Brain Research, 513 (1990) 60–66
Elsevier

BRES 15327

The arcuate nucleus of hypothalamus mediates low but not high frequency electroacupuncture analgesia in rats

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(Accepted 29 August 1989)

Key words: Electrolytic lesion; Kainic acid; Electroacupuncture analgesia; Tail flick latency; Arcuate nucleus; Rat

Electrolytic, kainic acid or sham lesions were made in the arcuate nucleus of the hypothalamus (ARH) in female Wistar rats to investigate the putative role of the ARH in the organization of low (2 Hz) or high (100 Hz) frequency electroacupuncture (EA) analgesia. Both electrolytic and chemical lesions lead to an almost total suppression of the low frequency EA analgesia as measured 4 and 6 days following the surgical intervention, leaving high frequency EA analgesia unaffected. In sham-operated animals, the antinociceptive effect induced by low or high frequency EA was essentially intact. These data indicate that neurones of the ARH most likely play an important role in mediating low, but not high frequency EA analgesia.

INTRODUCTION

The findings that low and high frequency electroacupuncture (EA) stimulation administered to the same stimulation sites was capable of activating different peptidergic systems in the spinal cord to exert analgesic effect[7,13,30,31] have raised the supposition that analgesia induced by EA with different frequencies may be mediated by different neural circuits in the central nervous system[3,12,24]. This hypothesis was strongly supported by the recent findings in rats that ablation of the forebrain via a transection rostral to the midbrain produces an almost total abolishment of low frequency EA-induced analgesia, without affecting high frequency EA analgesia. This indicates that low frequency EA utilizes neural substrata located cephalic to the midbrain[33]. Since the antinociceptive effect induced by low frequency EA stimulation was unaffected following the removal of the telencephalon, the diencephalon might play an important role for mediating low, but not high frequency EA analgesia[33]. Among the neural structures present in the diencephalon, the arcuate nucleus of the hypothalamus (ARH) is the one deserving serious consideration. Localized electrical or chemical activation of the ARH produces a profound and long-lasting antinociceptive effect comparable to those obtained after peripheral EA stimulation[9,26,29]. These effects of EA-induced analgesia were markedly attenuated by electrical or chemical lesions in the hypothalamic arcuate areas[10,11,26]. The results suggest that the arcuate nucleus of the hypothalamus (ARH) in the diencephalon serves as the main neural substratum for the mediation of the low frequency EA analgesia. In the present study, restricted electrolytic lesion of the ARH was made to determine its relevance in mediating low and high frequency EA analgesia. In another group, kainic acid, a cytotoxin which has been shown to selectively destroy cell bodies leaving intact the passing fibers[5,16,22], was injected into the ARH to differentiate whether the destruction of cell bodies or fibers of passage are responsible for the observed effect.

MATERIALS AND METHODS

Subjects

Female Wistar rats weighing 200–250 g were supplied by the animal center of the Beijing Medical University. They were randomly assigned into one of four groups: electrolytic lesion group, kainic acid lesion group, sham-operated electrolytic control group, and vehicle solution control group.

Surgery

Rats were anesthetized intraperitoneally with 10% chlorohydrate (0.3 ml/100 g) and placed in a stereotaxic instrument. Small burr holes were made on the skull directing to the arcuate nucleus of the hypothalamus at the stereotaxic coordinates of A 4.4–6.2 (1.4 mm caudal and 0.4 mm rostral to the bregma), L or R 0.0 and H 9.8–10.2 according to the atlas of Pellegrino and Cushman[23]. For the electrolytic lesion a unipolar electrode made by insulated stainless steel wire (0.1 mm in diameter, bared only at the tip) was inserted

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into the ARH area and the indifferent electrode was connected with the skin of wound. A 0.5-mA anodal current from a DC generator (WYJ-30, China) was delivered through the electrode for 20 s. Sham-operated controls had the electrode placed 1.0 mm dorsal to the ARH and no current was passed through the electrode. Kainate lesions were made by injecting 0.3 μl of kainic acid (Sigma Chemical Co.) dissolved in 0.1 M phosphate buffer solution (2 μg/μl, pH 7.4) through a stainless steel tubing of 0.25 mm external diameter. The amount of injection (0.3 μl) and the rate at which it was injected (0.05 μl/min) was controlled by a slow injection apparatus (Palmer). After finishing the injection, the needle was remained in the brain for 5 further min to reduce backflow of the solution along the tract of the injection. For control, animals were given same amount of vehicle solution (phosphate buffer solution) into the hypothalamic arcuate areas.

