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Diencephalon as a cardinal neural structure for mediating 2 Hz- but not 100 Hz-electroacupuncture-induced tail flick reflex suppression

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Transections at two levels of the forebrain were undertaken in the rat to investigate the neural structures indispensable for the mediation of tail flick reflex suppression (TFRS) induced by low (2 Hz) and high (100 Hz) frequency electroacupuncture (EA) stimulation. Removal of the telencephalic structures did not affect TFRS produced by high frequency EA, although a mild and temporal attenuation was observed for low frequency EA-induced TFRS. Ablation of the whole forebrain (the telencephalon and diencephalon) resulted in a total abolishment of 2-Hz EA effect as measured 5, 24 and 72 h after surgery. In the meantime there was a moderate attenuation (-32.8%, $P < 0.05$) of 100-Hz EA effect, which appeared 5 h after the operation and recovered after 24 h. The results indicate that (1) high frequency EA effect persisted in animals devoid of the whole forebrain structures; (2) an intact diencephalon is indispensable for the neural circuitry controlling low frequency EA-induced TFRS.

INTRODUCTION

The difference between low frequency (1–4 Hz) and high frequency (60–200 Hz) electroacupuncture (EA) analgesia was originally noticed in the experiments using various species of animals by the observations that opiate receptor antagonist naloxone was capable of blocking only low frequency, but not high frequency EA analgesia[3,24,26,27]. Similar results were reported more recently by Han and co-workers, showing that a dose of 0.5 mg/kg and 20 mg/kg of naloxone was needed for 50% reversal of 2 Hz and 100 Hz EA analgesia respectively[14]. In the cross-tolerance studies, rats made tolerant to 100 Hz EA were still effective to 2 Hz, and vice versa[6]. Since the discovery of dynorphin[11,12] and the findings that its analgesia was mediated by kappa opioid receptors[13,32] which are relatively resistant to naloxone blockade[2,8], it was speculated that different parameters of EA stimulation may activate various peptidergic systems in the central nervous system[14,15]. This concept was confirmed by fellow experiments: (1) intrathecal injection of delta receptor antagonist ICI174864 or Met-enkephalin antiserum blocked 2 Hz but not 100 Hz EA analgesia; kappa receptor antagonist Mr2266 or dynorphin antiserum, on the contrary, blocked analgesia induced by 100 Hz but not 2 Hz EA stimulation[6,31]. (2) Radioimmunoassay of spinal

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perfusate revealed that Met-enkephalin and dynorphin A were preferentially released by 2 Hz and 100 Hz, respectively[7]. However, little is known regarding the neural structures in the central nervous system where the EA inputs from 2 Hz or 100 Hz or both were received and integrated to produce an analgesic effect. In the present study, removal of the telencephalon or the telencephalon plus diencephalon was made surgically in different groups of rats to explore the possible involvement of forebrain in the central mechanisms integrating the analgesic effect induced by EA of different frequencies.

MATERIALS AND METHODS

Animals, surgery and postoperative care

Female Wistar rats (weighing 200–250 g) supplied by the animal center of the Beijing Medical University were used and anaesthetized intraperitoneally with 10% chlorohydrate (0.3 ml/100 g). The head of the animal was placed in a stereotaxic head-holder in a prone position such that the interaural line (an imaginary line that passes through the centers of the ear plugs) is exactly 5 mm below the level of the upper incisor bar. To ablate the telencephalon, a small patch of parietal bone was removed by a fine rongeur. With the aid of the Pellegrino et al. atlas[23], under direct visual observation, decortication was made by transecting the brain with a thin plastic plate which was obliquely inserted with a backward slanted angle of 50° from the vertical line. Such transection usually went downwards from the exposed area (2.5–3.0 mm caudal to the bregma) to the anterior border of the optic chiasma[28] (see Fig. 1, upper panel, section I). Thus most of the cerebral cortex, hippocampus, septum, caudate-putamen, accumbens and basal forebrain regions were separated from the underlying brain structures. The ablation of the diencephalon in addition to the telencephalon was made by the same plate which was inserted vertically (Fig. 1, upper panel, Section II). The skull was finally closed with dental cement. In the case of control animals receiving the sham-lesion, the skull was opened and then closed with dental cement.

