EFFECTS OF ELECTROACUPUNCTURE AND NALOXONE ON MOUTH OPENING REFLEX

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Abstract

전기침과 Naloxone이 개구반사에 미치는 영향

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악안면 영역에 동통이 가하여지면 회피반사의 일종인 개구반사가 유발되며, 개구반사의 크기는 뇌간의 중추에 전달된 동통의 크기에 비례한다. 따라서 동통의 정도를 악이복근의 근전도를 이용하여 정량화 할 수 있고 동통의 지표로 이용할 수 있다. 본 실험은 악안면동통에 의하여 유발된 개구반사의 크기가 침점(족삼리) 전기자극으로 감소되는지와 이러한 전기침의 효과가 나타나는데 opioid물질이 관여하는지에 대하여 연구하고자 하였다.

8주 이상, 150g이상의 Sprague-Dawley계 쥐 34마리를 암수 구별없이 이용하여 실험하였다. 복강내 Urethane용액(1.5g/kg)을 주입 전신마취하고 악이복근을 노출시켜 근전도 기록을 위한 한쌍의 선전극을 삽입하였다. 동통유발을 위하여 구강내 하악 이공주변에 0.1mm의 선전극을 한쌍 삽입하고 전기자극기에 연결하였다. 유해자극 조건은 duration 100㎲ec, interval 5sec의 pulse로 정하고 자극의 크기는 개구반사를 일으키는 역치의 2배 크기로 하며 매 측정시마다 동일 자극을 10회식 가하여 평균하였다. 침점의 전기자극을 위하여 침점의 하나인 족삼리(Zusanli)에 표면전극(넓이 0.4cm^2 정도)을 부착하고 자극부위가 약한 근수축을 일으키는 강도인 100㎲ec, 5V, 2Hz의 자극을 20분간 가하였다. 악이복근의 근전도는 교류증폭기(Dam80, WPI, USA)에서 1000배 증폭하여 유해자극이 가해진 순간 oscilloscope에서 관찰하여 그 크기를 측정하였다. 침점의 전기자극으로 나타나는 진통효과가 opioid의 분비와 관련있는지 알아보기 위하여 opioid의 길항제인 naloxone(0.2mg/kg)을 복강내로 투여하였다.

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실험군을 4군(group Ⅰ - Ⅳ)으로 분류하였고 각 군에서 근전도를 측정한 단계는 다음과 같다.
group Ⅰ : control, 침점에 전기자극 20분간 가한후(EA), 20분후(EA20)
group Ⅱ : control, 침점에 전기자극 20분간 가한후(EA), naloxone 투여 20분후(NX)
group Ⅲ : control, naloxone 투여 5분후 침점에 전기자극 20분 가한후(NxEA20)
group Ⅳ : control, naloxone 투여 20분후(NX20)
구강내에 가해진 유해자극에 의하여 발생하는 악이복근 근전도는 족삼리 침점의 전기자극으로 그 크기가 감소하였고 이러한 침점자극의 효과는 naloxone의 투여로 인하여 억제되었다. 이와 같은 결과는 침점자극으로 진통작용이 나타나는데에 opioid물질이 관여하고 있음을 시사한다.

Ⅰ. INTRODUCTION

Numerous researches show that acupuncture stimulation increase pain threshold in man and that analgesic effects probably analogous to those in man are produced by its stimulation in various animal species. In some parts of the world, acupuncture has been employed as a method of obtaining analgesia for thousands of years without the mechanism of its action being understood. The theory of energy, called chi, set forth in the ancient Chinese text is quite foreign to western medical thought. According to the Chinese hypothesis, the body is endowed with a fixed energy quotient at birth. At the same time that this is depleted through the vicissitudes of daily living, it is augmented and transformed by energy obtained from food and air. Energy imbalance is the root of illness; its absence is death. This energy is considered to circulate throughout the body in a well defined cycle, moving in a prescribed meridian to meridian and from organ to organ.

