this effect diminished gradually with decreasing frequency, reaching zero at 4 Hz [23] (Fig. 1). A similar approach was used to study the possible effect of β -endorphin in mediating EA analgesia. Injection of β -endorphin antiserum into rat periaqueductal grey matter resulted in an 88% decrease of analgesia at 2 Hz EA and a 61% decrease in analgesia at 15 Hz EA, with no blockade of the analgesic effect of 100 Hz EA [24].

Another example is endomorphin, a small peptide composed of only four amino acid residues that has been recognized as an endogenous opioid peptide with highly selective affinity for the μ -opioid receptors [25]. Antibodies against endomorphin injected into the cerebral ventricle [26] or the spinal subarachnoid space [27] dose-dependently reduced the analgesia induced by 2 Hz EA stimulation, but not that induced by 100 Hz EA stimulation. This result is very similar to that obtained with the other two agonists of μ and δ receptor already mentioned, enkephalin and β -endorphin. Taken together, these studies support the proposition that, although high-frequency stimulation is

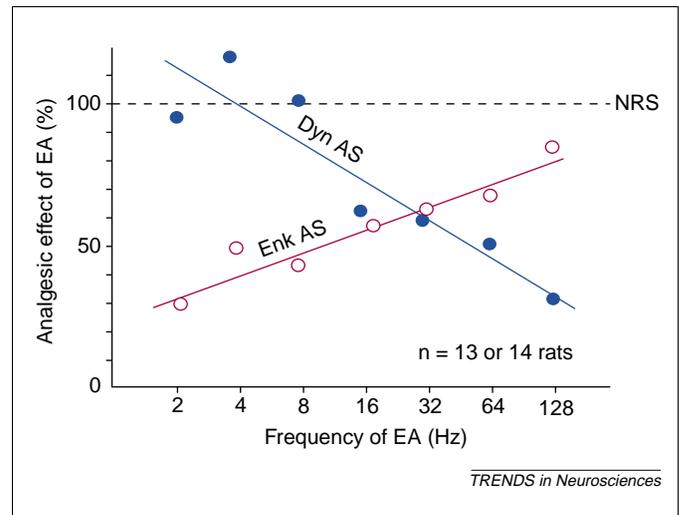


Fig. 1. Antibody-microinjection study investigating the roles played by spinal met-enkephalin and dynorphin A in mediating the analgesic effects that are induced by electroacupuncture (EA) of different frequencies. Rats were given intrathecal injection of normal rabbit serum (NRS) or antisera against either met-enkephalin (Enk AS, red) or dynorphin A (Dyn AS, blue), 30 minutes before the administration of EA. The analgesic effect was measured by the percentage change of tail-flick latency (data were normalized with NRS control group as 100%). The analgesic effects of low- or high-frequency EA were blocked by Enk AS or Dyn AS, respectively. Modified, with permission, from Ref. [23].

preferable for the release of many CNS peptides, it should not be taken as a gold standard in determining the parameters of electrical stimulation for activating a specific neuropeptide for either experimental or therapeutic purposes.

Putative neural pathways mediating low- and high-frequency electroacupuncture-induced analgesia

The afferent impulses induced by acupuncture have been characterized to be mainly transmitted by A β and A δ fibres [28]. Wang and colleagues have conducted a series of experiments to analyze the possible neural pathways responsible for the frequency-specific release of different kinds of opioid peptides in rat CNS [29] (Fig. 2). Lesion of the arcuate nuclei of the hypothalamus abolished analgesia induced by low-frequency EA but not that induced by high-frequency EA, whereas selective lesion of the parabrachial nuclei of the brainstem attenuated the effects of high-frequency EA but not those of low-frequency EA. The periaqueductal grey matter is a common element for both of the descending pain inhibitory systems. These findings have been partially supported by subsequent morphological studies using *fos* gene expression as marker of brain activation in the rat [30], and functional magnetic resonance imaging (fMRI) study in human volunteers (W.T. Zhang, *et al.*, unpublished).

