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Original Article

# Dose-effect relationship between electroacupuncture with different parameters and the regulation of endogenous opioid peptide system☆
# 不同参数电针对内源性阿片肽系统调节的量效关系

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ABSTRACT

Endogenous opioid peptides (EOP) are the neurochemical basis of the anesthetic and analgesic effects of acupuncture, and the quantity of acupuncture stimulus can be controlled accurately by using electroacupuncture (EA). The present study explores the dose-effect relationship between EA with different parameters and the regulation of EOP system. In this paper, the intervention effects of EA on EOP system were specially discussed in terms of the single factor and the different combinations of the frequency, waveform and current intensity. This study shows that EOP system presents a frequency-response specificity. The low frequency of EA promotes the release of enkephalin, $\beta$-endorphin and endomorphin, the high one activates the dynorphin system selectively, and the intermediate frequency works on promoting the release of enkephalin and $\beta$-endorphin, as well as dynorphin. Sparse-dense wave of EA may induce the release of enkephalin, $\beta$-endorphin, endomorphin and dynorphin, presenting a synergistic effect. However, the waveform of EA should be selected flexibly in clinical practice. Sometimes the better therapeutic effect can also be obtained with the continuous wave of EA. EOP system is involved in mediating appropriate intensity of EA, while the acupuncture effect generated by an extra strong EA stimulation refers to a kind of stress response of non-opioid mechanism. The different combinations of EA parameters result in various effects. The combination of EA parameters should be optimized in accordance with different diseases, which is valuable for guiding clinical practice and the development of EA therapy.



The origin of acupuncture can be traced back to the clan community period in the primitive society in China. "*Bian* stone" is the prototype of acupuncture device, the production of metal needling device greatly promotes the development of acupuncture techniques [1]. *Deqi* (arrival of *qi*) is essential for acupuncture effect in treatment of diseases. "*Deqi* determines the therapeutic effects", and "the quick onset of effect depends on the rapid *deqi*". It was not until the early 1950s that people began to intensify or maintain *deqi* with electric stimulation instead of manual twisting-needle technique so as to improve the curative effect of acupuncture. In 1953, the researchers confirmed the effects of EA therapy through experimental studies and clinical observations [2]. Since then, EA technology is popularized and developed rapidly in China. When *deqi* is obtained after needles inserted at acupoints, the electric stimulus is delivered through the needles with a very little electric current in EA therapy. Such operation generates a dual stimulation by both acupuncture and electricity in treatment of diseases [3]. With the development of modern science and technology, transcutaneous electrical acupoint stimulation (TEAS) becomes a new technology of treatment, in which, the non-invasive skin electrodes are used instead of conventional needling of acupuncture. The constant electrical current stimulation breaks through the high resistance of the keratoderma and reach the deep layer of acupoints in treatment [4]. At present, there are various means

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of acupuncture such as manual needling, EA and TEAS. EA and TEAS have been gradually accepted by the majority of physicians and patients because of their significant therapeutic effect and accurate control of stimulation parameters [5,6]. The setting of different stimulation parameters is crucial for EA effect, therefore, the selection of a proper parameters becomes the key topic of clinical trial and mechanism study of acupuncture. The exact material basis has been determined in the generation of acupuncture effects through the research of acupuncture mechanism [7]. The neurochemical mechanism dominated by endogenous opioid peptides (EOP)system is one of the mainstream theories. Besides, monoamine neurotransmitters are also involved, represented by thrombocytin and norepinephrine; amino-acid neurotransmitters (glutamate) and neuropeptides (substance P) [8]. This paper expounds the dose-effect relationship between EA of different parameters and the regulation of EOP system.

## 1. Endogenous opioid-peptide system

Opioids are the oldest and most effective drug for the treatment of severe pain throughout human history [9–10]. But the receptors that bind to opioids and are responsible for their physiological effects were not confirmed in studies until the 1960s. Assumed logically, these receptors are not present to bind plant compounds, but rather to bind endogenous molecules [11]. Hence, scientists intend to find opioid receptors in the body, and then to search for their endogenous ligands. These EOP and their homoreceptors are collectively known as endogenous opioid system [10].