The burr holes were filled with sterile bone wax, the incision was sutured and testing commenced 4 and was repeated 6 days following surgery.

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 at 5-min intervals obtained prior to the EA stimulation were averaged and taken as basal pain threshold, usually in the range of 4–6 s. The values of the subsequent measurements after EA administration were expressed as percentage changes from the basal TFL. An elevation over 150% of the basal pain threshold was taken as the cut-off limit to avoid unnecessary skin damage. Details of the method have been described elsewhere[27].

Two stainless steel needles were inserted into each hind leg at a depth of 5–9 mm, one in the point Zusanli (S_{36}, 5 mm lateral to the anterior tubercle of the tibia) and the other in the point Sanyingjiao (Sp_6, 3 mm proximal to the medial malleolus, at the posterior border of the tibia). Bidirectional stimulating pulses of 2 Hz (one positive pulse was followed by an identical negative pulse, each pulse lasted for 0.3 ms) from a constant current programmed pulse generator (HGC-24, China) were given via two needles inserted into S_{36} and Sp_6 respectively for a total of 20 min. The intensity of stimulation was increased stepwise from 1 mA to a maximal of 3 mA with steps of 1 mA lasting for 5 min each. The TFL was measured immediately after the cessation of EA stimulation. A session of EA with frequency of 100 Hz (bidirectional, 0.3 ms pulse width) was applied to the same rats after an interval of 30 min. The intensity of stimulation (1-2-3 mA) was the same as in 2 Hz EA. While 2 Hz EA produced rhythmic contractions of the hind legs, 100 Hz EA produced tetanic contraction. Squeaks occurred at the beginning of a new stimulation intensity and subsided in 2–3 min. No significant behavioral effects have been observed during the administration of either low or high frequency EA stimulation.

Histology

Upon conclusion of the testings, all animals were sacrificed with an overdose of anesthetic. Brains were removed and stored in 10%

