Involvement of endogenous beta-endorphin in antinociception in the arcuate nucleus of hypothalamus in rats with inflammation

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Abstract

Although exogenous administration of beta-endorphin to the arcuate nucleus of hypothalamus (ARC) had been shown to produce antinociception, the role of endogenous beta-endorphin of the ARC in nociceptive processing has not been studied directly. The aim of the present study was to investigate the effect of endogenous beta-endorphin in the ARC on nociception in rats with carrageenan-induced inflammation. The hindpaw withdrawal latency (HWL) to noxious thermal and mechanical stimulation was assessed by the hot-plate test and the Randall Selitto Test. Intra-ARC injection of naloxone had no significant influence on the HWL to thermal and mechanical stimulation in intact rats. The HWL decreased significantly after intra-ARC injection of 1 or 10 mg of naloxone in rats with inflammation, but not with 0.1 mg of naloxone. Furthermore, intra-ARC administration of the selective mu-opioid receptor antagonist beta-funaltrexamine (beta-FNA) decreased the nociceptive response latencies to both stimulation in a dose-dependent manner in rats with inflammation, while intra-ARC administration of the selective delta-opioid receptor antagonist naltrindole or the selective kappa-opioid receptor antagonist norbinaltorphimine (nor-BNI) showed no influences on the nociceptive response latency. The antiserum against beta-endorphin, administered to the ARC, also dose-dependently reduced the HWL in rats with inflammation. The results indicate that endogenous beta-endorphin in the ARC plays an important role in the endogenous antinociceptive system in rats with inflammation, and that its effect is predominantly mediated by the mu-opioid receptor.

Keywords: Arcuate nucleus; Inflammation; Beta-endorphin; Hyperalgesia; Beta-funaltrexamine; Mu-opioid receptor

1. Introduction

Beta-endorphin is an endogenous opioid peptide synthesized almost exclusively by the cells in the arcuate nucleus (ARC) (Bloom et al., 1978). Another group of betaendorphin containing neurons are located in the nucleus tractus solitarius (Bronstein et al., 1992; Maley, 1996). The neurons containing beta-endorphin in the ARC project widely in...
the brain (Finley et al., 1981; Sim and Joseph, 1991). The major fiber bundles terminate in the midbrain periaqueductal gray matter (Pilcher et al., 1988), which plays an important role in the descending antinociceptive pathway (Behbehani, 1995; Sandkühler, 1996; Wang et al., 1999, 2000; Xu et al., 2000). Administration of beta-

endorphin to various brain areas, including the ARC, induced strong analgesic effects (Bloom et al., 1976; Kuraishi et al., 1980; Tseng and Wang, 1992; Suh et al., 1999).

It is well known that opiates are the most powerful analgesic substances to relieve chronic pain in clinic (Sandkühler, 1996). In a recent study, the released opioid peptides reduced the response to sustained pain (Zubieta et al., 2001). In addition, the concentration of beta-endorphin in the cerebrospinal fluid was shown to increase when the ARC received either electrical stimulation or chemical stimulation by glutamate (Bach, 1997). At the same time, these stimulations were shown to induce antinociceptive effects (Wang et al., 1990; Bach, 1997), indicating an involvement of beta-endorphin in antinociception. The nocifensive responses to formalin were enhanced by intracerebroventricular injection of the anti-

serum against beta-endorphin (Wu et al., 2001). The same research group also demonstrated that the mu-opioid receptor antagonist enhanced the nocifensive responses in formalin test (Wu et al., 2002). Taken together, these results strongly suggest the involvement of beta-endorphin and the mu-opioid receptor in the endogenous antinociceptive system in the brain. In another report, it was shown that peripheral pain stimulus increased the level of beta-endorphin in the ARC of rats (Zangen et al., 1998), suggesting that the endogenously released beta-endorphin in the ARC may play an antinociceptive role, although there is no direct evidence to support this till now.

The carrageenan-induced inflammation model can closely reproduce some human pain syndromes. Thus it has been frequently used in the field of pain research. The present study was designed to demonstrate the antinociceptive effect of endogenous beta-endorphin in the ARC of rats with carrageenan-induced inflammation. In order to investigate the effect of endogenous beta-endorphin and the opioid receptor in intact rats and rats with carrageenan-induced inflammation, naloxone, three types of selective opioid antagonists and the antiserum against beta-endorphin were administered to the ARC.

