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# Analgesia from electrical stimulation of the hypothalamic arcuate nucleus in pentobarbital-anesthetized rats

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Inhibition of noxious heat-induced tail flick by electrical stimulation of the arcuate nucleus of the hypothalamus (ARH) was examined and characterized in pentobarbital-anesthetized rats. Systematic mapping studies revealed that inhibition of the tail flick reflex could be induced by stimulating widespread areas in the ventromedial parts of the hypothalamus, which include the paraventricular nucleus, ventromedial nucleus, dorsomedial nucleus, anterior hypothalamic area as well as the ARH areas. The ARH stimulation-produced tail flick suppression could be completely blocked by systemic naloxone (2 mg/kg) which shows the involvement of an opiate mechanism in this effect. Although the tail flick reflex in the lightly anesthetized state is of significantly shorter latency than in the unanesthetized state, thresholds of the ARH stimulation for suppressing spinal nociceptive reflexes in the lightly anesthetized state were not significantly different from the thresholds at the same ARH sites in the awake state.

## INTRODUCTION

The implication of the arcuate nucleus of the hypothalamus (ARH) in the pain modulation has been well established in the previous studies performed in this and other laboratories[18,42,47]. For example, electrical lesion or chemical destruction of the rat's ARH by administration of kainic acid, a cytotoxin which selectively destroys neuronal perikarya[8,25,30], substantially attenuated or even totally abolished analgesia induced by low-frequency electroacupuncture stimulation of peripheral acupoints[47]. Similar results were also obtained in the adult rat by neonatal administration of monosodium glutamate which has been shown to destroy $\beta$-endorphin containing neurons within the ARH region[10,16]. Alternatively, direct electrical or chemical activation of this nucleus in the lightly pentobarbital-anesthetized and acutely prepared[18,48,49] or unanesthetized and chronically prepared rat[17] elicited a long-lasting inhibition of spinal nociceptive reflex. In electrophysiological studies, stimulation in the ARH has been shown to significantly suppress the noxious-evoked excitatory responses of neurons in the periaqueductal gray of rats[43]. In the current investigation, using the tail flick latency as pain index in lightly anesthetized rats, the inhibition of the spinal nociceptive reflex by the ARH stimulation was further examined and a special emphasis was put on the question whether the ARH is the most effective area for antinociception.

## METHODS

### *Animals*

Experiments were performed in adult Wistar female rats weighing 250–350 g on the day of surgery. Rats were initially anesthetized with 55–65 mg/kg of pentobarbital sodium (Du-pont) administrated intraperitoneally. A supplementary dose of 10 mg/kg was added as needed to maintain the animal within a light state of anesthesia throughout the experiment, which prevented gross bodily or facial movements while maintaining corneal, auricular and flexion reflexes and stable nociceptive withdrawal threshold. The animal's rectal temperature was kept within normal physiological limits by a heating lamp.

### *Noxious stimulation*

The nociceptive threshold of the animal was assessed by measuring the latency of the tail withdrawal from noxious thermal irradiation, which has been described elsewhere in detail[35]. In brief, the tail was placed over a hole (0.6 mm in diameter) through which light from a projector bulb was directed, thus the distance between the heat source and the tail was constant. Tail flick latency (TFL) was recorded automatically by an automatic electronic counter which displayed the time for which the heat had been applied. As the effect of the anesthetic subsided, the baseline TFL became stable at approx. 2.5–4.0 s and remained at this level throughout the experiment. The results of the 3 successive measurements with 3 min apart were averaged and taken as baseline level of the nociceptive threshold. The values of the subsequent measurements after brain stimulation (see below) were expressed as percentage changes from the baseline TFL. An elevation over 150% of the basal nociceptive threshold, i.e. 250% referring to the basal TFL,

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was imposed as cut-off limit to minimize possible damage to the skin. When brain stimulation was effective in inhibiting the tail flick reflex, control TFL measurements in the absence of brain stimulation always followed at 3 min intervals.