Postoperatively, the animals that survived were tube-fed twice daily with a dry baby-food mixture dissolved in normal saline (10 ml per day). They were kept in individual cages at an ambient temperature of 20–25 °C under a 12 : 12 h light/dark cycle. Body weight and rectal temperature were monitored and maintained within normal physiological limits.

Nociceptive test and EA stimulation

Rats were kept in special holders for testing tail flick latency (TFL) with thermal irradiation given to the lower third of the tail. The results of the 3 successive measurements with 5 min apart obtained at the beginning of the EA stimulation were averaged and taken as the baseline level, usually in the range of 4–6 s. The values of the subsequent measurements after EA administration were expressed as percent changes from the basal TFL. An elevation over 150% of the basal level was taken as the cut-off limit to avoid unnecessary skin damage. Details of the method have been described elsewhere[25].

Two stainless steel needles were inserted into each hind leg, one in the point Zusanli (S36, 5 mm lateral to the anterior tubercle of the tibia) and the other in the point Sanyingjiao (Sp6, 3 mm proximal to the medial malleolus, at the posterior border of the tibia). Electrical stimulation (0.3 ms duration and 2 Hz) from a HGC-24 programmed pulse generator was given via two needles for a total of 20 min. The intensity of the stimulation was increased stepwise from 1 mA to 3 mA with each 1-mA step lasting for 5 min. The TFL was measured immediately after the termination of EA stimulation. Another EA trial at the frequency of 100 Hz was applied to the same rats with 30-min intervals between the two trials.

Histology

The animals that survived and completed the tests 5 or 24 or 72 h after the operation were sacrificed (injected with excessive chlorohydrate) and decapitated. The head of the animal was removed and immersed in 10% formaline for 4–7 days. Frozen serial sections of 100 μm were made in the mid- and forebrain in the frontal plane stained with Neutral red and inspected microscopically.

Statistical analysis

Data are shown as mean and its S.E.M. Significance of differences between groups were tested with Student's *t*-test (two-tailed).

RESULTS

Histology

The reconstruction of the brain was based on the microscopical outline of the remaining structures; damage due to degenerative changes was not considered. The lesion in the detelencephalated rats showed that all parts of the nucleus accumbens and the majority of the cerebral cortex, hippocampus, septal area and caudate-putamen had disappeared bilaterally in all preparations. In the animals devoid of the telencephalon plus diencephalon, similar lesions were produced except that most of the thalamus and hypothalamus was absent. In most of the animals the boundaries of the transection were located in the preoptic region in the detelencephalated rats, and in the area anterior to the interpeduncular nucleus in the whole forebrain-ablated rats (see upper panel in Fig. 1). A typical example of the detelencephalated animal is illustrated in Fig. 1.

Tail flick reflex suppression (TFRS) produced by EA of different frequencies

TFRS induced by EA of 2 Hz and 100 Hz was measured respectively in sham-operated, decerebrated and the whole forebrain-transected groups of animals before they were operated upon. As shown in Figs. 2–4, the extent of in-