Optimization of peripheral electrical stimulation for maximal release of central opioid peptides

From the research already mentioned, stimulation at a single frequency, whether low or high, would not be sufficient to trigger the full release of all four kinds of opioid peptide together. To elicit the maximal release of all four, two models have been considered. Model A involves stimulation at low (2 Hz) and high (100 Hz) frequencies alternately (referred to as '2/100'), optimally spaced so that

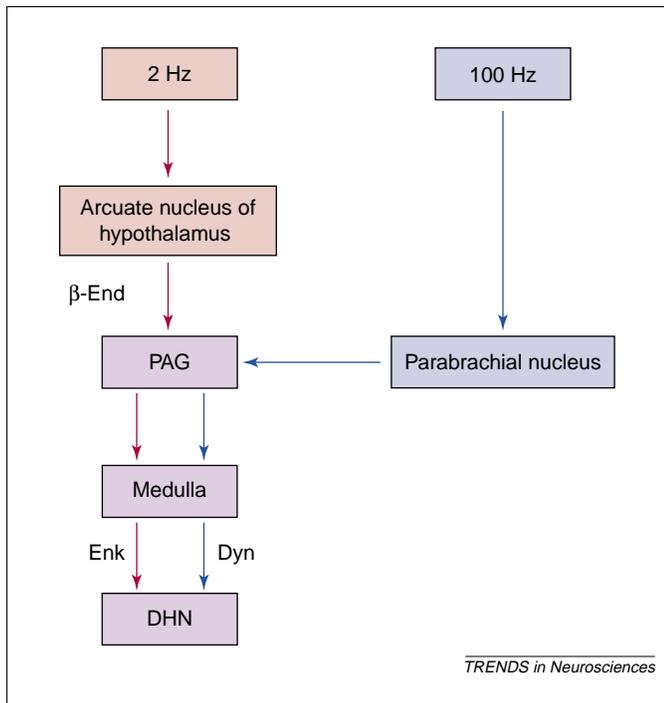


Fig. 2. Neural pathways mediating the analgesic effect elicited by low-frequency (2 Hz, red) or high-frequency (100 Hz, blue) electroacupuncture stimulation. Abbreviations: DHN, dorsal horn neuron of the spinal cord; Dyn, Dynorphin A; β -End, β -endorphin; Enk, enkephalin; PAG, periaqueductal grey matter. Modified, with permission, from Ref. [23].

the residual effect produced by the low frequency stimulation could overlap with that produced by the high frequency and, therefore, elicit an synergistic effect [31]. Model B involves stimulation at 2 and 100 Hz simultaneously (referred to as '2 + 100') applied at different parts of the body, in which case all four kinds of opioid peptide might be released simultaneously (Fig. 3).

Model A has been tested carefully [32], showing that automatic shifting between low- and high-frequency stimulation for three seconds each (i.e. 2/100 stimulation) did, indeed, produce a simultaneous activation of the enkephalin and dynorphin systems, inducing a much more potent analgesic effect than that induced by a constant frequency stimulation.

For model B (2 + 100), two possibilities exist. One (B1) is that the brain is capable of clearly distinguishing two different frequencies of stimulation (2 Hz versus 100 Hz) and induces the two efferent systems to work simultaneously. The other (B2) is that two different signals (2 and 100 Hz), coming from two different sites, merge in the reticular formation of the brainstem so that they are received as a stimulation of 102 Hz, which is indistinguishable from a stimulation of 100 Hz. Model B2 is supported by at least three observations [33]. First, an increase of the content of dynorphin IR in the spinal fluid (representing an increase in release of the dynorphin peptide) was observed in both the 2/100 and 2 + 100 modes, yet an increase of the release of endomorphin IR was observed only in rats treated with 2/100 mode. Second, intrathecal injection of κ opioid-receptor antagonist norbinaltorphimide (Nor-BNI) suppressed the analgesic effect of both the 2/100 and 2 + 100 modes, whereas the μ opioid-receptor antagonist