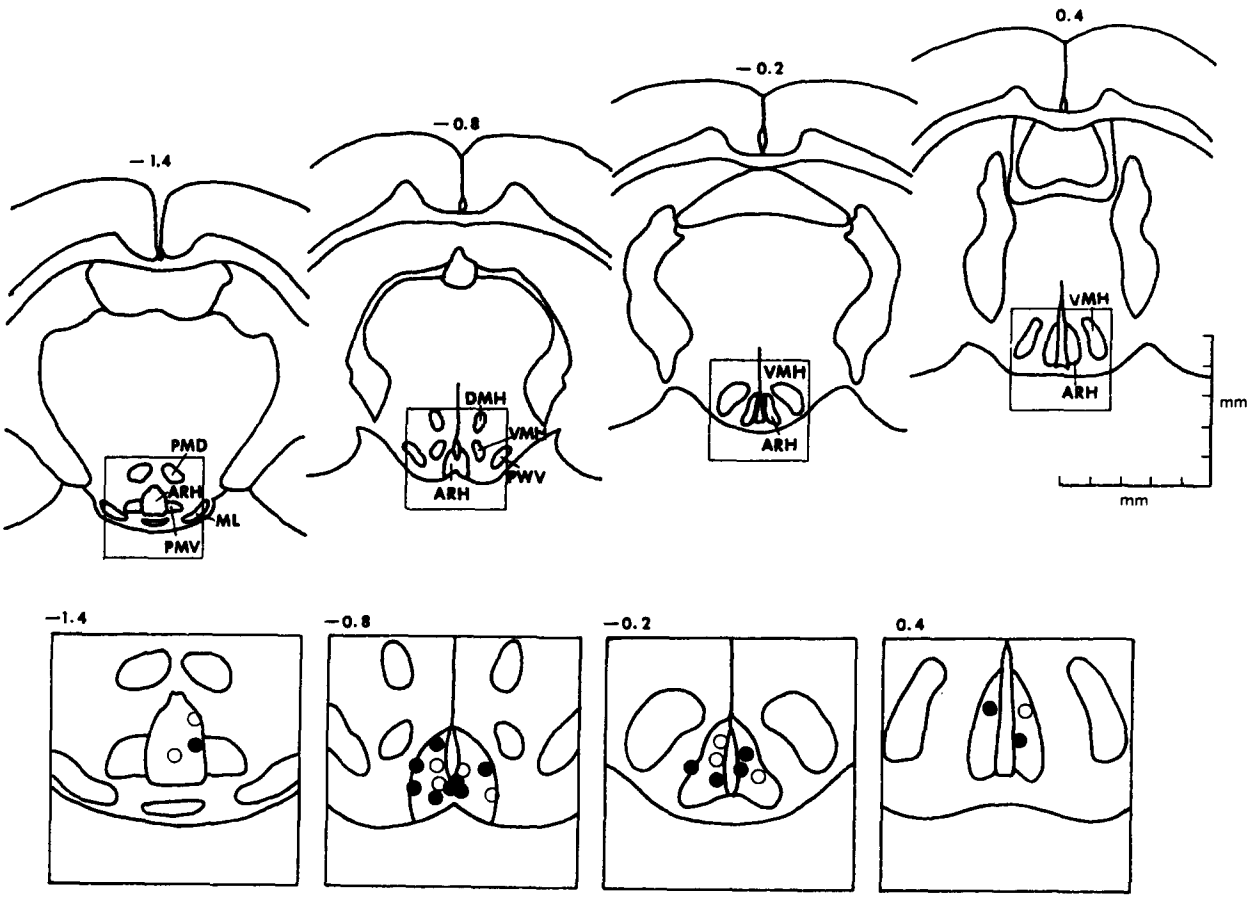

Fig. 1. Anatomical locations of electrolytic (solid circles) and kainic acid (open circles) lesions within the arcuate nucleus displayed on enlarged standard sections of the ventromedial parts of the hypothalamus. Rostrocaudal coordinates are indicated in relation to bregma. ARH, arcuate nucleus of the hypothalamus; DMH, dorsomedial nucleus of the hypothalamus; ML, lateral mamillary nucleus; PMD, dorsal premamillary nucleus; PMV, ventral premamillary nucleus; VMH, ventromedial nucleus of the hypothalamus (according to the atlas of Pellegrino and Cushman[23]).