### *1.1. Opioid receptors*

In 1973, Pert, Simon and Terenius used different radioligand-binding assays to confirm the opioid receptors in the brain [12–14]. In 1992, the delta opioid receptor (DOR) was cloned from the NG108-15 cell line of mouse brain [15–16]. In 1993, Chen et al. first reported mu opioid receptor (MOR) cloned from cDNA library of rat brain [17], and in the same year, kappa opioid receptor (KOR) was cloned from striatal cDNA library of rat brain by Meng, et al. [18]. In 1994, Mollereau et al. cloned a completely new receptor whose amino acid sequence shared 49% to 50% identity with rat MOR, DOR, and KOR. However, the affinity of selective ligands for these three classical opioid receptors is very low, indicating that this receptor is similar to the discovered opioid receptor structure, but has different functional properties, thus it is named opioid receptor-like 1 (ORL 1) [19].

### *1.2. Endogenous opioid peptides*

EOP refers to small molecules that are naturally produced in the various glands (such as the pituitary and adrenal glands) in the central nervous system and the body, and can be used as both hormones and neuromodulators. EOP, as the hormone, is secreted from the glands into the circulation and delivered to various remote target tissues where the responses are initiated. As the neuromodulator, EOP is produced and secreted by neurons and works on the brain and spinal cord to regulate the actions of other neurotransmitters [20]. The so-far discovered EOP includes enkephalin, endorphin, dynorphin, norphin and orphorphin.

In 1975, Hughes, et al. first isolated and purified enkephalin from the pig brain, and reported two forms of enkephalin, i.e. methionine enkephalin (MEK) and leucine enkephalin (LEK) [21]. In the following year, Cox et al. found that the C-terminal 31 peptide of $\beta$-lipolysin in the pituitary, which was composed of 91 amino acid residues and had an opioid function. They named it $\beta$-endorphin [22]. In 1979, Goldstein's research team extracted an extremely active opioid substance from the pig pituitary, named dynorphin, and the first 13-amino acid sequence was determined initially [23]. The 17-amino acid sequence of dynorphin was determined completely in 1981 [24]. In 1982, the the dynorphin of 13 peptides discovered were designated dynorphin B; and the previously discovered 17-peptide dynorphin was designated dynorphin A [25].

The study shows that enkephalin is the endogenous ligand, while dynorphin is the endogenous ligand of KOR. But $\beta$-endorphin has relatively similar affinity for MOR and DOR, which cannot be identified as the specific ligand of MOR. Until April 1997, Zadina et al. reported that two 4-peptides acting like morphine were isolated from bovine brain, named endomorphine-1 and endomorphin-2, which are the bioactive peptides with the highest affinity and selectivity for MOR known currently [26]. Orphanin-FQ (OFQ) was isolated from rat and pig brain tissue in Meunier and Reinscheid laboratories in 1995. It is EOP definitely, but different from the classical opioid peptide in structure and function, and it refers to the endogenous ligand of ORL 1 receptor [27,28].