*Brain stimulation*

Rats were mounted in a stereotaxic head-holder in a prone position and a small patch of parietal bone above the coordinate of the ARH region was removed by a fine rongeur. A unipolar stimulating electrode made of insulated stainless steel wire (0.1 mm in diameter, only bared over the cross section of its tip) was stereotaxically lowered into the hypothalamus. The initial coordinates were aimed at A 6.0 (0.2 mm rostral to bregma), R 0.2, and H 7.7 according to the brain atlas of Pellegrino et al.[33]. Seven sites from H 7.7 down to H 10.5 with a step of 0.4 in the sagittal plane of R 0.2 were stimulated. After data collection, the electrode was removed and reinserted at A 6.0, R 0.7, and H 7.7 to H 10.5, and A 6.0, R 1.2, and H 7.7 to H 10.5. The same systematic mapping was also performed at a more caudal level (A 5.1, 0.7 mm caudal to bregma). A dorsoventral order of stimulation in a given electrode track was always followed by opposite order (ventrodorsal order) of stimulation in another track. Focal electrical brain stimulation consisted of cathodal pulses of 32 Hz and 300 $\mu$s duration from a constant current stimulator (SEM-3201, Nihon Kohden). Brain stimulation was started 5 s before and continued during noxious heating of the tail until the tail flick reflex was evoked or cut-off limit had been reached. The stimulation current was increased stepwise until the threshold for inhibition of the TFL was determined or non-antinociceptive effects of stimulation were observed (e.g. apnea, body movement, facial cramping, etc.). At each effective stimulating site where analgesia could be elicited, the minimum current intensities to increase TFL up to the cut-off level were conventionally measured. In some cases, after the threshold current for cut-off at a given locus in the ARH area was determined, naloxone hydrochloride (Dupont laboratories) at a dose of 2 mg/kg was administered i.p. TFLs were monitored thereafter and recorded continuously until it returned to baseline level.

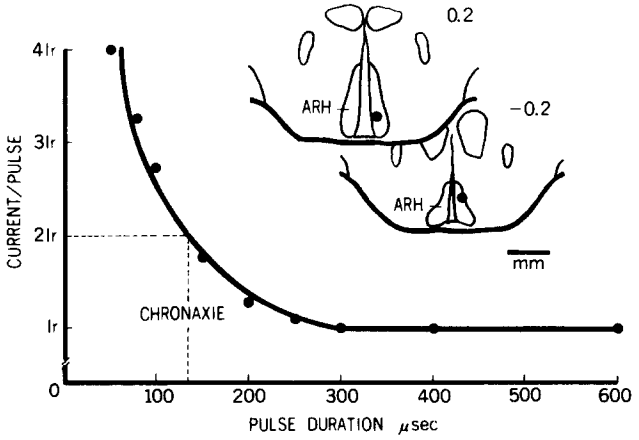


Fig. 1. Strength–duration curve for stimulation in the arcuate nucleus of the hypothalamus (ARH) in the pentobarbital-anesthetized rat. Stimulation sites are indicated on representative coronal brain sections modified from the atlas of Pellegrino et al.[33]. Figures in the upper right of each section indicate the rostrocaudal coordinates referring to bregma. The rheobasic current intensity ($I_r$, 35 $\mu$A) was determined from the asymptotic minimal current inhibiting the tail flick reflex at long pulse durations. Other data points are portrayed as multiples of the $I_r$. The average chronaxie of stimulation for the two sites in the hypothalamic arcuate region was 129 $\mu$s.

*Histology*

At the end of each experiment, an anodal current of 1 mA intensity and 5 s duration was applied to the stimulating electrode tip. Rats were sacrificed with an overdose of pentobarbital given i.p. The brain was removed and fixed with a mixture of 1% potassium ferrocyanide in 10% formaline for at least 4 days; 50-$\mu$m frozen serial sections of the ventromedial parts of the hypothalamus were taken and stained with Neutral red. The locations of the blue spots in the hypothalamus were plotted onto standard sections of the hypothalamus taken from the atlas of Pellegrino et al.[33].