2. Materials and methods

2.1. Animals

All experiments were performed on freely moving male Wistar rats weighing from 200 to 250 g (Experimental Animal Center of Peking University, Beijing, China). The rats were housed in cages with free access to food and water, and maintained in a climate-controlled room on a normal day/night cycle. All experiments were conducted according to the guidelines of the animal ethical committee of Karolinska Institute and every effort was made to minimize both the animal suffering and the number of animals used.

2.2. Intra-ARC injection

The animals were anesthetized by intraperitoneal pentobarbital (50 mg/kg) and mounted on a stereotaxic instrument. A stainless steel guide cannula of 0.8 mm outer diameter was directed into the ARC (AP: 2.40, L: 0.5, V: 9.8 mm; AP, anterior (↑) or posterior (↓) to Bregma; L, lateral to midline; V, ventral to the surface of skull) according to Paxinos and Watson (1998) and fixed to the skull by dental acrylic. Intra-ARC injections were performed 2–3 days after surgery. On the day of experiment, a stainless steel needle with 0.4 mm diameter was directly inserted into the guide cannula, with 2.8 mm beyond the tip of the guide cannula. One microliter of solution was thereafter infused into the ARC over 1 min.

2.3. Carrageenan-induced inflammation
Animals received a unilateral injection of carrageenan (2 mg/100 ml per paw) into the left hindpaw. The contralateral paw was untreated. Three hours after the injection of carrageenan, hindpaw withdrawal latencies (HWLs) were measured by the hot-plate test and the Randall Selitto Test as the basal levels. Then each animal received an intra-ARC injection of either vehicle or drug. The HWL of each animal was then assessed at 5, 10, 20, 30 and 60 min after intra-ARC injection.

2.4. Nociceptive tests

Rats were habituated to handling and the testing equipment for 5 days before surgery. The HWL in response to noxious thermal and mechanical stimulation was tested as described before (Yu et al., 1996, 1999). Briefly, the HWL to noxious heat stimulation was tested by a hot plate maintained at a temperature of 52°C (51.8–52.48°C). The time to hindpaw withdrawal was measured in seconds to be referred to as the HWL to thermal stimulation. The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess the HWL to mechanical stimulation. A wedge-shaped pusher at a loading rate of 30 g/s was applied to the dorsal surface of the manually handled hindpaw and the latency required to initiate the struggle response was assessed and expressed in seconds. The measurement was performed on both hindpaws at each time point. The average values obtained before intra-ARC injection were regarded as the basal HWLs in both tests. The HWLs recorded during subsequent measurements were expressed as percentage changes from the basal level for each rat. Each rat was tested with both stimulations. A cut-off limit of 15 s was set up in both tests to avoid tissue damage.

2.5. Radioimmunoassay for beta-endorphin-like immunoreactivity

Intact rats and rats with carrageenan-induced inflammation (3 days after carrageenan injection) were killed by decapitation. The entire rat brain was rapidly removed and placed in a mold where it could be sliced in 2.5 mm coronal sections by a thin (0.07 mm) stainless steel wire. The tissue samples were quickly dissected out from the slice, numbered, weighed and immediately frozen on dry ice. The tissue was stored at 270°C until extraction and analysis.

A combined neutral and acid extraction of dissected tissue was chosen. The samples of frozen hypothalamus tissue were transferred to tubes containing 2 ml of boiling 0.05 mol/l phosphate buffer, pH 7.4, for 10 min. Then the samples were cooled before being homogenized on a vortex mixer with a steel rod in the tube. The samples were centrifuged (48C, 2800 g) for 10 min. The supernatant was taken off and poured into other tubes. The pellet was dissolved and mixed in 2 ml of 1.0 mol/l acetic acid. The solution was again incubated at 1008C for 10 min. The procedure with centrifugation, cooling and mixing was repeated. The supernatant from the extraction with acetic acid was pooled with the one from the neutral and the samples were lyophilized overnight. The lyophilized sample was dissolved in 1 ml of phosphate buffer and stored at 2 208C until radioimmunoassay (RIA).

