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Effect of low- and high-frequency TENS on Met-enkephalin-Arg-Phe and dynorphin A immunoreactivity in human lumbar CSF

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Summary Transcutaneous nerve stimulation (TENS) treatment was given for 30 min to 37 patients divided into 3 groups of 10 patients and 1 group of 7 patients. Two groups received low-frequency (2 Hz) and the other 2 groups high-frequency (100 Hz) stimulation. A diagnostic lumbar cerebrospinal fluid (CSF) sample was obtained immediately before and after stimulation. The CSF samples were subjected to analysis of immunoreactive (ir) opioid peptides, Met-enkephalin-Arg-Phe (MEAP) from preproenkephalin and dynorphin A (Dyn A) from preprodynorphin, respectively.

Low frequency TENS applied on the hand and the leg resulted in a marked increase (367%, $P < 0.05$) of ir-MEAP but not ir-Dyn A, whereas high-frequency (100 Hz) TENS produced a 49% increase in ir-Dyn A ($P < 0.01$) but not ir-MEAP. This is the first report in humans that 2 Hz and 100 Hz peripheral stimulation induces differential release of peptides from preproenkephalin and preprodynorphin, respectively.

Key words: Transcutaneous electrical nerve stimulation (TENS); Opioid peptide; Met-enkephalin-Arg-Phe; Dynorphin A; Electroacupuncture; (Human)

Introduction

There is a substantial literature suggesting that the analgesic effect produced by acupuncture and related stimulation techniques at least partly is due to the release of endogenous opioids at CNS sites relevant to pain control [9].

Cheng and Pomeranz were the first to show that antinociception produced by 4 Hz electroacupuncture (EA, electrical stimulation applied via the needles inserted into so-called acupuncture points) in mice was readily blocked by the opioid antagonist naloxone at 2 mg/kg, suggesting an 'endorphin' mechanism, while 200 Hz EA-induced antinociception was naloxone resistant and hence categorized as 'non-endorphinergic' [2]. However, it was later found that high frequency (100 Hz) EA-induced analgesia in the rat was, in fact, also naloxone reversible provided a higher dose (10 mg/kg) was used [10]. More extensive work showed that the mechanisms for low- and high-frequency EA analgesia are different. In the rat low-frequency EA appears to be mediated via proenkephalin-derived peptides acting on mu receptors whereas high-frequency EA appears dynorphinergic [5] and probably mediated via kappa opioid receptors which are relatively resistant to naloxone blockade [7,11]. This explains why larger doses of naloxone are needed for blocking the analgesic effect induced by high frequency EA [8].

In the clinic, classic acupuncture with manual rotation of needles, EA or transcutaneous nerve stimulation (TENS) are used in otherwise therapy-resistent chronic pain. The widespread use of these techniques testifies to patient compliance and clinical effectiveness. However, the response rate is not uniform depending on the type of stimulation variables and kind

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of pain condition. Evaluation of therapeutic effectiveness is not trivial due to the inadequacy of placebo controls. It could be argued that treatment effects are not dependent on physical variables such as stimulation characteristics and that similar results might have been effectuated by massage or purely psychologic intervention.

The clinical practice of the different stimulation techniques is also varied. Probably, the TENS procedures can be used more objectively than other techniques and are more easily optimized since stimulation frequency and intensity can be varied over a wide range. Clinical studies have indicated a major mechanistic difference between low-frequency TENS, which at least partly appears to work through endogenous opioid systems, since the effect is readily reversed by naloxone and high-frequency TENS which seems to act via non-opioid systems which are not affected by such doses of naloxone [16]. It has also been investigated directly whether opioid peptides are released into the cerebrospinal fluid (CSF) by acupuncture or TENS. An early study indicated release of unspecified opiate receptor active material during low-frequency TENS [13]. Later studies have focused on individual peptides. Both Met-enkephalin and beta-endorphin have been reported to be released by classic, manual acupuncture and by EA [3,4,12]. However, there are not comparative data in which one type of stimulation or set of stimulation conditions have been compared with another. In this communication, a comparison is made between the 2 commonly used forms of TENS (2 Hz and 100 Hz, respectively). Two representative peptides have been measured, each from one of the major opioid systems in the spinal cord and in brain: Met-enkephalin-Arg-Phe (MEAP) from preproenkephalin and dynorphin A (Dyn A) from preprodynorphin.