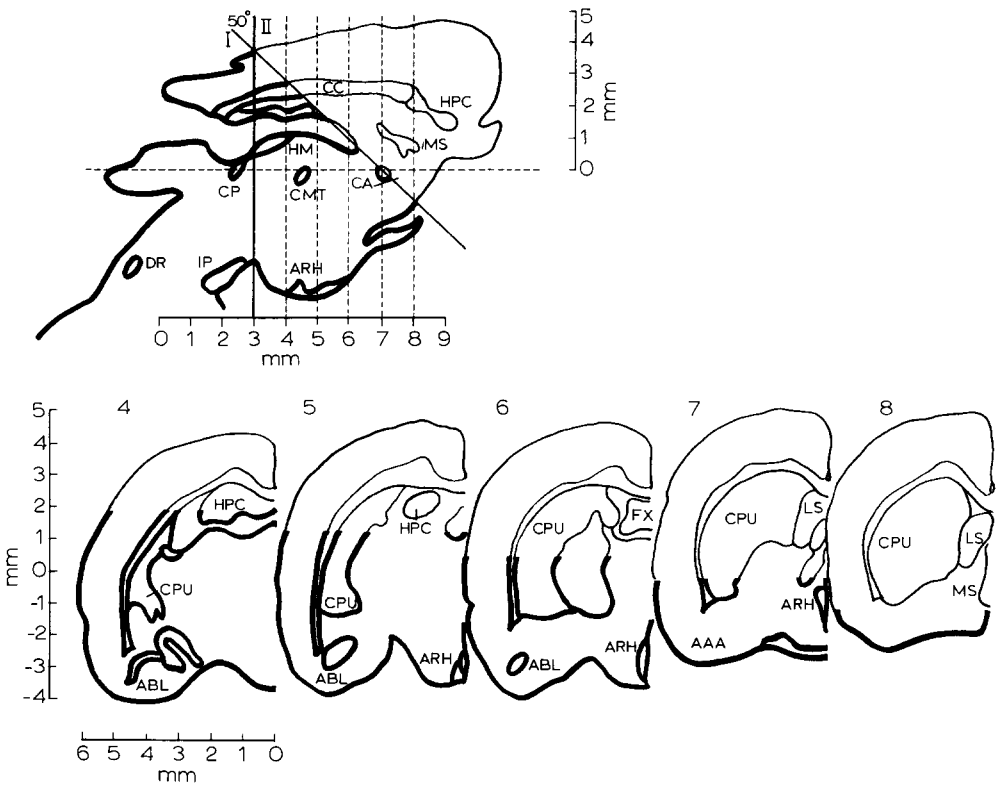

Fig. 1. Upper panel: the midsagittal drawing illustrating the removal of the telencephalon (section I) and the whole of forebrain (the telencephalon plus diencephalon, section II). Lower panel: reconstructions of brain in a detelencephalon rat. Parts of 5 serial sections in the frontal plane were shown rostrocaudally. The thin lines represent ablated brain structures. Figures give distance from the vertical zero plane determined by the atlas of Pellegrino et al.[23]. AAA, anterior amygdaloid area, ABL, basal amygdaloid nucleus, lateral part; ARH, arcuate nucleus of the hypothalamus; CA, anterior commissure; CC, corpus callosum; CMT, centromedian nucleus of the thalamus; CP, posterior commissure; CPU, caudate nucleus putamen; DR, dorsal raphe nucleus; FX, fornix; HM; medial habenular nucleus; HPC, hippocampus; IP, interpeduncular nucleus; LS, lateral septal nucleus; MS, medial septal nucleus.