Beta-endorphin-like immunoreactivity levels were determined by RIA using rabbit antiserum solution and tracer solution [125I]-beta-endorphin. Cross-reactivity with [Arg8]-Vasopressin was 0%, [Lys8]-Vasopressin, 0.01%, LH–RH 0% and with Oxytocin (human, rat) 0%. A standard curve of beta-endorphin was prepared. The antiserum solution was incubated with 100 ml of standard solution or extracted sample at 48C for 24 h. One hundred microliters of the tracer solution was added and incubated at 48C for another 24 h. Separation of the bound fraction from the unbound fraction was done by incubating the samples together with 500 ml of a second rabbit antibody, decanting Suspension 3 (Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden), for 30 min in room temperature. Incubation of the samples were interrupted by adding 1 ml of water (milli-Q) to the tubes, the samples were then centrifuged for 17 min (48C, 2800 g) and the supernatant decanted. The radioactivity in the precipitate was measured in a gamma counter for 6 min/sample. The limit of the assay was 7.8 pmol/l. All samples were assayed in duplicate.

2.6. Chemicals
Solutions for intra-ARC administration were prepared with sterilized saline, each with a volume of 1 ml containing: (1) 0.1, 1 or 10 mg of naloxone (naloxone hydrochloride, Sigma Chemical Company, St. Louis, MO); (2) 0.1, 1 or 5 nmol of beta-funaltrexamine (beta-FNA hydrochloride; Tocris Cookson, Bristol, UK); (3) 1 nmol of nor-binaltorphimine (nor-BNI hydrochloride; Tocris Cookson); (4) 1 nmol of naltrindole (naltrindole hydrochloride; Tocris Cookson); (5) 0.01, 0.05 or 0.1 ml of the antiserum against beta-endorphin (polyclonal rabbit antiserum, Peninsula Lab., San Carlos, CA).

2.7. Statistical analysis

At the end of the experiments, the location of the tip of the injection needle was verified. The results are shown in Fig. 1. Only the results from nociceptive tests where the tips of the injection needle were within the ARC were used for statistical analysis. Data from nociceptive tests were presented as mean ^ SEM. Differences between groups were determined by two-way analysis of variance (ANOVA) for repeated measurements in behavioral experiments and Student’s t-test (two-tailed) in RIA to determine the change of beta-endorphin. P-values less than 0.05, 0.01 and 0.001 were considered as significant differences.

Fig. 1. Illustration of the location of the tip of the injection needle in the ARC.

3. Results

3.1. Influence of intra-ARC administration of naloxone on HWLs in intact rats

It is not clear whether there is a tonic release of endogenous opioid peptides producing an antinociceptive effect in the ARC of intact rats. In this part of the experiments, intact rats received intra-ARC injection of 10 mg of naloxone n = 8, or 1 ml of 0.9% saline as a control n = 7. There were no significant changes in HWLs

\[ F_{1;13} = 0.05, P = 0.83; \]  
\[ F_{1;13} = 0.57, P = 0.46. \]
3.2. Inflammation-induced hyperalgesia was enhanced by intra-ARC administration of naloxone

To investigate the involvement of endogenous opioid peptides in the ARC responding to inflammation, the broad spectrum antagonist of opioid receptors, naloxone, was used. Rats with inflammation received intra-ARC injection of 0.1 n 8, 1 n 7 or 10 mg of naloxone n 8, or 1 ml of 0.9% saline as a control n 8. The results are shown in Fig. 2.

Compared with the control group, the HWL to thermal and mechanical stimulation decreased significantly after intra-ARC injection of 1 mg (left HWL in thermal test: F\textsubscript{1;13} 52:49, P 0:001; right HWL in thermal test: F\textsubscript{1;13} 24:68, P 0:001. Left HWL in mechanical test: F\textsubscript{1;13} 74:62, P 0:001; right HWL in mechanical test: F\textsubscript{1;13} 80:18, P 0:001) or 10 mg of naloxone (Left HWL in thermal test: F\textsubscript{1;14} 88:53, P 0:001; right
Fig. 2. Effects of intra-ARC injection of 0.1, 1 or 10 mg of naloxone on HWLs to thermal (A and B) and mechanical stimulation (C and D) in rats with inflammation. A and C: HWL of left hindpaw; B and D: HWL of right hindpaw. Intra-ARC administration of 1 ml of 0.9% saline served as the control group. HWL, hindpaw withdrawal latency; ARC, the arcuate nucleus. Data are presented as mean ± SEM. The statistical difference between groups was determined by two-way ANOVA.