Materials and methods

Subjects and CSF collection

The subjects were 37 inpatients of 2 hospitals in Beijing, the First University Hospital and the Xuan-Wu Hospital, a municipal hospital specialized in neurology. Patients were all Han-Chinese, aged 21–62 years with neurological disorders including tumors, head trauma, lumbar disc herniation. They had been selected for routine diagnostic lumbar puncture and were asked to participate in the study. Informed consent was obtained from all of them prior to initiation of the study. TENS was not offered as a treatment potentially relieving pain but only as an investigational procedure. This probably reduced any anticipatory placebo response. The patients were randomly distributed into 4 groups of 10 patients each. A standard lumbar puncture was performed at 9.00–11.00 h taking a 6 ml sample, which was divided into 3 ml for routine laboratory diagnosis and 3 ml collected in polyethylene tubes containing 90 μg of bacitracin (Sigma) and 100 μl of 1 M HCl for peptide analysis. While resting on the side contralateral to the side of stimulation the patient was given TENS for 30 min and then subjected to a second lumbar puncture. Each patient only participated once in the study. By a technical error samples from 3 patients in one of the groups were lost.

Stimulation procedures

A skin electrode made of conductant rubber 24 mm in diameter was fixed with EEG gel to the dorsum of one (randomly selected) hand, on the radial aspects of the middle of the second metacarpal bones, corresponding to the acupoint Hegu or LI 4, while another electrode was fixed onto the palmar surface opposite to the Hegu point to complete a circuit. Another pair of electrodes was attached onto the ipsilateral leg, one 2–3 cm lateral and 2–3 cm below the anterior tubercle of the tibia, corresponding to the Zusanli point or ST 36, and the other on the belly directly below the gastrocnemius muscle corresponding to the Chengshan point or UB 57. The 2 pairs of electrodes were connected to the dual channel electronic stimulator HAN ACUTENS model WQ 1002 which delivers bidirectional square waves of 0.3 msec duration, such that 1 positive wave was followed by 1 negative wave of identical intensity. The frequency was set at 2 Hz or 100 Hz. The amplitude was manually adjusted to ensure a visible contraction of the underlying muscle, giving a current of 26–30 mA. The doctor doing these adjustments was blinded as to the clinical status of the patients or to the potential outcome of the chemical measurements. After the cessation of the 30 min stimulation period, a second sample of CSF was taken. The CSF samples were coded, put immediately into a freezer of $-20\,^{\circ}$C and were lyophilized within 2 weeks. The lyophilized samples were kept at $-20\,^{\circ}$C or lower until they were assayed.

Radioimmunoassay

The CSF samples were reconstituted in 16 ml of a buffer (0.01 M pyridine, 0.1 M formic acid) and run through a 1 ml SP-Sephadex ion exchanger column equilibrated in the same buffer. The column was washed with 16 ml of a 0.1 M pyridine, 0.1 M formic acid buffer, and then eluted with 4 ml 0.35 M pyridine, 0.35 M formic acid buffer for MEAP and then with 4 ml of a 1.6 M pyridine, 1.6 M formic acid buffer eluting Dyn A. The respective fractions were taken to dryness in vacuo. Samples to be assayed for MEAP were oxidized by 3% H_2O_2 in 1 M acetic acid for 30 min at $37\,^{\circ}$C.

Radioimmunoassays for MEAP [13] and Dyn A [14] followed previously published procedures. The Dyn A

TABLE I

THE CONTENT OF IMMUNOREACTIVE (ir) MET-ENKEPHALIN-ARG-PHE (MEAP) AND DYNORPHIN A (DynA) IN THE CEREBROSPINAL FLUID (CSF) OF PATIENTS, SUBJECTED TO TRANSCUTANEOUS NERVE STIMULATION (TENS)

Mean values ± S.E.M. are given. NS = not significant.