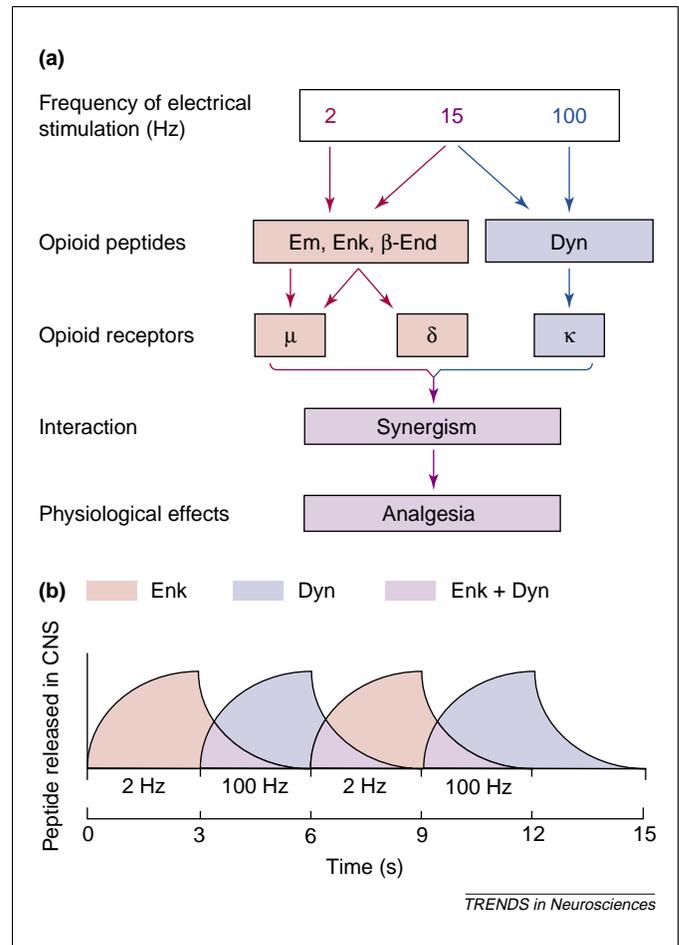


Fig. 3. Possible mechanisms for the analgesic effects of acupuncture. (a) Opioid peptides and opioid receptors involved in analgesia elicited by electroacupuncture of different frequencies. Opioids and receptors involved at 2 Hz are in red, those involved at 100 Hz, in blue. At 15 Hz, there is a partial involvement of components involved at both of the other two frequencies (purple). Abbreviations: Dyn, dynorphin A; β -End, β -endorphin; Em, endomorphin; Enk, enkephalins. Simultaneous activation of all three types of opioid receptor elicits a synergistic analgesic effect. Note that simultaneous receptor activation does not necessarily mean that the opioids are released simultaneously – it could be that the residual presence of one opioid overlaps with newly induced release of another. (b) Model for the synergistic analgesic effect produced by alternating low and high frequency stimulation (referred to as model A in the text). Stimulation at 2 Hz facilitates the release of enkephalin (red); that at 100 Hz stimulates the release of dynorphin (blue). The overlapping areas (purple) indicate the synergistic interaction between the two peptides. Modified, with permission, from Ref. [32].

D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr amide (CTOP) produced a selective blockade of the analgesia only in the 2/100 mode. Third, these results have been validated by the antibody microinjection experiment. Taken together, the 2/100 mode seems to activate both the μ/δ and κ opioid systems to induce a synergistic analgesic effect, whereas the 2 + 100 mode activates only the κ opioid system. In accordance with this hypothesis, the analgesic effect induced by 2/100 Hz was significantly stronger than that induced by 2 + 100 Hz [33]. A recent study using molecular biology has supported the concept that endogenously released dynorphin does indeed possess a strong antinociceptive effect in the spinal cord [34].

Clinical verification of laboratory findings

The findings obtained in experimental animals have since been confirmed in humans in clinical practice. White *et al.*

at the University of Texas Southwestern Medical Center (TX, USA) performed a series of studies to determine whether peripheral electrical stimulation of the alternating-frequency mode would produce a significantly stronger analgesic effect than that produced by stimulation of fixed frequency in various clinical settings. Observations on the post-operative requirement of opioid analgesics [35] revealed that the alternating-mode stimulation reduced morphine requirement by 53%, whereas a constant low (2 Hz) or constant high (100 Hz) frequency produced only a 32 or 35% decrease, respectively. Ghoname *et al.* [36] made similar observations in patients with chronic lower-back pain and found that the alternating mode of stimulation was the most effective in decreasing pain, increasing physical activity and improving the quality of sleep (when compared with the pure low- and pure high-frequency stimulation). Because the alternating mode produced a more potent analgesic effect, it was used as a standard mode of stimulation for further studies searching for the optimal intensity [37] and optimal stimulation duration [38]. Thus, controlled clinical studies performed in the past six years using peripheral electrical stimulation for the control of various forms of acute [35,37] and chronic [36,38,39] pain have elegantly replicated what we have found in animal studies over the past two decades.

Results obtained in EA-induced analgesia have been applied to the treatment of heroin addiction with considerable success. The withdrawal syndrome observed in rats dependent on morphine can be effectively suppressed by 100 Hz EA, which accelerates the release of dynorphin in the spinal cord [40,41]. By contrast, morphine-induced conditioned-place preference (CPP), an experimental model simulating the craving of heroin addicts, can be successfully suppressed by 2 Hz EA but not 100 Hz EA [42,43]. This effect can be blocked by a small dose of naloxone, indicating the involvement of endogenous opioid peptides interacting with μ and δ opioid receptors [42,43]. As would thus be expected, in clinical practice the alternating mode of stimulation has shown strong therapeutic effects for both physical and psychological dependence in heroin addicts [44,45].