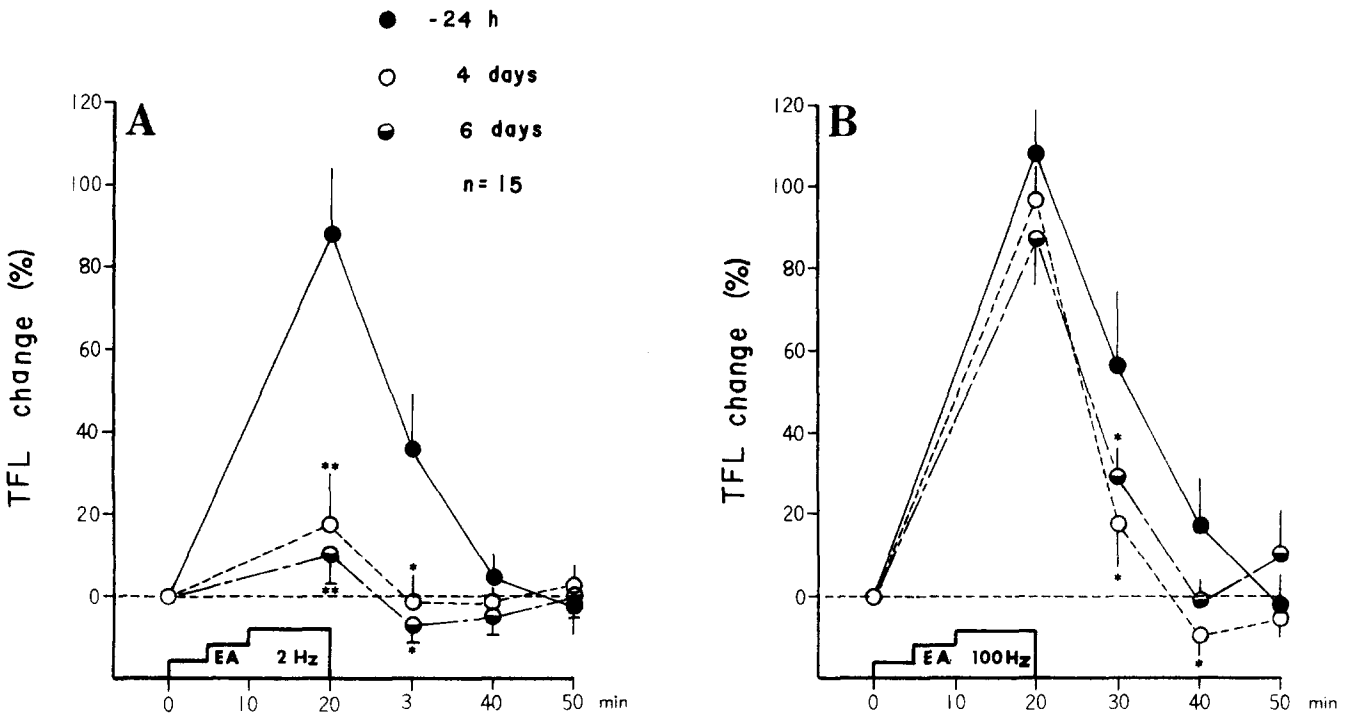

Fig. 2. The analgesic effect of 2 Hz (A) and 100 Hz (B) electroacupuncture stimulation in arcuate nuclei (ARH)-lesioned rats. Electroacupuncture analgesia was measured 24 h before and 4 and 6 days after the ARH lesion. The values are expressed as mean percentage increase over basal TFL. Vertical bars indicate standard errors. $*P < 0.05$ and $**P < 0.01$, as compared with analgesic effect taken prior to lesion.