## 2. EOP system is a key mediator of acupuncture effects

The acupuncture anesthesia emerged in the late 1950s and it promotes the research on the mechanism of acupuncture analgesia in China [29]. Acupuncture anesthesia is a special operation. The various systems of the human body are regulated through acupuncture analgesia, due to which, hypalgesia or the increase of pain threshold occurs after stimulating some acupoints when undergoing surgery. In August 1958, Professor Hui-zhu YIN, from Shanghai First People's Hospital successfully performed the first surgery undergoing acupuncture anesthesia in the world [30]. In December of the same year, the operation with EA anesthesia was successfully delivered in Xi'an Fourth People's Hospital [31]. In 1965, research on the mechanism of acupuncture analgesia was launched in Shanghai, Beijing and other places [29]. In 1974, Professor Ji-sheng HAN and his team reported that the pain threshold of the recipient rabbits was increased significantly after injecting the cerebrospinal fluid (CSF) of "the CSF-supplied rabbits" into the ventricles of the "the CSF-received rabbits". It indicates that neurochemicals with analgesic effect are produced in the brain during acupuncture [7]. In 1977, Mayer et al. published that the analgesic effect of acupuncture on pulp pain could be blocked by the intravenously-injected morphine receptor antagonist, naloxone. This finding first proves that endogenous opioid substance is involved in acupuncture analgesia in human body [32]. More importantly, in 1978, Peets et al. reported that the analgesic effect of EA was reduced in CXBK mice with lower density of opioid receptor. It shows that endogenous opioids are crucial in acupuncture analgesia [33]. Professor Xiao-ding CAO and his team have engaged in the study of acupuncture analgesia mechanism for over 20 years since the end of 1964. Through multidisciplinary studies in neurophysiology, neuropharmacology and neuromorphology, it is proved that the afferent impulse after stimulating the acupoints with acupuncture goes up along the conduction pathway of pain and temperature perception to the brain, triggering the positive actions of the transmitter system dominated by EOP, and realizing acupuncture analgesia through the descending inhibition system [34]. In addition, the researchers also observed that the tolerance to acupuncture is similar to morphine, and such phenomenon may be related to the anti-opiate substance generated along with endogenous opioids during acupuncture [35].

Through more than half a century of practice and development, it is proved that acupuncture analgesia activates the endogenous pain perception modulation system chiefly mediated by the opioid peptide system, which has been recognized in the field of global scientific field. In the study by Han JS, et al., among 3 975 articles related to acupuncture, published from 1991 to 2009, there were 1 647 articles associated with analgesia, and about 22% of those ar-

ticles referred to the studies of the opioid mechanism [36]. As the major action, EOP system participates in the modulation of pain information, but it is also equally important on the regulation of the endocrine and immune network, cardiovascular function, digestive system and respiratory system [37]. In 2020, Qi JL et al. took the target protein of acupuncture effect, the key protein of EOP system as the source node, and accurately predicted the potential indications of acupuncture based on the human gene database MalaGards. A total of 63 dominant diseases of acupuncture are concluded, such as polycystic ovary syndrome, primary hypertension, digestive disorders and asthma, involving various systems of the body. It is found that EOP system is the key medium of acupuncture effect [38].

## 3. EOP system and dose-effect of EA

### 3.1. Dose-effect of EA

In general, the quantity of acupuncture stimulus is the comprehensive value of external physical factors exerted during acupuncture. The quantity of EA stimulus is in reference to acupuncture stimulus. Such concept refers to the comprehensive value relevant with the electric pulse stimulation during EA [39]. The relationship between the quantity of acupuncture stimulus and efficacy constitutes the dose-effect relationship of acupuncture [40]. This relationship is also presented in EA. The selection of EA parameters is the key to the successful delivery of EA or TEAS. The main factors affecting the EA stimulus quantity include the frequency, waveform and intensity of the output of EA apparatus, and these three parameters are interacted. Hence, the study should focus not only on the effects of individual parameter, but also on the responses to different combinations.

### 3.2. Frequency of EA

The EA frequency is the number of pulses per second, measured in Hz [41]. Nerves conduct information by discharging individual impulse. The coding of the transmitted information on a single nerve fiber is reflected by the frequency of the pulse, meaning the interval distance of the 2 pulses. Hence, the frequency of electrical stimulation is regarded as the most important determinant for neuroregulation [42]. In clinic and experiment studies, 2 Hz, 15 Hz and 100 Hz are identified as the standard setting for low, medium and high frequency of EA stimulus, respectively [43].