Responses of other neuropeptides to peripheral electrical stimulation

Orphanin FQ (OFQ, also known as nociceptin) [46,47] is another opiate-related neuropeptide that modulates nociception. Recent studies describe apparent paradoxical effects of OFQ on pain modulation – analgesia in the spinal cord and pronociception (an increase in pain sensitivity) in the brain [48–52]. Analgesia induced by 100 Hz EA can be potentiated by antibodies to OFQ injected into the cerebral lateral ventricle and suppressed by the same antibodies injected into the spinal arachnoid space [53], suggesting that endogenous OFQ released by 100 Hz EA plays opposite roles in brain and spinal cord.

Cholecystokinin octapeptide (CCK-8) has been recognized as an anti-opioid peptide in the CNS [54]. The most effective method for stimulating the release of CCK-8 in the spinal cord with peripheral stimulation is to use higher frequencies (15 or 100 Hz), whereas 2 Hz is only marginally effective [55]. Liu *et al.* [56] measured the

amount of CCK-8 in rat spinal perfusate as an indicator of CCK-8 release and found that those rats showing a significant increase in CCK release during 100 Hz EA stimulation were low responders (i.e. exhibited weak EA analgesia), whereas rats showing little increase in CCK release were high responders (i.e. exhibited strong EA analgesia). Moreover, the speed of response also plays an important role. It seems that the effect of EA analgesia is determined by, among other things, the magnitude and the rapidity of CCK release in the spinal cord in response to peripheral stimulation. This has been confirmed by the finding that a rat that is not responsive to 100 Hz EA can be transformed into a responder by injection of antisense oligonucleotides to CCK mRNA into the cerebral ventricles, which suppresses the expression of CCK in the brain [57]. Furthermore, a responder rat can be changed into a non-responder by inducing overexpression of CCK in the brain [58].

Substance P mediates nociception at the first synapse in the spinal cord. *In vivo* study revealed that peripheral stimulation in the 8–100 Hz range elevated the content of SP in rat spinal perfusate, with maximal effect at 15 Hz [59]. Similar results were obtained in cats (maximal release at 20 Hz) [60]. By contrast, 2 Hz peripheral stimulation produced a 50% decrease in the SP content of the spinal perfusate [59], possibly owing to the release of enkephalin [21], which in turn suppressed the release of SP [61].

Angiotensin II (AII) is another neuropeptide with anti-opioid activity [62]. The release profile is unique, with a significant decrease (+62%, $P < 0.01$) at 15 Hz and a significant increase (+60%, $P < 0.05$) at 100 Hz [63]. The decrease of AII release can be reversed by the μ -preferring opioid antagonist naloxone, which changed the 62% decrease into a 125% increase. These results suggest that opioid peptides are important modulators affecting the release of other neuropeptides: 2 Hz EA releases enkephalin, which activates AII and, thus, a negative feedback control [63]; 100 Hz EA releases dynorphin, which activates CCK-8 and, thus, another feedback control [64]. These can be considered as examples of the fine-tuning that is achieved by interactions among peptides.

Last, but not least, is the finding that brain-derived neurotrophic factor (BDNF) can be released by peripheral stimulation of 100 Hz bursts, but not by pure low- (1 Hz) or pure high- (constant 100 Hz) frequency stimulation [65]. This has been verified in primary cultures of hippocampal neurons, in which high-frequency bursts of stimuli evoke instantaneous secretion of BDNF together with the induction of long-term potentiation (LTP) [66]. The ability of peripheral stimulation to accelerate the release of nerve growth factors has obvious clinical implications.

Concluding remarks

It has long been a dream to cure diseases by non-invasive measures that activate self-healing mechanisms, without using drugs or surgical operations. One recent effort along these lines was the use of repetitive transcranial magnetic stimulation (rTMS) to stimulate certain areas of the cerebral cortex; this has achieved limited success in the treatment of depression [67]. Evidence presented in the present

review demonstrates that it is possible to facilitate the release of certain neuropeptides in the CNS by means of peripheral electrical stimulation. In contrast to magnetic stimulation, which stimulates the superficial areas of the brain (i.e. the cortex) [67], peripheral stimulation of the skin or deeper structures activates various brain structures and/or the spinal cord via specific neural pathways (Fig. 2). Any predictions made at this stage should not be overly optimistic. But the clinical efficacy demonstrated using frequency-specific parameters to ease post-operative pain [35,37], lower-back pain [36,38] and diabetic neuropathic pain [39], and the successful application of 100 Hz (but not 2 Hz) stimulation for treating muscle spastic pain of spinal origin [68], certainly hold exciting promise for the future.

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