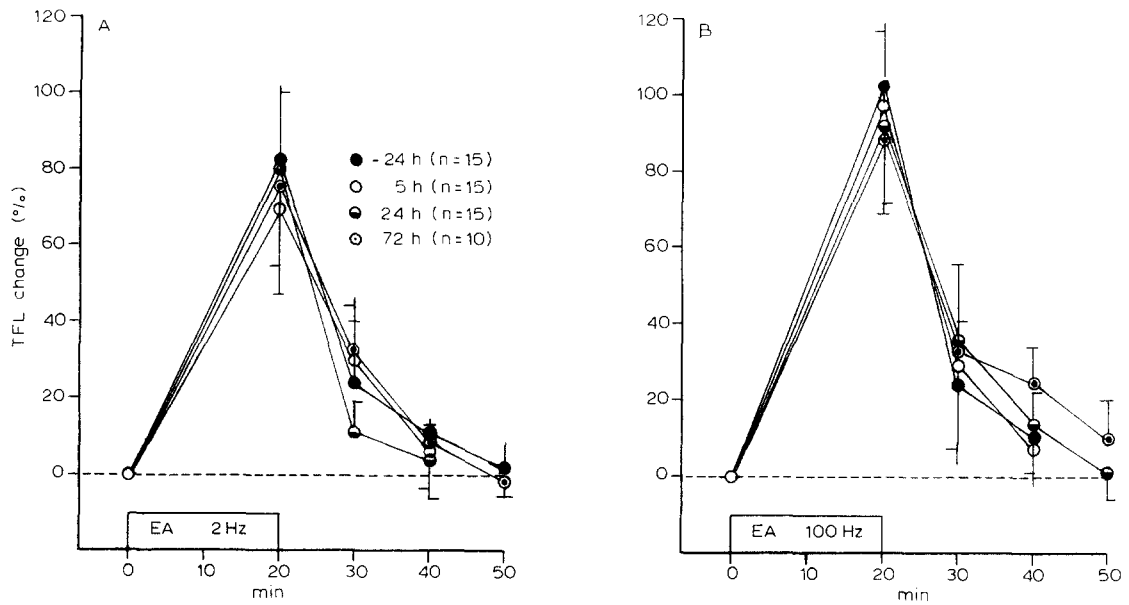

Fig. 2. The effect of 2 Hz (A) and 100 Hz (B) electroacupuncture stimulation on tail flick reflex in mock-operated animals. Electroacupuncture analgesia was examined 24 h before and 5, 24 and 72 h after operation, respectively. The effects of both 2 Hz and 100 Hz electroacupuncture observed 5, 24 and 72 h later were not significantly different from those before operation. The values are expressed in terms of mean percentage over basal TFL. The vertical bars indicate standard errors.

crease in TFL varied with the frequency of EA stimulation, being 80–90% at 2 Hz and 100–110% at 100 Hz. Recovery of the TFL to the basal level usually took place within 20–30 min after cessation of EA stimulation.

Sham-operated animals

In this group of animals, the effect of 2 Hz and 100 Hz electroacupuncture was examined 24 h before and 5, 24 and 72 h after the operation. As shown in Fig. 2A,B, only a small attenuation of EA effect was noticed after the sham lesion, which did not exhibit a statistically significant difference when compared with those taken prior to the surgical preparation.

Detelencephalated animals

In a group of 13 animals, the mean effect of EA-induced TFRS as measured 24 h prior to the surgery was 91 ± 15% (mean ± S.E.M.) and 115 ± 9% for 2 Hz and 100 Hz EA, respectively. Ablation of the telencephalon resulted in a very mild suppression of the EA effect as measured 5, 24 and 72 h after the operation for both low and high frequency EA (Fig. 3A,B). It was only at the point 5 h after the lesion that the effect of 2 Hz EA (67 ± 14%) was significantly lower than the control value taken 24 h before the operation ($P < 0.05$).

Animals devoid of the telencephalon plus diencephalon

As shown in Fig. 4, the TFRS effect induced by 2 Hz EA was almost totally abolished as tested 5 (17 ± 10%, $P < 0.01$), 24 (5 ± 9%, $P < 0.01$) and 72 (11 ± 4%, $P < 0.01$) h after the surgical intervention. In the same group of animals, there was also a mild attenuation of the TFRS induced by 100 Hz EA, reaching a statistically significant level 5 h ($P < 0.05$) after the operation. Table I summarizes the percentage reversal of 2 Hz and 100 Hz EA analgesia in various lesion groups.

Changes in basal TFL

Basal TFL tested 5 h after operation was slightly shortened in the animals devoid of the telencephalon. Such changes, however, were not significant when compared with the preoperative