*Statistical analysis*

Data are shown as means ± S.E.M. Statistical analysis of

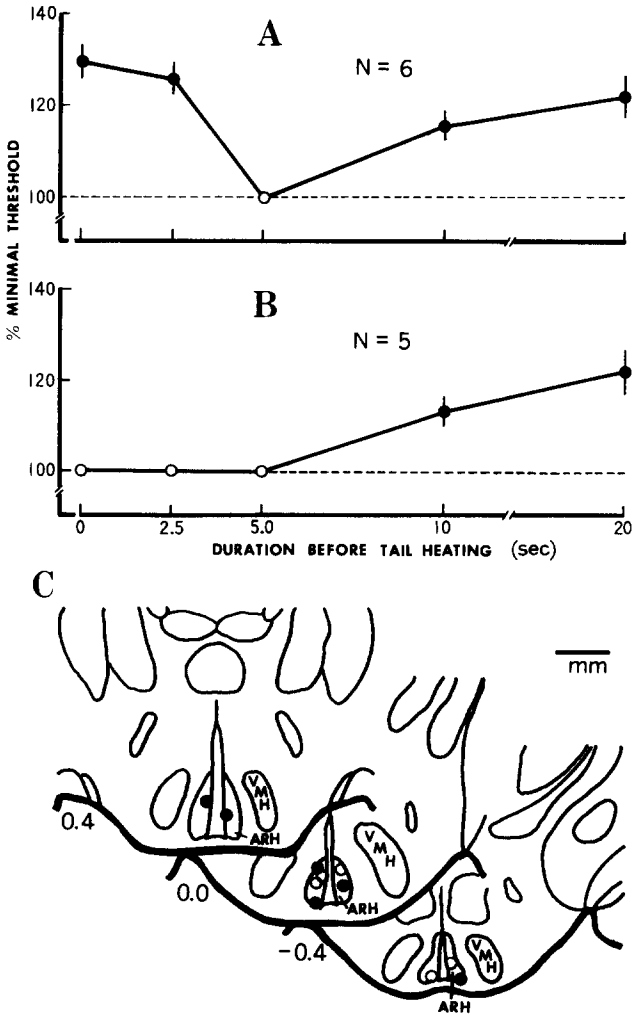


Fig. 2. A and B: effects of varying the duration of conditioning electrical stimulation of the hypothalamic arcuate nucleus before initiation of testing tail heating on the threshold for inhibition of the tail flick reflex. Brain stimulation continued during heating of the tail until the cut-off level had been reached. Data points (filled circles) represent the mean percentage of the minimal threshold (open circles) and vertical lines the ± S.E.M. The mean minimal current intensity for inhibition of the tail flick was 60.1 $\mu$A in panel A and 57.5 $\mu$A in panel B. C: distributions of electrical stimulation sites within the ARH areas. Filled and open circles indicate the sites from which the data in panels A and B were obtained. Figures in the bottom left of each section give the rostrocaudal coordinates relating to bregma. Abbreviations: ARH, arcuate nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus (according to the atlas of Pellegrino et al.)[33].