Frequency (Hz)	No.	Immunoreactivity	Pre-TENS (fmol/ml)	Post-TENS (fmol/ml)	P (paired t)
	10	MEAP	129 ± 56	603 ± 229 (+367%)	< 0.05
2	7	DynA	4.2 ± 0.8	5.4 ± 1.3 (+29%)	NS
	10	MEAP	121 ± 51	70 ± 18 (−58%)	NS
100	10	DynA	4.1 ± 0.4	6.1 ± 0.6 (+49%)	< 0.01

antiserum is directed against the C-terminus of the molecule and will recognize C-terminal fragments. However, there is no (< 0.1%) cross-reaction with other opioid peptides including dynorphin B, α-neoendorphin, Met- or Leu-enkephalin, enkephalyl hexapeptides or beta-endorphin. The MEAP antiserum shows no (< 0.1%) cross-reaction with any of the peptides mentioned. The antiserum is directed to the C-terminus of the peptide and will, therefore, recognize N-terminally elongated peptides. However, the MEAP heptapeptide is selectively eluted in the ion-exchange preseparation [6].

Results

The pre-TENS levels of MEAP and Dyn A did not differ between groups. Levels of MEAP showed more variation than those of Dyn A. Low frequency (2 Hz) TENS produced a 367% increase in the content of ir-MEAP in the spinal fluid ($P < 0.05$). The inter-individual variation in response was considerable with 5 patients showing very marked increases. There was only a very modest (29%) increase in ir-Dyn A ($P > 0.05$). In contrast, high-frequency (100 Hz) TENS produced a moderate (49%) yet statistically significant ($P < 0.01$) increase in ir-Dyn A content. Nine out of 10 patients showed an increase in ir-Dyn A. There was a slight decrease in the content or ir-MEAP, although statistically not significant (Table I).

Discussion

There are several earlier clinical reports that TENS or classic acupuncture release opioid peptides. Thus, acute low-frequency TENS was found to produce an increase in the CSF content of a pool of opioid peptides measured in a receptor assay. The peptides appeared to be of segmental origin [17]. Early studies on EA in heroin addicts indicated release of Met-enkephalin (a peptide from preproenkephalin) but not of beta-endorphin [3] whereas the reverse was observed in chronic pain [4]. More recent work from the same group indicated increase in total opioid activity by 2–3 Hz EA but no change in beta-endorphin or Dyn A [12]. These latter negative findings are in full agreement with the present data.

A particularly interesting observation in the present study is the frequency-dependent differential release of opioid peptides from 2 different systems. When naloxone was used to probe the mechanisms of TENS-induced analgesia, only the effect of low-frequency stimulation was affected [16]. These and other studies favored the interpretation that analgesia induced by low-frequency TENS or classic acupuncture at least partly are mediated via opioid peptides, whereas high-frequency TENS induced analgesia works by some non-opioid and not further specified gating mechanisms. However, the different kind of opioid peptides, i.e., the dynorphins, released by high-frequency stimulation, would be less readily reversed by naloxone [7,11]. In fact, 1 week of daily treatment with high-frequency TENS has been found to increase levels of opioid peptides measured by receptor assay. The increase was only observed in patients who had initial levels below those measured in healthy volunteers. The responding patients had pain of neurogenic origin and were also the best long-term responders to treatment [1].

It is comforting that the distinctions observed here between mechanisms of low- and high-frequency stimulation are entirely parallel to those previously observed in animal experiments. In rats subjected to spinal superfusion, low-frequency stimulation releases Met-enkephalin and high-frequency stimulation Dyn A [5]. The hypoalgesia induced by low-frequency stimulation was readily reversed by naloxone and, therefore, probably mediated via mu-receptors, whereas hypoalgesia induced by high-frequency stimulation required much higher doses of naloxone for reversal but was easily reversed by a kappa-receptor antagonist, indicating that the effects were mediated via kappa-receptors [8]. Since dynorphins are preferred ligands for kappa-receptors, experimental data strongly suggest that the opioid mechanism stimulated by high-frequency TENS is dynorphinergic.

The peptide(s) mediating the response to low-

frequency TENS cannot be acting on delta-receptors which are at least as insensitive to naloxone as kappa-receptors and preferred by enkephalins. A potential candidate peptide deriving from the preproenkephalin gene is methorphamide, which is selective for mu-receptors [18]. The identification of this or related peptides as mediators will have to await further investigation.

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