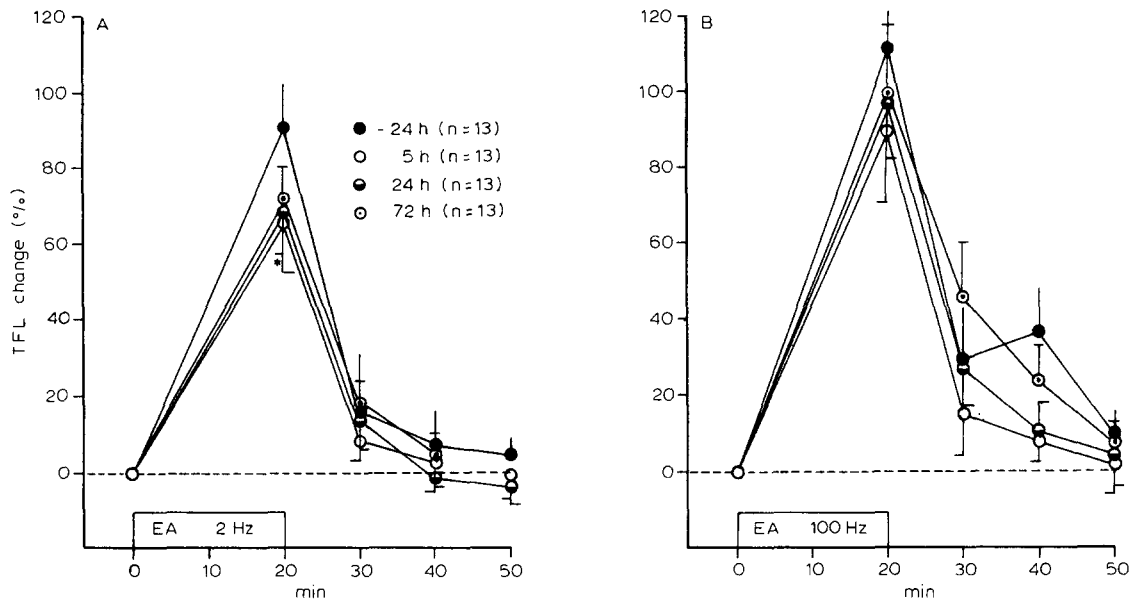

Fig. 3. The effect of 2 Hz (A) and 100 Hz (B) electroacupuncture stimulation on tail flick reflex in the detelencephalated animals. Electroacupuncture analgesia was measured respectively 24 h before and 5, 24 and 72 h after operation. The values are expressed as mean percent increase over basal TFL. Vertical bars indicate standard errors. $*P < 0.05$ as compared with EA effect taken prior to operation.