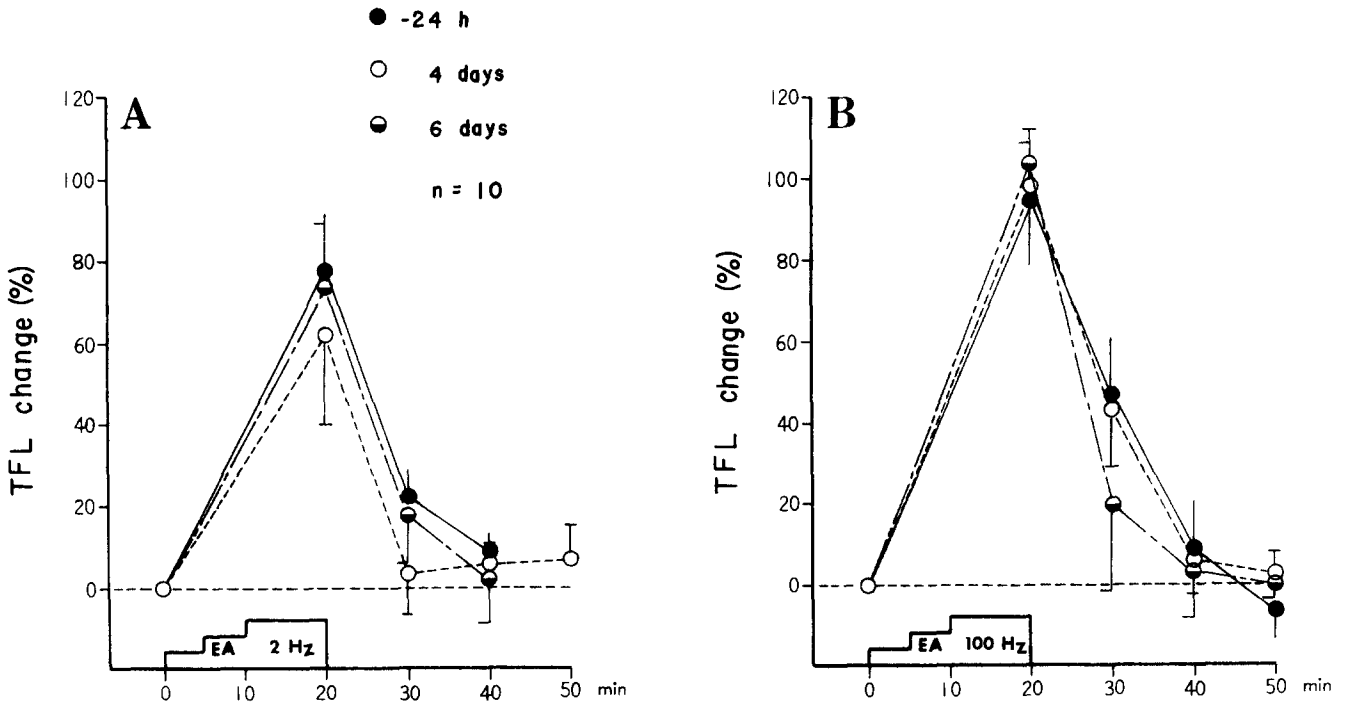

Fig. 3. The analgesic effect of 2 Hz (A) and 100 Hz (B) electroacupuncture stimulation in sham-operated rats. Electroacupuncture analgesia was examined 24 h before and 4 and 6 days after the sham operation. The effect of both 2 Hz and 100 Hz electroacupuncture tested 4 and 6 days later was not significantly different from those taken prior to the operation. The values are expressed in terms of mean percentage increase of TFL over baseline level. The vertical bars indicate standard errors.

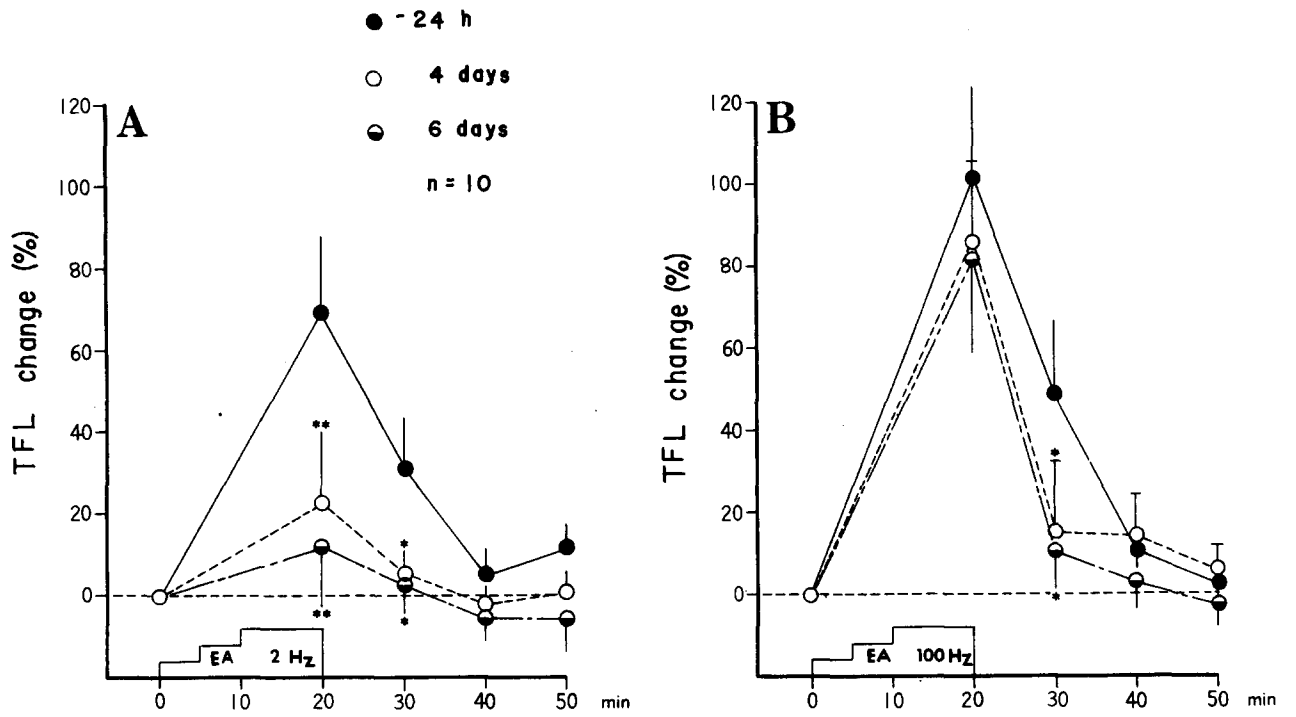

Fig. 4. The analgesic effect of 2 Hz (A) and 100 Hz (B) electroacupuncture stimulation in rats subjected to kainic acid lesion in the ARH. Annotations as in Fig. 2.

formaldehyde solution for 4–7 days, and then sectioned in 50-μm slices which were stained with Neutral red and examined under a light microscope to determine the extent of the damage. The microscopic images thus obtained were transferred to standardized diagrams of the arcuate nucleus area in the Pellegrino and Cushman atlas[23].