The study by Ji JH, et al. showed that 2 Hz EA promoted the release of the peripheral and central $\beta$-endorphin and enkephalin, and effectively relieved visceral pain in the rat model of irritable bowel syndrome [44]. Cheng and Pomeranz reported that the intraperitoneal injection of naloxone (1 mg/kg) in mice could be against analgesia obtained by low frequency (4 Hz) EA, but had no effect on the analgesic effect of high frequency (200 Hz). It is proposed that analgesia only under low-frequency EA belongs to the opioid mechanism [45]. This finding promotes a further discussion about the frequency specificity of naloxone for blocking EA analgesia. Ji-sheng HAN and his team conducted the research for the effects of subcutaneous injection with naloxone on EA analgesia in the rats. The results showed that analgesia under 2 Hz, 15 Hz and 100 Hz of EA was reversed by 50% corresponding to 0.53 mg/kg, 1.02 mg/kg and 24 mg/kg of naloxone, respectively. It is pointed out that the conclusion is limited for the study undergoing single-dose naloxone. In fact, naloxone can also reverse the analgesic effect of high frequency of EA. The opioid peptides are involved for the analgesic effect with either the low or high frequency of EA. Compared with low-frequency EA stimulus, the dose of naloxone required for 50% blockage of the analgesic effect is varied by over 40 times in high-frequency EA. It is suggested that EA stimulus at different frequencies may exert analgesic effects by releasing different endogenous morphine-like substances [46]. Because the sensitivity to naloxone is not same among different opioid receptors, the complexity of naloxone for blocking the opioid peptides is diverse. In the in vitro experiment of the ileum method in guinea pigs by Goldstein, et al, the results showed that the apparent affinity of naloxone for completely blocking dynorphone is 1/13th that for blockage of LEK or normorphine [23].

The above assumption has been verified by using the antibody microinjection method. The results indicated that the intrathecal injection of enkephalin antibody reduced the analgesic effect of 2 Hz EA by over 70%. The blockage of antibody decreased with increasing the frequency of EA and the blockage was no longer generated when the EA frequency was up to 128 Hz. The intrathecal injection of dynorphin antibody reduced the analgesic effect of 128 Hz EA by over 70%, but there was no any influence on 2 Hz and 4 Hz EA [47]. Injection of $\beta$-endorphin antibody into rat midbrain periaqueduct gray matter reduced analgesia by 88% at 2 Hz of EA, by 61% at 15 Hz; and the analgesia was unaffected at 100 Hz [48]. After the injection with endomorphine peptide antibody in the spinal subarachnoid space, EA analgesia was blocked at 2 Hz, but without affected at 100 Hz [49]. The injection of OFQ antibody into the lateral ventricle of rat had a synergistic effect on analgesia under 100 Hz EA [50]. Besides, the 2/100 Hz stimulation mode was adopted in the experiment. One electrode was set to be 2 Hz and another one was at 100 Hz to stimulate two different acupoints simultaneously, but the brain center may accept the stimulation as 102 Hz, which can only cause the increasing release of dynorphin in spinal perfusion fluid, rather than the increase of endomorphine peptide induced by 2/100 Hz EA [51].

In the study by Fei H, et al, DOR antagonists and KOR receptor antagonists were injected intrathecally in the rats, and it was found that DOR antagonists only reduced analgesia of EA at 2 Hz. KOR antagonist could be antagonistic on analgesia of 100 Hz EA. It is suggested that DOR mediated the effect of low-frequency EA and KOR mediated the effect of high frequency [52]. Through cross-tolerance experiment, EA was operated when the rats presented a tolerance to MOR agonist. The results showed that the analgesic effect was weakened under 2 Hz and density wave 2/15 Hz EA, and that of 100 Hz EA was not affected. It means that MOR is involved in mediating the analgesic mechanism of EA at 2 Hz and density wave 2/15 Hz [53].

Different opioid receptors and their endogenous ligands have the specificity for the responses to different frequencies. The low-frequency (2 Hz) EA promotes the release of enkephalin, $\beta$-endorphin and endomorphin, which acts on DOR and MOR. The high-frequency (100 Hz) EA selectively activates the dynorphin system, releasing dynorphin and acting on the KOR. The intermediate frequency (15 Hz) EA may function somehow in between and it may promote the simultaneous release of enkephalin, $\beta$-endorphin and dynorphin.