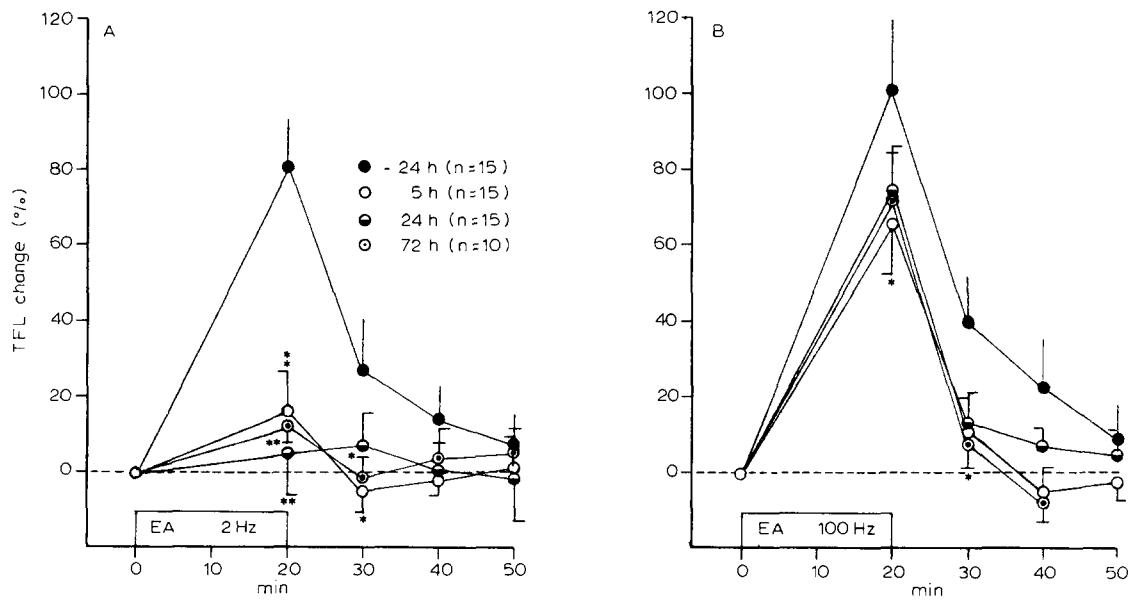

Fig. 4. The effect of 2 Hz (A) and 100 Hz (B) EA stimulation in the animals on tail flick reflex devoid of the telencephalon plus the diencephalon. EA analgesia was measured respectively 24 h before and after 5, 24 and 72 h after preparation. The values are expressed as mean percent increase over basal TFL. Vertical bars indicate standard errors. $*P < 0.05$ and $**P < 0.01$ as compared with EA effect taken prior to preperation.

TABLE I

Reversal of low and high frequency EA induced tail flick reflex suppression in the mock-operated, detelencephalated and the whole forebrain transected animals

The percentage of reversal is calculated from the equation $1-V_a/V_b$, where V_b and V_a represent percent increase in tail flick latency tested before and after operation.

Type of operation	*EA frequency (Hz)*	*After operation (h)*		
		5	*24*	*72*
Mock	2	3.7	0.4	10.8
	100	2.6	7.3	8.2
Detelencephalon	2	25.5*	24.2	21.0
	100	21.1	14.2	12.5
Ablation of the forebrain	2	78.8*	93.4**	85.7**
	100	32.8*	25.3	25.6

* ($P < 0.05$) and ** ($P < 0.01$) indicate significant blockade of the effect of EA analgesia as compared with the value taken 24 h before the operation.

TABLE II

Tail flick latencies (s) assessed before and after intracranial operation in rats (means ± S.E.M.)

Type of operation	*Before operation*	*After operation (h)*		
		5	*24*	*72*
Sham	4.4 ± 0.2	4.4 ± 0.3	4.4 ± 0.6	4.7 ± 0.3
Detelencephalon	4.9 ± 0.3	4.5 ± 0.6	4.6 ± 0.5	4.8 ± 0.4
Ablation of forebrain	5.3 ± 0.3	4.7 ± 0.2	5.2 ± 0.2	5.4 ± 0.3

level, and there was a tendency to recover within 24 and 72 h. Similar results were found in rats devoid of the telencephalon plus the diencephalon.

DISCUSSION

The application of electroacupuncture (electrical stimulation via acupuncture needles) instead of manual needling greatly facilitated the study of the mechanisms of acupuncture effects[5,17]. It was soon revealed that, just as different ways of needle manipulation may produce different therapeutic effects, different parameters of electrical stimulation can produce quantitatively, or even qualitatively different results. While there is a general agreement on the naloxone reversibility and opioid nature of the low frequency EA analgesia[1,18,20], much controversy has been imposed on the mechanisms underlying high frequency EA analgesia. The resistance of high frequency EA analgesia to naloxone blockade has been explained by its non-opioid nature[3,24,26,27], or by the mediation of dynorphin[11,12] which is relatively resistant to naloxone. Since these studies were targeted on the chemical nature of the synaptic transmission, the solution of the problem can certainly be helped by the identification of the reflex arcs involved in these two categories of EA effects.

High frequency EA analgesia has been suggested as a segmental phenomenon[19,29,30]. However, we have shown in previous studies in the rat that high thoracic spinal transection abolished not only low, but also high frequency EA analgesia[16]. Besides, stimulation at point S36 mainly aroused the deep peroneal nerve which belongs to L_4–L_6[9] and did not fall into the segments receiving afferents from the tail[10] (sacral 3

through coccygeal 3); therefore the suppression of tail flick reflex by EA at S36 and Sp6 cannot be regarded as a segmental phenomenon. From the results obtained in the previous and the present studies, we can infer that the central mechanism integrating high frequency EA effect should be mainly located supra-spinal and infra-diencephalon, i.e. in the lower brainstem.