During the past two decades, evidence has accumulated indicating that acupuncture activates an intrinsic neural network which modifies the activity of pain transmitting neurons. The inhibiting action is partly mediated by endogenous opioid peptides and monoamines. The system is organized at three levels of the neuroaxis:spinal cord, medulla and the midbrain[13]. The raphe magnus nucleus and the spinal cord constitute a fundamental circuit while the PAG funnels the influences from the more rostral structures and collects information from the spinal cord[2]. PAG initiates descending and ascending inhibition resulting in the reduction of pain. The endogenous pain control system may be elicited by other physiological stimuli and the effect of acupuncture is scarcely specific.

The observation that the narcotic antagonist naloxone could inhibit analgesia[10] produced by electrical stimulation of the brain indicated the involvement of an endogenous chemical in the relief of pain[3,6,11]. Multiple endogenous opioid peptides have been identified that have similar phar macological properties to known narcotic analgesics. The biosynthesis, release, and degradation of opioid peptides have been studied in order to better understand how the manipulation of endogenous opioid systems can be used to produce or augment analgesia. Those studies reveal that various conditions and manipulations, such as electrical brain stimulation, acupuncture, stress, and the administration of opioid analgesics, can cause the release of endogenous opioid peptides and possibly endogenous nonpeptide substances[12]. It has also been discovered that nonopioid peptides, such as cholecystokinin, calcitonin, and

angiotensin II, can alter the action of opioid analgesics by antagonizing or potentiating their effects. An understanding of the role of endogenous peptides in endogenous opioid mechanisms is necessary for the development of new ways to treat pain and such other disorders as sleep apnea in children(sudden infant death syndrome), head injury, and opioid addiction that involve the activation or alteration of endogenous opioid systems.

The present study was designed to observe (1) whether electrical stimulation at acupuncture point could produce antinociceptive effects on oral pain; (2) whether the antinociceptive effects could be blocked by intraperitoneal injection of the opioid receptor antagonist naloxone. The analgesic effect of acupuncture was estimated by the degree of the jaw opening reflex evoked by noxious electrical stimuli around mental foramen which was measured by the digastric muscle electromyogram.

Ⅱ. METHODS

Experiments were carried out with 34 Sprague Dawley rats weighing 180-200g. Each ainimal was anesthetized with intraperitoneal urethane in an initial dose of 0.15g/100g. A pair of stimulating electrodes(interpolar distance of 3mm) of wires, 0.1mm in diameter, insulated except for the tips, were inserted into the area around mental foramen for noxious stimulation. Rectangular constant current pulses(100㎲sec duration, 2Hz) with intensity of about 2 times threshold for evoking the jaw opening reflex.

Anterior belly of digastric muscle was exposed and a pair of 0.1mm wire electrodes were inserted for EMG recording. The magnitude of the jaw opening reflex was estimated by averaging the amplitudes of digastric EMG measured from oscilloscope.

Surface electrode with 0.49㎠ was located on the acupuncture point Zu sanli(ST36, lower lateral to the knee joint) for acupuncture stimulation(100㎲, 5V, 2Hz). This stimulation was maintained for 20min. Weak muscular con tractions were being elicited during the stimulation. Naloxone hydrochloride(0.2㎎/㎏) was given intraperitoneally to observe whether the suppressive effect involves the endogenous opioid neurotransmitter activated by electroacupuncture stimulation.

Experimental groups were classified as follows;digastric EMG was measured on each step.

group Ⅰ:Control, Electroacupuncture stimulation on Zusanli for 20 min(EA), 20 min after cessation of EA.

group Ⅱ:Control, Electroacupuncture stimulation on Zusanli for 20min(EA), treatment with naloxone for 20min(NX).

group Ⅲ:Control, electroacupuncture stimulation on Zusanli for 20min after 5min from naloxone injection (NXEA20).

group Ⅳ:Control, After 20min from naloxone injection only(NX20).

The data were expressed as the means and standard errors of the relative values compared to control. Statistical analysis of difference was assessed with a two-way ANOVA.