### 3.3. Waveform of EA

The common modulated pulse waveforms are sparse-dense wave and intermittent wave, and the continuous wave cannot be modulated. The continuous wave refers to a fixed frequency, with a same period of each pulse. Sparse-dense wave is a composite wave, meaning, sparse and dense waves are exerted alternatively. Discontinuous wave is a composite wave that is exerted intermittently and rhythmically [54].

The human body is prone to adapt to the electrical stimulation produced by the continuous wave, and the modulated pulse wave (e.g. sparse-dense wave, discontinuous wave) relatively reduces the adaptive response of the above electrical stimulation [41]. The effect of EA of sparse-dense wave and 2/15 Hz or 2/100

Hz is stronger than that by EA of the single frequency and continuous wave. The optimal selection of waveform determines the influence of EOP system on acupuncture effect [55]. EA of the sparse-dense wave and 2/100 Hz exerts the stimulation of low frequency (2 Hz) and high frequency (100 Hz) alternatively, it may induce the simultaneous release of enkephalin, $\beta$-endorphin, endorphin and dynorphin, activates MOR, DOR and KOR, and generates synergistic effects [56]. The results of cross-tolerance experiment showed that the sparse-dense wave and 2/15 Hz EA presented analgesic effects through three opioid receptors, i.e. MOR, DOR and KOR; and increased the release of both central enkephalin and dynorphin [57]. It is suggested that the EA stimulation under sparse-dense wave and 2/15 Hz contains two kinds of information: first, low frequency (2 Hz) and intermediate frequency (15 Hz) stimulation promotes the release of enkephalin and dynorphin respectively; second, the alternative waves produces a similar effect as high frequency one, which enhances the release of dynorphin [46].

Hamza, et al. conducted the clinical research to verify the stimulation effects of EA under sparse-dense wave and at single frequency with continuous wave, and observed the use dose of morphine for controlled analgesia after operation in patients. Morphine consumption decreased by 32%, 35% and 53% in the continuous wave 2 Hz group, the continuous wave 100 Hz group, and the sparse-dense wave 2/100 Hz group, respectively [58]. Based on the synergistic effect of various types of EOP, the sparse-dense wave of EA stimulus presents some advantages. However, it should be selected flexibly depending on the individual case in clinic. For example, neuropathic pain must be treated with EA under 2 Hz continuous wave, and muscle spasm pain caused by central nervous system damage should be treated with EA under 100 Hz continuous wave, otherwise, the treatment is ineffective [59]. Regarding pain relief in the acute stage of lumbar disc herniation, the effect of the stimulus under sparse-dense wave is better than that under continuous wave and the intermittent wave, but for improving the lumbar functions, the therapeutic effect is the best with the stimulus under continuous wave [60].

### 3.4. Intensity of EA

The pulse amplitude represents the stimulation intensity of EA, which generally refers to the difference between the maximum and the minimum values of the pulse voltage or current, and also refers to the jump amplitude value during the change in a pulse wave [2].

The EA strength affects various systems, tissues and diseases relatively, thus it cannot be expressed by absolute quantity. Besides, the tolerance of either patients or animals to EA should be considered during study [41]. The sensory threshold is determined when the output intensity of the current induces a gentle muscle jumping at the needling insertion area, or the patient feels mild tingling and vibration in the local area. The slight rigidity or contraction of the muscle induced by the increase of the current intensity, stress response in experimental animal or stabbing pain in patient shows the pain threshold [61]. In the study by Duanmu CL, et al. by determining the thresholds of A and C fibers with C fiber reflex, EA intensity was distinguished, which provides a reference for the standardization of EA intensity [62]. *A neuroanatomical basis for electroacupuncture to drive the vagal–adrenal axis* was published on *Nature*, October 2021, which is a milestone event in the field of basic research of acupuncture and moxibustion. The study proved that EA of high intensity (3 mA) at “Zusanli (ST36)” and “Tianshu (ST25)” activated the sympathetic efferent activity and exerted the immunomodulatory effect through the spleen function. The low-intensity (0.5 mA) EA at “ST36” only presented its anti-inflammatory effect through activating the vagus-adrenal axis mediated by PROKR2-Cre nerve fibers [63]. These findings highlight the importance of the choice of current intensity in the effect onset of acupuncture.