Low frequency EA analgesia has been characterized by the widespread distribution of its analgesic effect, and hence would not be considered as a segmental phenomenon. That the TFRS of 2 Hz EA could be abolished by the ablation of the the diencephalon but not of the telencephalon provides strong evidence for the importance of the diencephalic structures in mediating 2 Hz EA analgesia. Lo et al. have reported in awake and Flaxedil-immobilized cats that the centromedian nucleus of the thalamus plays an important role in the process of low frequency EA analgesia, since (1) the neurones within this nucleus could be activated effectively by 4–8 Hz, but not by 60 Hz electrical stimulation delivered to the deep peroneal nerve[22]; and (2) low, rather than high frequency stimulation of the centromedian nucleus was most effective in suppressing the nociceptive discharges of parafascicular neurones[21]. More evidence supporting the existence of a diencephalic mechanism for processing low frequency EA analgesia was that chemical destruction of the neonatal beta-endorphinergic neurones in the arcuate nucleus of hypothalamus attenuated 2-Hz, but not 100-Hz EA analgesia[4]. In a recent experiment, direct electrolytic lesion was applied to the arcuate nucleus of adult rats, which blocked substantially low frequency EA analgesia without marked influence on high frequency EA analgesia (Wang et al., unpublished observations). Studies are under way exploring the role played by the centromedian nucleus of thalamus and arcuate nucleus of hypothalamus in mediating low frequency EA analgesia.

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REFERENCES

1 Besson, J.M. and Chaouch, A., Peripheral and spinal mechanisms of nociception, *Physiol. Rev.*, 67 (1987) 67–186.
2 Chavkin, C., James, I.F. and Goldstein, A., Dynorphin is a specific endogenous ligand of the k-opioid receptor, *Science*, 215 (1982) 413–415.
3 Cheng, R.S.S. and Pomeranz, B., Electroacupuncture analgesia could be mediated by at least two pain-relieving mechanisms: endorphin and non-endorphin systems, *Life Sci.*, 25 (1979) 1957–1962.
4 Fan, S.G., Wang, X.J., Ding, X.Z. and Han, J.S., Attentuation of low but not high frequency electroacupuncture analgesia by injection of monosodium glutamate in neonatal rats, *Clin. J. Appl. Physiol.*, in press.
5 Fan, S.G., Qu, Z.C., Zhe, Q.Z. and Han, J.S., GABA: antagonistic effect on electroacupuncture analgesia and morphine analgesia in the rat, *Life Sci.*, 31 (1982) 1225–1228.
6 Fei, H., Sun, S.L. and Han, J.S., New evidence supporting differential release of enkephalin and dynorphin by low and high frequency electroacupuncture stimulation, *Kexue Tongbao*, 33 (1988) 703–705.
7 Fei, H., Xie, G.X. and Han, J.S., Differential release of Met-enkephalin and dynorphin in spinal cord by electroacupuncture of different frequency, *Kexue Tongbao*, 31 (1986) 1512–1515.
8 Gilbert, P.E. and Martin, W.R., The effect of morphine- and nalorphine-like drugs in the non-dependent, morphine-dependent and cyclazocine-dependent chronic spinal dog, *J. Pharmacol. Exp. Ther.*, 198 (1976) 66–82.
9 Greene, E.c., *Anatomy of the Rat*, Hafner, New York, 1955, pp. 127–133.
10 Grossman, M.L., Spinal segments receiving the afferent inputs from the tail, *J. Comp. Neurol.*, 206 (1982) 9–16.
11 Goldstein, A. and Ghazarossian, V.E., Immunoreactive dynorphin in pituitary and brain, *Proc. Natl. Acad. Sci. (Wash.)*, 77 (1980) 6207–6210.
12 Goldstein, A., Tachibana, S., Lowney, L.I., Hunkapiller, M. and Hood, L., Dynorphin (1–13), an extraordinary potent opioid peptide, *Proc. Natl. Acad. Sci. (Wash.)*, 76 (1979) 6666–6670.
13 Han, J.S. and Xie, C.W., Dynorphin: potent analgesic effect in spinal cord of the rat, *Life Sci*, 31 (1982) 1781-1783.
14 Han, J.S., Ding, X.Z. and Fan, S.G., The frequency as the cardinal determinant for electroacupuncture analgesia to be reversed by opioid antagonists, *Acta Physiologica Sin.*, 38 (1986) 475–482 (Chinese, with English abstr.).