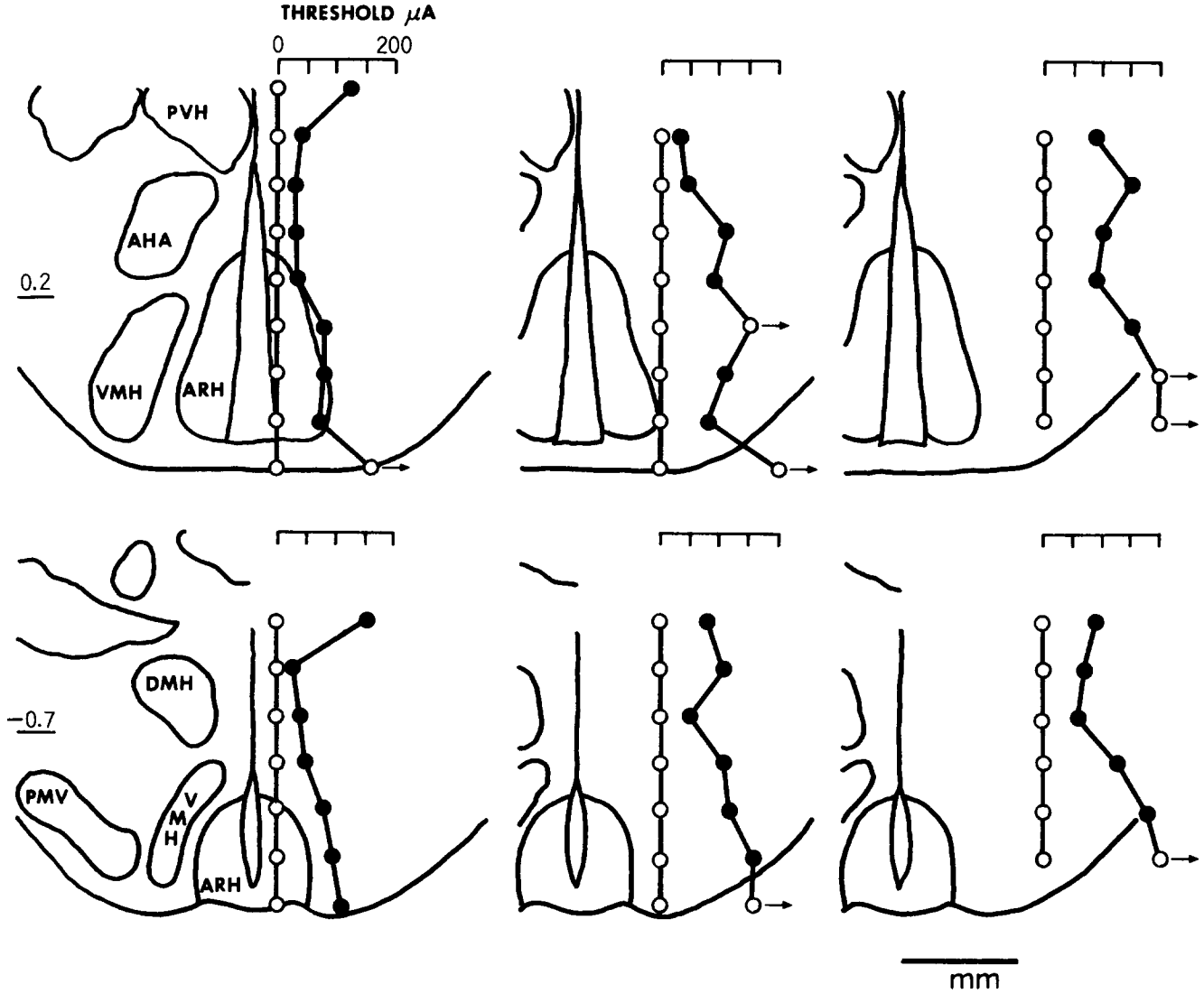


Fig. 3. Histologic reconstruction of electrode tracks in the ventromedial parts of the hypothalamus and the threshold of stimulation displayed on representative coronal brain sections[33]. Filled circles indicate thresholds for inhibition of the tail flick latency at the sites of stimulation (open circles) portrayed along the vertical electrode tracks. Open circles with arrow indicate that non-antinociceptive effects were produced at the indicated stimulation intensity.

difference between groups was assessed with Student's *t*-test (two-tailed). $P < 0.05$ was taken as significant level of difference.

## RESULTS

### *Brain stimulation*

Brain stimulation in the ARH was characterized by its strength–duration relationship and by the optimal interval between the onsets of brain stimulation and noxious radiant heating of the tail. The strength–duration relationship was evaluated by systematically varying the pulse duration ($\mu$s) and determining the threshold constant current strength ($\mu$A) required to inhibit the spinal nociceptive reflex (i.e. no tail flick within 8 s). From the strength–duration curve in Fig. 1, the chronaxie was determined, which is defined as the time on the strength–duration curve for twice the asymptotic minimal current (i.e. the rheobasic current, $I_r$) required to inhibit the TFL at long pulse durations. Chronaxie for monopolar focal electrical brain stimulation in the ARH was found to be 129 $\mu$s (Fig. 1).

We examined the effects of varying the interval between the onsets of conditioning brain stimulation and heating of the tail on brain stimulation-induced analgesia and results are illustrated in Fig. 2. In some stimulation sites (Fig. 2A, $n = 6$), an optimal interval of 5 s was obtained with a minimal threshold of 60.1 $\mu$A. Intervals shorter or longer than 5 s needed higher intensity to elicit analgesia. Stimulation at other sites (Fig. 2B, $n = 5$) showed an optimal interval in the range between 0–5 s.