Ⅲ. RESULTS

Group Ⅰ

The amplitude of digastric EMG was decreased to 40% of control after EA stimulation for 20min and this decrement lasted (41% of control) 20min after cessation of EA (Fig. 1).

Group Ⅱ

The amplitude of digastric EMG was de-

creased to 38% of control after EA stimulation for 20min and recovered to 60% of control after naloxone application(Fig. 2).

Group Ⅲ

The amplitude of digastric EMG was decreased to 82% of control after EA stimulation for 20min under the naloxone application(Fig. 3).

Group Ⅳ

The amplitude of digastric EMG was not changed significantly(106% of control) after simple naloxone application(Fig. 3).

The analgesic effect of electroacupuncture stimulation on oral pain was found markedly in group I and II, and also observed in group III. That kind of analgesic effect was blocked by naloxone application in NX in group II and in NXEA20 in group III as compared with EA in group I or II(Fig. 4).

Ⅳ. DISCUSSION

Transcutaneous electrical nerve stimulation (TENS), acupuncture-needling, and electroacupuncture are useful non-ablative methods in medical practice for relief of pain. These procedures appear to work by causing an increased discharge in afferent nerve fibers which in turn modifies the transmission of impulses in pain pathways[17]. It is known that the mechanism of analagesic effect via these maneuvers are variable depending on the stimulating parameters. For example, the endogenous opioid system is profoundly related to the mechanism when a peripheral nerve stimulation is applied with parameters of low frequency and high intensity[15,16]. However, when stimulated with parameters of high frequency and high intensity, the reduced activity of dorsal horn neurons are only slightly reversed by a systemic administration of naloxone, a specific opiate antagonist. The involvement of endogenous opioids in antinociception induced by electrical stimulation has been well documented[7]. Several experiments have shown that electrical stimulation of different frequencies at traditional acupuncture sites induces the release of different opioid peptides in the spinal cord of rats and results in antinociceptive effects via different opioid receptors[18].

It has been reported that electrical stimulation at traditional acupuncture sites in healthy males significantly reduced the pain induced by immersion of fingers into a cold bath at 0 ℃. Morphine produced an effect similar to that of acupuncture. Both clinical and laboratory research has shown that the latency to achieve analgesia after onset of acupuncture stimulation is approximately 20 to 30 min. In the present study, the earliest antinociceptive effect that we measured was at 20 min poststimulation. The antinociceptive effect lasted about 20 min after cessation of stimulation (Fig. 1).

According to the traditional Chinese merid ian theories, stimulatioion of the particular points on the body surface exerts a specific analgesic effect on the structures some dis tance away. Stimulation of Yin-Hisiang, Ho-Ku or Shou-Sanli produced the suppressive effects on the pulp evoked jaw opening reflex[5,14], suggesting that the effects depend on complex mechanisms in the central nervous system involved intrasegmental and extrasegmental structures and on neurohumoral factors, such as endorphines or enkephalins, because the effects are blocked by naloxone injection.

Opioid receptors in the central nervous system may be classified according to phar macological, behavioral, or binding studies[1]. Classical μ-receptors probably have beta-en-

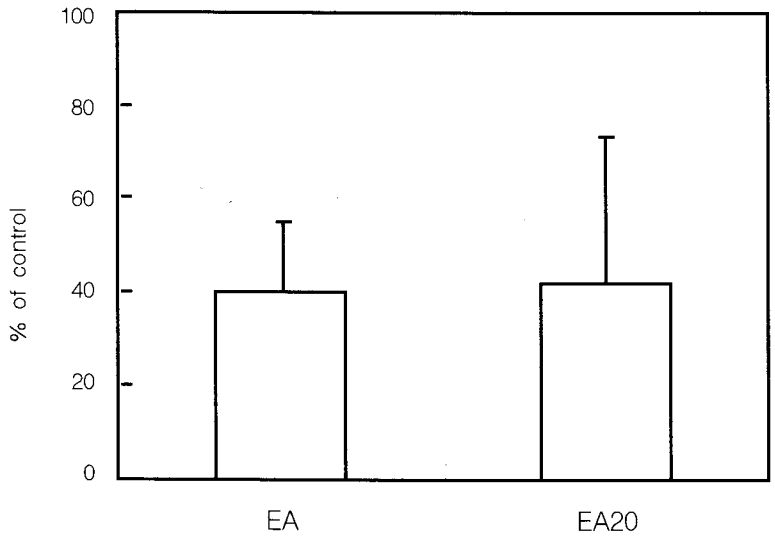

Fig. 1 Antinociceptive effect of electroacupuncture.