15 Han, J.S., Xie, G.X., Ding, X.Z. and Fan, S.G., High and low frequency electroacupuncture analgesia are mediated by different opioid peptides, *Pain*, Suppl. 2 (1984) 543–544.
16 Han, J.S., Zhang, M. and Ren, M.F., The effect of spinal transection on acupuncture analgesia and morphine analgesia, *Kexue Tongbao*, 31 (1986) 710–715.
17 Han, J.S. and Xie, G.X., Dynorphin: important mediator for electroacupuncture analgesia in the spinal cord of the rabbit, *Pain*, 18 (1984) 367–376.
18 Han, J.S. and Terenius, L., Neurochemical basis of acupuncture analgesia, *Ann. Rev. Pharmacol. Toxicol.*, 22 (1982) 193–220.
19 Handwerker, H.O., Iggo, A. and Zimmermann, M., Segmental and supraspinal action on dorsal horn neurons responding to noxious and non-noxious skin stinuli, *Pain*, 1 (1975) 147–165.
20 He, L.F., Involvement of endogenous opioid peptides in acupuncture analgesia, *Pain*, 31 (1987) 99–121.
21 Lo, F.S., Yuan, C.S., Yang, S.L. and Zhang, X.T., Inhibitory effect on nociceptive discharges of parafascicular neurones of thalamus by electrical stimulation of nucleus centrum medianum at different frequency, *Acta Physiol. Sin.*, 37 (1985) 1–9 (in Chinese, with English abstract).
22 Lo, F.S., Yuan, C.S., Yang, S.L. and Zhang, X.T., Responses of nucleus centrum medianum of the thalamus to stimulation of deep somatic nerves in cats, *Acta Physiol. Sin.*, 37 (1985) 113–119 (in Chinese, with English abstract).
23 Pellegrino, L.J., Pellegrino, A.S. and Cushman, A.J., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979.
24 Pertovaara, A. and Kemppainen, P., The influence of naloxone on dental pain threshold elevation produced by peripheral conditioning stimulation at high frequency, *Brain Res.*, 215 (1981) 426–429.
25 Ren. M.F. and Han, J.S., Rat tail flick acupuncture analgesia model, *Clin. Med. J.*, 92 (1979) 576–582.
26 Sjolund, B.H. and Eriksson, M.B.E., Endorphins and analgesia produced by peripheral conditioning stimulation, *Adv. Pain Res. Ther.*, 3 (1979) 587–592.
27 Sjolund, B.H. and Eriksson, M.B.E., The influence of naloxone on analgesia produced by peripheral conditioning stimulation, *Brain Res.*, 173 (1979) 295–301.
28 Wang, Q., Guo, X.Q. and Li, P., The blocking effect of somatic inputs on the bradycardia induced by stimulation of the nucleus tractus solitarius in rabbits, *Chin. J. Physiol. Sci.*, 3 (1987) 366–373.
29 Wall, P.D. and Sweet, W.H., Temporary abolition of pain in man, *Science*, 155 (1967) 108–109.
30 Walf, C.J., Transcutaneous and implanted nerve stimulation. In P.D. Wall and R. Melzack (Eds.), *Textbook of Pain*, Churchill Livingstone, London, 1984, pp. 678–690.
31 Xie, G.X. and Han, J.S., Analgesia produced by electroacupuncture of different frequencies are mediated by different varieties of opioids in the spinal cord, *Kexue Tongbao*, 30 (1985) 385–391.
32 Xie, G.X. and Han, J.S., Dynorphin: analgesic effect via kappa-receptors in spinal cord of rats, *Acta Pharmacol. Sin.*, 5 (1984) 231–234.