In the study by Huang, et al. [64], the increased percentage of tail-flick latency after EA was used to measure EA-induced analgesic effect. It was observed that the analgesic effect was positively correlated with the current intensity when EA was set between 0.5 mA to 2.0 mA. When the current intensity increased to 3.0 mA from 2.0 mA, the analgesic effect was remained stable. Naloxone, 1 mg/kg reversed the analgesic effect by 68% in 2 Hz EA of 2.0 mA group, but it had no effect in 2 Hz EA of 3.0 mA group. Naloxone, 25 mg/kg blocked the analgesic effect by 67% in 100 Hz EA of 2.0 mA group, but it had no effect in 100 Hz EA of 3.0 mA group. It is concluded that opioids are not involved in EA mechanism with the current intensity over 2.0 mA. In the study by Zhang AZ, et al [65], the defensive movement response and cortical pulp evoked potential of rabbits were taken as the indicators of pain response. They found that the intravenous injection of naloxone (0.4 mg/kg) could resist significantly the analgesic effect of suitable EA (peak current 7.5 mA to 8.0 mA), but it could not reverse the analgesic effect of super-strong EA (peak current 12.5 mA to 15.0 mA). It is suggested that opioid receptors may be involved in mediating the analgesic effect of suitable EA, and the analgesia produced by super-strong EA is a stress response of a non-opioid mechanism.

Feng JJ, et al. [66] compared the assisted analgesic effect and the impacts on EOP among low (5 mA), moderate (12 mA) and high (26 mA) intensities in TEAS, as well as 5 mA EA. The results showed that the plasma enkephalin level was increased in the low-intensity TEAS group; the release of plasma $\beta$-endorphin and enkephalin was promoted in the moderate-intensity TEAS and EA groups. The analgesic effect of moderate-intensity TEAS was better when compared with the low and high-intensity TEAS. High-intensity TEAS did not significantly affect the content of $\beta$-endorphin and enkephalin in the venous blood. It is noteworthy that the analgesic effect of moderate-intensity (12 mA) TEAS is similar to that of EA at 5 mA. TEAS and EA are the common interventions for analgesia by stimulating the different hierarchical structures of acupoints. Duanmu CL, et al. [62] adopted two intensities of stimulation to activate A fiber threshold (Ta) or C fiber threshold (Tc) and compare the analgesic effects between EA and TEAS in rats with muscle inflammatory pain. The results indicated that TEAS at Tc intensity and EA at Ta intensity attenuated pain behavior and abnormal EMG distribution in model rats. Concerning the different levels of the pain source site, the analgesic effect can only be obtained by activating different afferent nerve fibers at different intensities. It is suggested that the afferent mechanisms of TEAS and EA effect are different, and the effects of EA stimulation are various by selecting different intensities.

In conclusion, there is a range of intensity at which the current intensity of EA works on the release of EOP in terms of underlying mechanism of EA. However, the measurement basis and method for the quantification of current intensity have not been unified in both animal experiment and clinical treatment currently. There is the divergence in quantifying the strength of electric current. It is suggested that the parameter range of electric acupuncture apparatus should be standardized in advance so as to contribute to the following study on quantifying the parameters of EA [67]. In animal experiment, if the selected EA intensity is too strong, A$\delta$ nerve fiber and C fiber could be activated, inducing a certain degree of stress analgesia [5], which may mobilize other non-opioid mechanism. It needs to be discussed additionally.