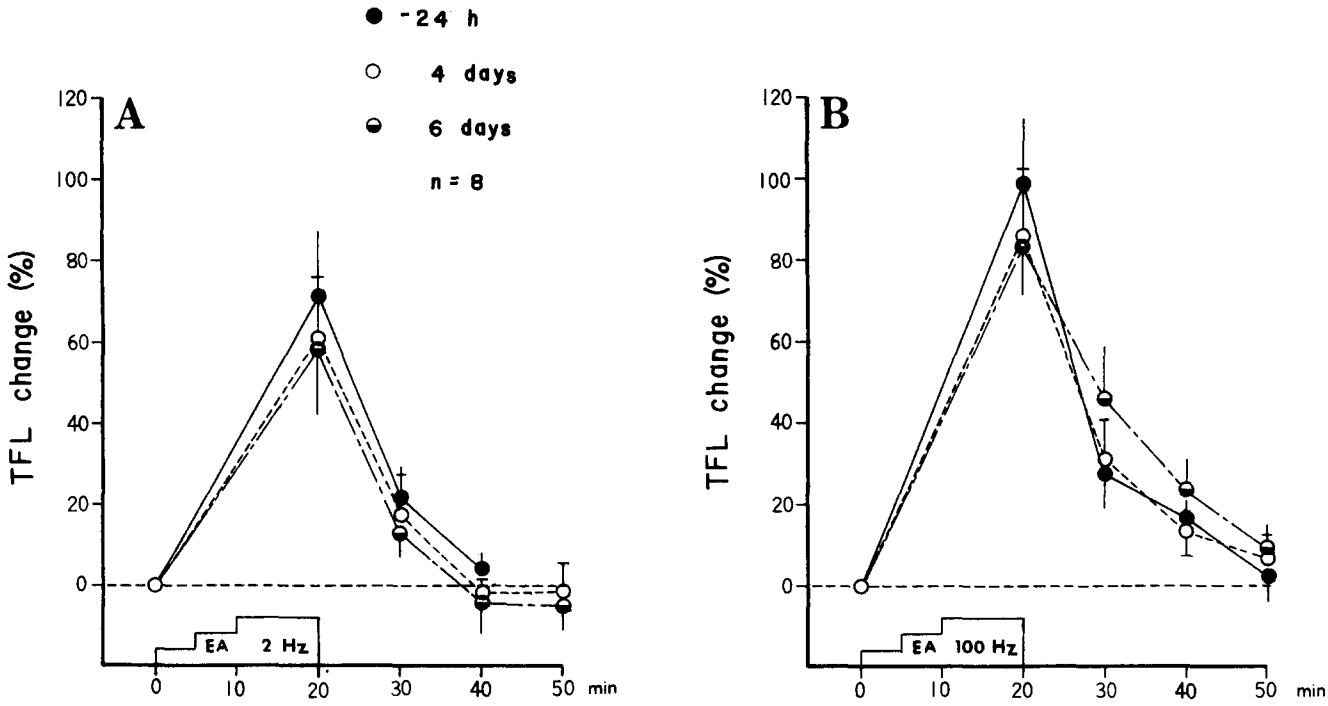

Fig. 5. The analgesic effect of 2 Hz (A) and 100 Hz (B) electroacupuncture stimulation in rats receiving vehicle injection into the ARH. Annotations as in Fig. 3.

TABLE I

Reversal of low and high frequency electroacupuncture (EA) analgesia in different groups of animals

The percentage of reversal is calculated from the equation $1\text{-}V_a/V_b$, where V_b and V_a represent percentage increase of tail flick latency tested before and 4 or 6 days after operation.

Type of treatment	*n*	*EA frequency (Hz)*	*After operation (%)*	
			4 days	*6 days*
Sham lesion	10	2	18.9	−3.0
		100	2.3	−10.8
Electrolytic lesion	15	2	79.6**	87.4**
		100	9.3	19.0
Vehicle solution	8	2	14.9	17.1
		100	12.5	14.2
Kainic acid	10	2	65.6**	81.7**
		100	17.1	20.9

**$P < 0.01$, indicates significant blockade of the effect of EA analgesia as compared with the value taken 24 h before the operation.