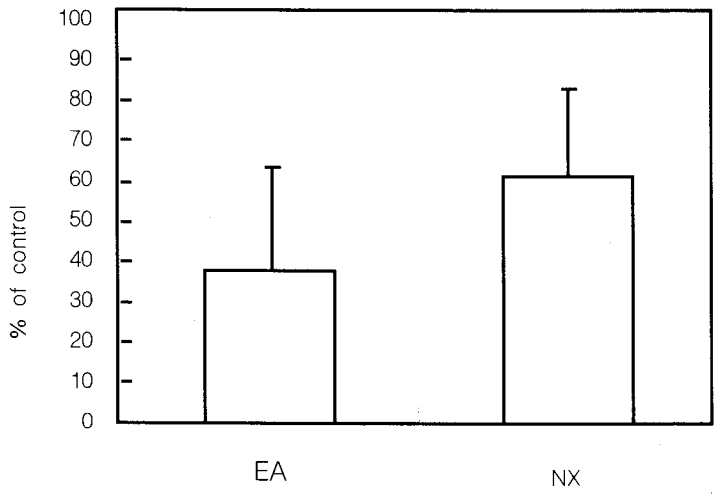

Fig. 2 Naloxone blockade of antinociception.

dorphin as an endogenous ligand, and seem to be involved in the modulation of pain perception, low frequency acupuncture analgesia, and the stimulation of prolactin, growth hormone and thyroid-stimulating hormone release. Met-enkephalin is likely to be an endogenous ligand for the δ-receptors, which predominate in the basal ganglia and limbic systems; such receptors may tonically inhibit the release of corticotrophin-releasing factor. It has been