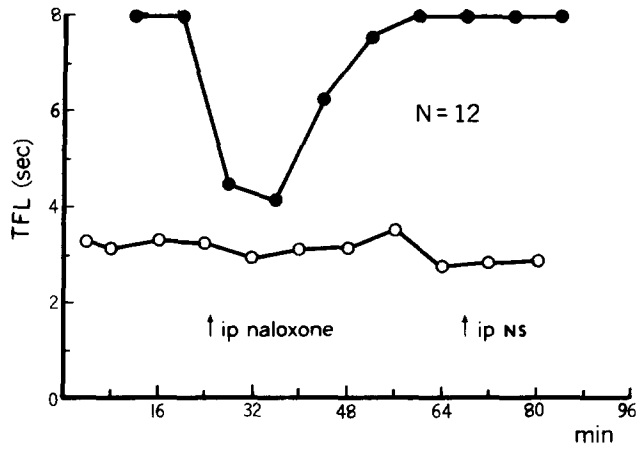


Fig. 4. Reversal of the ARH stimulation-produced analgesia following i.p. administration of naloxone (2 mg/kg) in pentobarbital-anesthetized rats. The filled and open circles represent the means of the tail flick latency with or without the ARH stimulation, respectively. 8 s was taken as the cut-off limit.

Longer intervals (10 or 20 s) needed higher stimulation intensity to produce the same degree of analgesia (Fig. 2A and B). However, no significant differences in the histological distributions were found between the two patterns of effect (Fig. 2C).

*Mapping experiments*

The ventromedial regions of the hypothalamus were systematically examined in 6 animals and examples of representative electrode tracks through these areas are presented in Fig. 3. In the ventromedial areas surveyed, stimulation of widespread areas reliably produced analgesia, which include the paraventricular nucleus, ventromedial nucleus, dorsomedial nucleus and anterior area of hypothalamus. Stimulation sites having relatively low thresholds (e.g. <50 $\mu$A) were principally found to be dorsal to the ARH, i.e. the ventral portion of the paraventricular nucleus, the medial portion of dorsomedial nucleus and the anterior area of the hypothalamus. Stimulation sites within the ARH inhibited the tail flick at a slightly higher threshold current intensity (83.3 ± 9.4 $\mu$A). Stimulation in ventrolateral hypothalamus (areas ventral to the ARH, a small ventromedial portion of the ventromedial nucleus and ventral premammillary nucleus) never suppressed the TFL at stimulation intensities up to 200 $\mu$A.

At threshold intensity, the inhibitory effect of stimulation either in the ARH or in the adjacent regions did not outlast the duration of stimulation. In some cases, a so called 'post-flick' phenomenon (i.e. a tail flick occurring immediately after the simultaneous offsets of brain stimulation and radiant heat at the 8 s cut-off time)[40] was observed. This post-flick presumably results from termination of brain stimulation while the skin temperature of the tail is still above the liminal temperature. Brain stimulation at intensities above the threshold for inhibition of the TFL were not characterized by post-flick, presumably because the effect of stimulation lasted for a sufficient time after its termination to allow the skin temperature of the tail to cool below the liminal temperature.

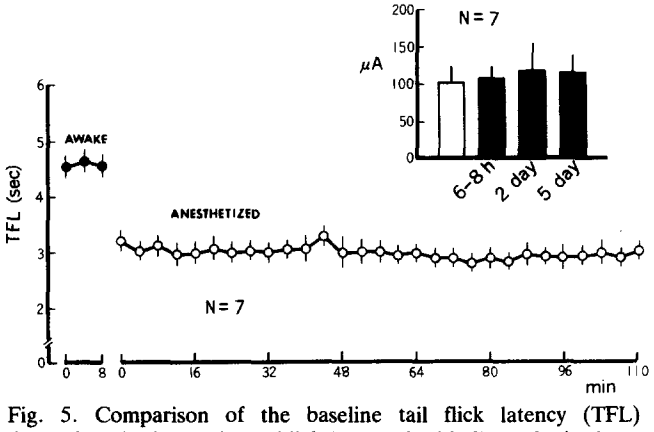


Fig. 5. Comparison of the baseline tail flick latency (TFL) determined in the awake and lightly pentobarbital-anesthetized rat. The TFL was measured at 4-min intervals 3 times before the i.p. injection of pentobarbital and 30–60 min after the completion of surgery when the rat showed spontaneous movements. The insert in the upper right corner represents a comparison of the threshold current intensities for inhibition of the TFL determined in the lightly anesthetized state and 6–8 h, 2 days and 5 days after electrode implantation. Values represent the means and vertical lines the ± S.E.M.

*Naloxone-reversible analgesia*

In all 12 rats, i.p. injection of naloxone (2 mg/kg) produced only a minimal fluctuation in baseline TFL (Fig. 4). However, naloxone almost totally reversed the ARH analgesia, i.e., decreased the TFL from above cut-off level to baseline values (Fig. 4). The reversal of analgesia by naloxone usually took place within 3 min after administration and lasted for approx. 18–30 min. In 4 rats tested, the same dose was repeated after the initial effects had worn off, and the effect was reproducible. As can be seen from Fig. 4, administration of saline had no significant effect on both baseline nociceptive threshold and the ARH stimulation-produced tail flick suppression.