Statistical analysis

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

RESULTS

Histology

Histological analysis of the electrolytic lesion group revealed that in 15 animals lesions had been placed in the ARH areas with predominant distribution in the middle parts of this nucleus (0.8 to 0.2 mm caudal to bregma, see Fig. 1). In two animals damage occurred ventral to the ARH and in one animal lateral to the ARH. They were not included in the group entitled ARH lesion group. The sites of injection of kainic acid in the ARH and its spread were also examined histologically from the frontal sections through the ventromedial parts of the hypothalamus. They ($n = 10$) exhibited similar distributions to those observed in electrolytic lesion group (Fig. 1), except for two other cases whose injecting sites were located in the area lateral to the ARH which were also expelled from statistical analysis. In both experimental groups the extent of the lesion comprised at least 80% of the ARH. In many cases, the lesion had a very small spread in the adjacent regions.

Effect of electrolytic lesions of the ARH on low and high frequency EA analgesia

In the group of 15 animals, the mean effect of EA analgesia as measured 24 h prior to the destruction of the ARH region was 87 ± 16% (mean ± S.E.M.) and 108 ± 12% for 2 Hz and 100 Hz, respectively, which gradually returned to the control value within 20–30 min after cessation of EA stimulation (Fig. 2A,B). Electrolytic lesion of the ARH resulted in an almost total suppression of 2 Hz EA analgesia as tested 4 (17 ± 14%, $P < 0.01$) and 6 (11 ± 6%, $P < 0.01$) days following damage to the ARH (Fig. 2A). On the contrary, a very mild attenuation of antinociceptive effect induced by 100 Hz EA was found in the same group of animals when tested 4 (98 ± 13%, $P > 0.05$) and 6 (87 ± 14%, $P > 0.05$) days after the ARH lesion (also see Fig. 2B). From Fig. 2B, it was also noticed that the ARH lesion resulted in a shortening of the after-effect of 100 Hz EA analgesia. The differences between the pre- and postoperative group were statistically significant ($P < 0.05$) at the point 10 min after the end of EA stimulation. In the sham-operated animals ($n = 10$), no significant changes in antinociceptive effect were observed between the pre- and postoperative groups (Fig. 3A,B).

TABLE II

Tail flick latencies (s) assessed 1 day before and 4 and 6 days after the treatment in rats (means ± S.E.M.)

Type of treatment	*n*	*Before treatment*	*After treatment*	
			4 days	*6 days*
Sham lesion	10	4.43 ± 0.18	4.34 ± 0.23	4.63 ± 0.27
Electrolytic lesion	15	4.71 ± 0.13	4.36 ± 0.17	4.56 ± 0.15
Vehicle solution	8	4.70 ± 0.18	4.14 ± 0.16	4.46 ± 0.21
Kainate lesion	10	4.68 ± 0.23	4.52 ± 0.19	5.15 ± 0.22

Effect of kainic acid lesion of the ARH on low and high frequency EA analgesia

Application of kainic acid into the ARH resulted in a significant blockade of low frequency EA analgesia, the mean percentage increase in TFL being 68.9 ± 18.6%, 24.6 ± 17.6 ($P < 0.001$) and 12.6 ± 16.7% ($P < 0.01$) as tested respectively before and 4 and 6 days after the lesion (Fig. 4A). In the same group of rats, high frequency EA-induced elevation of basal pain threshold showed only a mild attenuation following the ARH lesion, which is not significantly different compared to the preinjection level (Fig. 4B). However, for 100 Hz EA analgesia tested 4 and 6 days after the ARH lesion the point 10 min after cessation of EA stimulation was significantly lower than the control value taken prior to kainate lesion (Fig. 4B).

In control animals ($n = 8$), administration of the vehicle, phosphate buffer solution, produced no significant influence on the analgesia induced by either low or high frequency EA stimulation ($P > 0.05$, Fig. 5A,B). Table I summarizes the percentage reversal of the 2 Hz and 100 Hz EA analgesia in various lesion groups.

Changes in basal TFL

As shown in Table II, basal TFL tested 4 days later was slightly shortened in all groups of animals. Such changes, however, were of no statistical significance when compared with the preoperative control levels, and there was a tendency to recover 6 days after preparation.