### 3.5. Effect combination of different parameters of EA

The parameters of EA are interacted among each other, rather than worked individually. Exploring the effects of different parameter combinations on EOP is conductive to clinical practice of

EA. In the research by Kuai L, et al. [68], based on the orthogonal experimental design, the pain threshold and the content of $\beta$-endorphin in local inflammatory tissues were taken as the indicators in rat inflammatory pain model, and the best parameters of EA for treatment were 100 Hz in frequency, discontinuous waveform and 0.1 mA in current intensity. In the research [69], the analgesic effect and impact on peripheral $\beta$-endorphin content in rats with tibial cancer pain were compared between the 2/100 Hz + sparse-dense wave + 1 mA EA group and the 2/100 Hz + sparse-dense wave + 2 mA EA group. The results showed that the pain threshold was all increased in two groups, and EA of 2 mA promoted the release of $\beta$-endorphin into blood more significantly [69]. Wan Y, et al. [70] stimulated POMC knockout mice ($\beta$-endorphin deficiency) and wild-type mice with EA of different parameters. The results indicated that 2 Hz EA of low intensity (with 0.3 mA, 0.4 mA, 0.5 mA) could increase significantly the pain threshold of the wild-type mice, and the pain threshold in the gene knockout group fell rapidly after a slight rise at the beginning of EA. The 2 Hz EA of high intensity (with 0.8 mA, 1.0 mA, 1.2 mA) induced analgesia in both the gene knockout mice and the wild-type ones. With 100Hz EA of low intensity (with 0.3 mA, 0.4 mA, 0.5 mA), analgesia was not presented in both groups, but with the progressive increase of EA intensity (with 0.6 mA, 0.8 mA, 1.0 mA), the analgesic effect was re-obtained in two groups. From the perspective of gene, it is proved that $\beta$-endorphin and low-frequency (2 Hz) EA are involved in analgesia, rather than mediated by high-frequency (100 Hz) EA. However, it is also reflected that the analgesic effect of EA is determined by the comprehensive actions of frequency and intensity of EA. The combination of different EA parameters may lead to the release of various kinds of EOP. How to carry out the quantitative study of EA parameters based on the findings of single-factor research is the crucial direction of further research so as to guide the clinical practice.

In summary, we believe that EA stimulation is a holistic concept, and the multiple parameters constitute the basic elements of EA treatment and they interact with each other to influence its effect. Acupuncture and its related techniques induce the cascade reactions through the exogenous stimulation acting on specific parts of the body surface, and then activate the inherent regulatory system function in the body, and eventually achieve the disease prevention and treatment [71,72]. The information and energy carried by the quantity of EA stimulus are interactive in the body, and its intervention on endogenous substances is affected by the body conditions and disease characteristics [73]. It is proposed that the dose-effect research of EA should associate with the individual characteristics of diagnosis and treatment of traditional Chinese medicine and maintain the essence of traditional acupuncture besides focusing on the regularity between the stimulus quantity and effect size.

## 4. Conclusion and outlook

The study on the mechanism of acupuncture anesthesia and its analgesia has clarified that EOP is the neurochemical basis of anaesthetic and analgesic effect of acupuncture. During EA, the quantity of stimulus may be accurately controlled and the studies have found that EA of different parameters can promote the release of different kinds of EOP, and their effects are mediated by different types of opioid receptors. The study of EA parameters has been developed from single factor to the interaction of different parameters. It is believed that the stimulation delivered by EA of different parameters is similar to the action of different doses in medication. However, acupuncture is different from medication in action mode, with more complicated and subtle regulatory effects involved; it is mainly reflected in its specific selectivity. It needs to undertake a further study on its potential mechanism. For instance, as mentioned above, in the research by Qiu-fu MA's team in recent years, the neuronal level of specific labeling has been precisely determined on the base of anatomy, and it is the significant breakthrough in research of the dose-effect relationship of EA. Although analgesic effect is one of the main acupuncture effects, and EOP system has been involved in many studies, the other potential effects and the relevant functional regulatory mechanisms should be also worth considering in future. Zhang SL, et al. confirmed that met-enkephalin up-regulates opioid receptor expression in lung cancer cells via Bcl-2/Bax/caspase-3 signaling pathway, and subsequently enhances natural killer cell-driven tumor immunity [74]. The study by Chen MY, et al. showed that dynorphin A exerts the protective effects by intervening the oxidative stress and apoptosis in cerebral ischemia-reperfusion injury [75]. Studies have also shown that the activated DOR brings the protection in the ischemic damage of the brain and retina, while such effect induced