*The ARH stimulation in awake and anesthetized states*

The TFLs were both quantitatively and qualitatively changed in the lightly anesthetized rat (Fig. 5, $n = 7$). They were significantly shorter in the lightly anesthetized state than in the awake state (2.95 ± 0.09 s vs 4.55 ± 0.13 s; $P < 0.01$). The amplitude and vigor of the tail flick were remarkable more pronounced in the lightly anesthetized rat.

In 11 rats, a marked increase in the TFL was produced during the ARH stimulation and the threshold current intensity required to increase the TFL up to 8 s cut-off level ranged from 30 to 175 $\mu$A with the mean of 102.5 $\pm$ 13.7 $\mu$A (inset panel in Fig. 5). To compare the inhibitory threshold in awake and anesthetized state, implantation of the stimulating electrode was made in all animals. The threshold current intensity for inhibition in 7 rats was found to be 107.3 $\pm$ 16.3 $\mu$A, 119.5 $\pm$ 35.8 $\mu$A and 116.7 $\pm$ 24.1 $\mu$A as tested 6–8 h, 2 days and 5 days after electrode implantation respectively (inset panel in Fig. 5), all of which were not significantly different from the corresponding values taken in the anesthetized state in the same rats. In 4 of the 11 animals, the threshold for inhibition could not be determined in the awake state because of aversive effects (e.g. vocalization, attempt to escape).

## DISCUSSION

Electrical stimulation in the ARH area is characterized by large changes in the threshold for inhibition with very small changes in electrode position (e.g. see Fig. 3). It was not uncommon in this study for threshold to change 100 $\mu$A with a change in electrode position of only 0.4 mm. The abruptness of such changes suggests that current spread was not serious in the present experimental setup. Under the conditions of our experiment, the intensities of the deep microstimulus taken to suppress spinal thermal noxious reflex usually ranged from 25 $\mu$A to 90 $\mu$A, which could theoretically influence neural elements less than 400 $\mu$m[2,38,45,51]. Thus, lower thresholds for inhibition determined outside the ARH area emphasize the independence of these areas in descending inhibition of the spinal nociceptive reflex.

The strength–duration curve as shown in Fig. 2 yielded chronaxie of 129 $\mu$s. Myelinated fibers are known to have chronaxie of less than 100 $\mu$s, while cell bodies showed a much greater value[34,50]. Thus, the ARH stimulation-produced hypoalgesia can hardly be the result of activation of myelinated fibers passing in the immediate vicinity of the electrode tip. This result is quite compatible with the previous observations in both awake[11] and lightly-anesthetized rats[48] that a strong antinociception was invariably produced following intra-ARH administration of excitant amino acid L-glutamate.

Numerous studies have demonstrated the existence of endogenous pain control systems in humans and in animals. It is well established that electrical stimulation in the medial hypothalamus produced analgesia in monkey[3,32] and rats[26,36,37,39], as well as to inhibit evoked responses of spinal cord dorsal horn cell to noxious heat in the rat, cat and monkey[1,5,6,31]. The results in the present series of studies indicate that effective stimulation sites for inhibition of the tail flick were distributed in widespread areas in the ventromedial hypothalamus, including paraventricular nucleus, ventromedial nucleus, dorsomedial nucleus, anterior hypothalamic area as well as the ARH regions. The stimulation threshold for inhibition was relatively higher in the ARH areas and lateral hypothalamus and significantly lower in the areas immediately dorsal to the ARH and medial hypothalamus.