DISCUSSION

There is growing evidence showing that the hypothalamic arcuate region may serve as a cardinal link in the central pathway subserving EA analgesia. Immunocytochemical studies have shown that a dense distribution of β-endorphin-containing neurons exists in the ARH area[1,2,30,32], and selective destruction of these neurons by neonatal administration of monosodium glutamate into this areas produced a total abolishment of the analgesic effect induced by 14 Hz EA stimulation[10]. On the other hand, localized activation of the ARH area via electrical or chemical stimulation produced profound antinociceptive effect similar to those observed after EA stimulation[9,26,29]. In the present study, we put emphasis on the differential influences of the ARH lesions on low and high frequency EA-induced analgesia. The fact that the ARH lesion only blocked low frequency EA effect suggests that the ARH mainly mediates low, but not high frequency EA analgesia. Electrophysiological and histological studies have revealed a reciprocal connection between the ARH area and periaqueduct central gray (PAG)[21,35] which is the main structure where the descending inhibitory system for nociception originated. It is thus speculated that signals from low frequency EA stimulation may arrive at ARH which in turn activates the descending inhibitory system emanated from PAG to suppress spinal nociceptive reflex. The exact mechanisms await further investigation.

A serious handicap concerning electrolytic lesioning technique is the destruction of passing fibers as well as of the intrinsic neuronal elements[4,6,16,22]. This is especially important when the arcuate nucleus of the hypothalamus which is traversed or innervated by abundant extrinsic axons[21,35] is considered. The problem can be solved by using specific neurotoxins, such as kainic acid which seems to destroy the neuronal elements, leaving intact the passing fibers[5,16,22]. In the present study both electrical and kainic acid lesion were used, and the results were essentially the same, suggesting that it was the cell bodies rather than the passing fibers which mediate low frequency EA analgesia. Other strong support for this hypothesis comes from the study in the neonatal rat showing that destruction of beta-endorphinergic neurons in the ARH region by the application of monosodium glutamate into this nucleus elicited a marked attenuation of low but not high frequency EA analgesia[8].

Regarding neuronal elements indispensable for processing high frequency EA analgesia, there is some evidence showing that high frequency EA-induced analgesia was saved in rats devoid of the whole forebrain and missed in rats with transection of spinal cord at upper thoracic level[14,33]. It is thus obvious that the actual neural elements for mediating high frequency EA analgesia should be located supra-spinal and infra-diencephalon, i.e. in the lower brainstem.

Results of the present study showed that destruction of the ARH induced by either electrolytic or chemical lesions produced only a very small fluctuation of the TFL, which are consistent with previous observations in this[8] and other laboratories[10,11,22,24]. Thus, the ARH seems not to be important for the operation of the tail flick reflex.

Millan and his co-workers reported in the unanesthetized rat that a marked inhibition of the tail flick reflex to noxious heating of the tail could be invariably produced following the 5-min intermittent, inescapable scrambled foot-shock (3.5 mA, 300 ms delivered at 0.5 Hz)[17]. This stress-induced analgesia was partially attenuated by the localized electrolytic lesions within the ARH regions[17–20], indicating the involvement of this nucleus in processing the stress-induced analgesia. However, low frequency EA analgesia observed in the current study can be distinguished from the antinociception produced by stress of low frequency foot shock. The stress-antinociception seen by Millan et al. was only marginally affected by naloxone and does not show cross-tolerance to morphine[17–19], whereas low frequency EA analgesia was powerfully attenuated by low dose of naloxone[12]. Thus, the contribution of opioidergic mechanism to EA analgesia appears to be considerably greater than to the antinociception generated by a stressor. In line with this was the finding that low frequency EA analgesia was totally abolished by the ARH lesion, whereas low frequency stress shock-induced analgesia still existed in the ARH-lesioned rat, although it is significant smaller than those observed in the sham-operated animals[17]. Finally, the fact that ARH lesion selectively abolished analgesia produced by 2 Hz EA but not 100 Hz EA seems to argue against the notion that 2 Hz EA analgesia was mainly attributed to stress analgesia, because 100 Hz EA of the same intensity (1-2-3 mA) would have caused a similar degree, if not more severe, stress.

Acknowledgements. The authors wish to thank Mr. Shi Yushun and Mr. Zhao Chun for their excellent technical assistance and Dr. Ding Xuanzhou and Ma Qingping for their helpful suggestions and comments on certain aspects of the present investigation. This work was in part supported by National Natural Science Foundation of China and a grant from the National Institute of Drug Abuse, the United States of America (DA 03983).

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