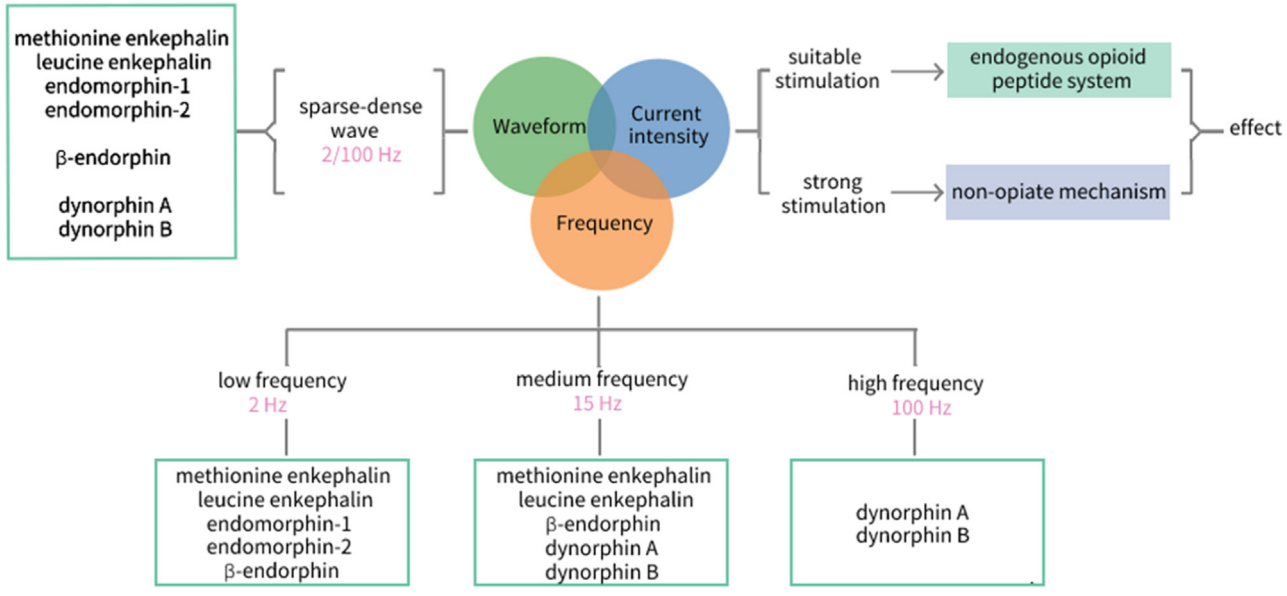


**Fig. 1.** EOP system and dose-effect relationship of electroacupuncture (frequency, waveform and intensity).

by EA is closely related to the activation of DOR [76–80]. Therefore, EOP system is a crucial medium of acupuncture effect, and understanding its potential mechanism is of great significance for expanding the dominant diseases of acupuncture and exploring the functions of EOP system.

The study of the dose-effect relationship of EA with different parameters based on EOP system is conductive to providing targeted and precise EA parameters for clinical applications. More quantitative studies of different stimulation parameters are required to ensure the effective control of them in practice. The achievement of basic researches needs to be transformed to guide clinical practice, and then improve the specificity and effect of treatment (Fig. 1).

## CRediT authorship contribution statement

Ping CHEN: Literature search, drafting of the manuscript, Revising the article. Hong XU: Participate in topic selection and design, revision of the content of the article. Ren ZHANG: Participate in topic selection and design, revision of the content of the article. Xue-song TIAN: Conceived of and designed the paper, providing guidance on the writing and revision of the full-text content, Secured the funding.

## Role of the funder/sponsor

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## Declaration of Competing Interest

The authors declare that there is no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## State of human/animal rights

No need to declare in this pape.

## Data sharing statement

You can contact the corresponding author for the data.

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