An interesting phenomenon relating to acupuncture analgesia was observed in rats, i.e. that administration of electroacupuncture of different frequencies may activate different peptidergic systems present in spinal cord to suppress spinal nociceptive reflex. For example, low (1–4 Hz)- and high (60–200 Hz)-frequency electroacunpuncture respectively activate spinal enkephalinergic and dynorphinergic system to exert a powerful antinociception[7,12,13,20,22,52]. Based on the fact that different spinal peptides were responsible for different frequency electroacupuncture analgesia, it could be speculated that brain mechanisms underlying high- and low-frequency electroacupuncture analgesia are most likely different[46]. More recent studies performed in this laboratory provide evidence for this hypothesis and reveal that the hypothalamic arcuate nucleus may serve as a cardinal neural component, selectively mediating low- but not high-frequency electroacupuncture analgesia since electrolytic or kainic acid lesions of this nucleus markedly reduced low-frequency electroacupuncture analgesia while leaving high-frequency electroacupuncture analgesia unaffected[47]. In the current study, effective stimulation sites for inhibition of TFL in the ARH coincide with those where electrolytic or chemical lesions resulted in a deficit of low-frequency electroacupuncture analgesia[47] and prolonged electrical stimulation produced a long-lasting hypoalgesia as those observed after administration of electroacupuncture[48].

To explain the significant reduction in the TFL in the lightly anesthetized rat, at least two mechanisms should be taken into consideration, i.e., facilitation of the spinal reflex by direct action of drug on the components of the reflex, or by removal of the tonic descending inhibition. The latter supposition has been supported by a vast amount of literature. Irwin et al.[24] and Han et al.[21] reported that the tail flick reflex in the rat was enhanced following spinal cord transection. Necker and Hellon[31] observed that afferent fibers from the rat's tail and the dorsal horn neurons excited by heating of the tail both exhibited lower threshold temperature for excitation when the spinal cord was reversibly blocked by cooling at the thoracic level. Mori et al.[29] concluded from their studies that pentobarbital in low doses (same as we employed) suppressed multisynaptic neural networks

such as descending inhibitory system much easier than suppressing simpler monosynaptic transmission.

While pentobarbital may release the spinal nociceptive reflex from tonic descending inhibition, it is also possible that the descending inhibitory system activated by the supraspinal stimulation may be suppressed by the drug in some extent and the stimulation threshold in the supraspinal structure may be elevated consequently. Frank and Ohta[15] and Taub[44] reported that pentobarbital abolished the inhibition produced by brainstem stimulation. However, we and others[9,40,41,53,54] found no significant differences in the threshold for inhibition of spinal noxious reflex in the same animals in the awake and lightly anesthetized states. Thus, in the lightly anesthetized rat pentobarbital produced a disinhibition of the nociceptive tail flick reflex from tonic descending control without affecting the threshold current intensity of ARH stimulation for inhibition of the spinal noxious reflex.

There is growing evidence showing that central endogenous opioid mechanisms are involved in mediating the ARH stimulation-produced analgesia. Among the family of opiates $\beta$-endorphin is an attractive candidate for managing ARH stimulation-produced antinociception since the clustering of $\beta$-endorphin synthesizing neurons have already been shown in the hypothalamic arcuate region[4,14]. Furthermore, bilateral radiofrequency lesion of the mediobasal arcuate hypothalamus greatly depleted immunoreactive $\beta$-endorphin from brain tissues and the degree of depletion of immunoreactive $\beta$-endorphin significantly correlated with the degree of attenuation of brain stimulation-induced antinociception[27]. Besides, fibers have been found to project from these arcuate perikarya to the ventral midbrain, periaqueductal gray[4,14,27,28], which was verified to be an important component in the central endogenous antinociceptive system and from which opioidergic analgesia can be readily attained[27]. In this laboratory, Fan and his co-workers reported in the conscious rat that the hypoalgesic effect induced by intra-ARH injection of L-glutamate could be markedly attenuated by microinjection of naloxone into the periaqueductal gray[11]. He and Han reported that analgesia induced by low-frequency electroacupuncture stimulation at hindleg points, possibly via the relay of the ARH neurons, was greatly reduced following direct administration of $\beta$-endorphin antiserum into the periaqueductal gray of the rat[23]. In the electrophysiological studies, systemic administration of naloxone completely reversed the inhibitory effect of the ARH stimulation on the evoked discharges of neurons in the periaqueductal gray to noxious stimulation[43]. Taken together, an endogenous opioid mechanism in ARH-periaqueductal gray pathway is probably involved in the ARH stimulation-induced analgesia.

In addition to the opioid mechanism in the midbrain area, spinal enkephalinergic system was also found to play an important role in this effect, since low-frequency electroacupuncture analgesia known to be mediated by ARH was almost totally abolished by intrathecal injection of the $\delta$ opiate blocker 1C1174,864 or met-enkephalin antiserum[13,52].

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