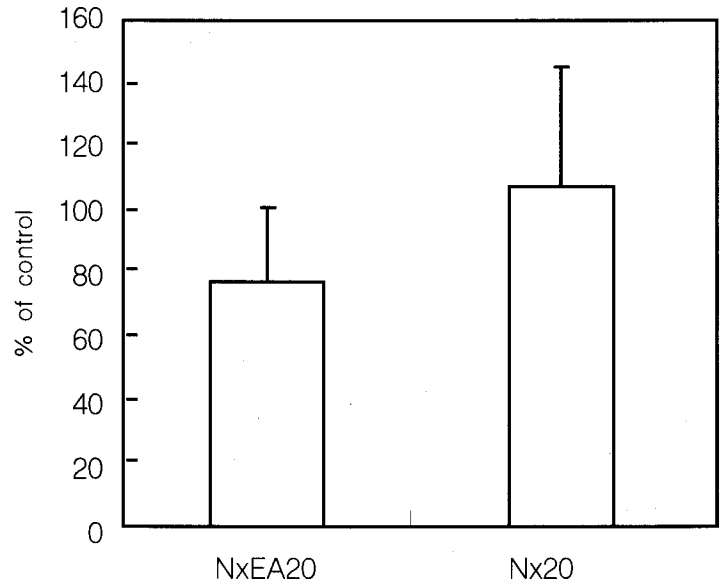

Fig. 3 Naloxone effects on nociception

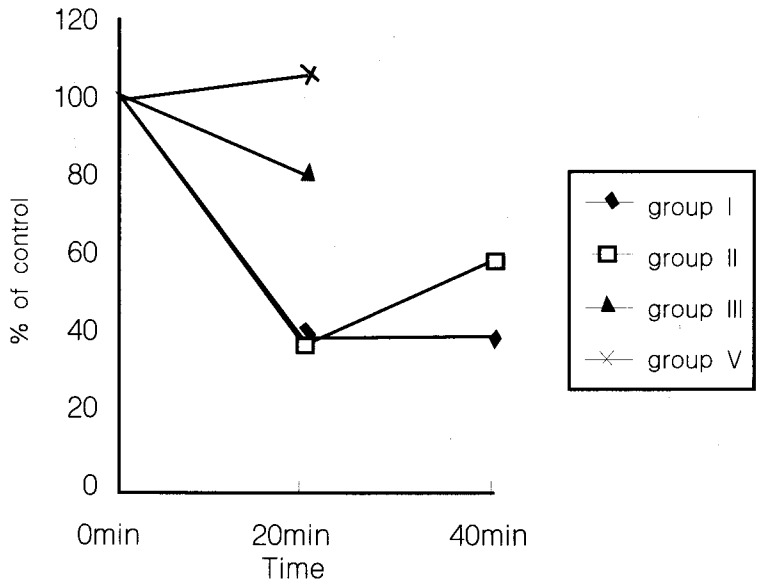

Fig. 4 Simultaneous array of the results from each groups according to the sequence

suggested that the newly described κ-receptors may inhibit the release of vasopressin and gonadotrophin-releasing factor and dynorphin may be their endogenous ligand.

It was reported that 2 Hz electrical stimulation at traditional acupuncture sites can induce the release of enkephalin the spinal cord of rats, resulting in antinociception[4]. Because both mu and delta opioid receptors can be activated by enkephalin, antinocicep-

tion induced by 2 Hz electrical stimulation would be expected to be partially blocked by either mu or delta opioid receptor antagonists[9]. Synergistic effects between μ and δ opioid agonists in the spinal cord and in the brain have been reported. Because antagonists at any one of the three opioid receptor types could reduce 30 Hz antinociception, it appears that all three opioid receptor types in the brain of rats are involved. This finding is similar to the conclusion that there was an interaction among mu, delta and kappa opioid receptors at the spinal cord level on the effect of 2/15 Hz electrical stimulation on the latency to tail flick with radiant heat. Previous reports measuring radiant heat tail-flick latency showed that 100 Hz electrical stimulation can accelerate the release of dynorphin in the spinal cord of rats, producing antinociception via the kappa opioid receptors[8].

In this experiments, 16 animals among 50 did not respond to acupuncture. So we studied this experiment with 34 animals which responded to acupuncture. These results is consistent with the report that differences in effectiveness of acupuncture analgesia might be attributed to individual variations in response; up to 30% of the test animals respond poorly. Therefore, this individual variation must be taken into consideration when applying clinically acupuncture to humans and animals. From an review of the use of acupuncture for relief of pain, it can be concluded that most studies on acupuncture suggested at 50-70% of patients with pain achieved clinically significant short term pain relief, compared with the placebo response rate of 30% commonly reported.

Although the traditional acupoints Zusanli was used in the present study, precision in electrode placement is not as important in electrical stimulation as in manual manipulation because the current spreads out from the electrodes.

The effective stimulation for producing acupuncture analgesia was found to be that which caused muscle contraction beneath the acupuncture point. Such muscles included tibial muscle beneath the Zusanli, the dorsal finger interosseous muscle beneath the Hoku point, and the levator auricular muscle beneath the Keimyaku point.

V. CONCLUSION

The present study was designed to observe (1) whether electrical stimulation at acupuncture point could produce antinociceptive effects on oral pain; (2) whether the antinociceptive effects could be blocked by intraperitoneal injection of the opioid receptor antagonist naloxone. The analgesic effect of acupuncture was estimated by the degree of the jaw opening reflex evoked by noxious electrical stimuli around mental foramen which was measured by the digastric muscle electromyogram.

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In regard to above results, stimulation of Zusanli resulted in reducing the amplitude of digastric EMG and these effects were inhibited by naloxone. It implies that effects of acupuncture stimulation on analgesia is mediated by endogenous opioid.

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