HWL in thermal test: $F_{1;14} = 57.80$, $P = 0.001$. Left HWL

in mechanical test: $F_{1;14} = 177.57$, $P = 0.001$; right HWL

in mechanical test: $F_{1;14} = 171.04$, $P = 0.001$), but not

0.1 mg of naloxone (left HWL in thermal test: $F_{1;14} = 1.97$, $P = 0.18$; right HWL in thermal test: $F_{1;14} = 0.17$, $P = 0.68$. Left HWL in mechanical test:

$F_{1;14} = 1.10$, $P = 0.31$; right HWL in mechanical test:

$F_{1;14} = 2.80$, $P = 0.11$). The HWL in response to 10 mg of naloxone decreased about 30% at 30 min after the injection, and the effect lasted for more than 60 min.
3.3. Influence of intra-ARC administration of beta-FNA on HWLs in rats with inflammation

Beta-FNA, a selective mu-opioid receptor antagonist, was employed to investigate whether the antinociceptive effect of endogenous opioid peptides in the ARC of rats was mediated by the mu-opioid receptor. Rats with experimentally induced inflammation received intra-ARC injection of 0.1 nmol (left HWL in thermal test: \( F_{1;14} = 19.98, P < 0.001 \) right HWL in thermal test: \( F_{1;14} = 19.98, P < 0.001 \)), 1 nmol of beta-FNA (left HWL in thermal test: \( F_{1;14} = 25.68, P < 0.001 \) right HWL in thermal test: \( F_{1;14} = 15.96, P < 0.01 \)), and 0.1 nmol of beta-FNA (left HWL in thermal test: \( F_{1;14} = 0.08, P > 0.78 \) right HWL in thermal test: \( F_{1;14} = 1.06, P > 0.32 \)). The results shown in Fig. 3.

Compared with the control group, the HWL to thermal and mechanical stimulation decreased significantly after intra-ARC injection of 1 nmol (left HWL in thermal test: \( F_{1;14} = 30.28, P < 0.001 \) right HWL in thermal test: \( F_{1;14} = 19.98, P < 0.001 \)), 5 nmol of beta-FNA (left HWL in thermal test: \( F_{1;14} = 25.68, P < 0.001 \) right HWL in thermal test: \( F_{1;14} = 15.96, P < 0.01 \)), but not \( 0.1 \) nmol of beta-FNA (left HWL in thermal test: \( F_{1;14} = 15.96, P < 0.01 \) right HWL in mechanical test: \( F_{1;14} = 131.73, P < 0.001 \)). The HWL in response to 5 nmol of beta-FNA decreased about 20% at 30 min after the injection, and the effect lasted for more than 60 min.

3.4. Influence of intra-ARC injection of nor-BNI or naltrindole on HWLs in rats with inflammation

In order to test whether delta- and kappa-opioid receptors were involved in the antinociceptive effect in ARC, rats with inflammation received intra-ARC injection of 1 nmol of nor-BNI (left HWL in thermal test: \( F_{1;14} = 1.06, P > 0.32 \) right HWL in mechanical test: \( F_{1;14} = 1.06, P > 0.32 \)), 1 nmol of naltrindole (left HWL in mechanical test: \( F_{1;14} = 1.06, P > 0.32 \) right HWL in mechanical test: \( F_{1;14} = 1.06, P > 0.32 \)). The HWL in response to 5 nmol of beta-FNA decreased about 20% at 30 min after the injection, and the effect lasted for more than 60 min.
0.9% saline as a control \( n = 8 \).

There were no significant changes in the HWL to thermal and mechanical stimulation after intra-ARC injection of 1 nmol of nor-BNI (left HWL in thermal test: \( F_{1;12} = 0.85 \), \( P = 0.37 \); right HWL in thermal test: \( F_{1;12} = 3.15 \), \( P = 0.01 \)); or 1 nmol of naltrindole (left HWL in thermal test: \( F_{1;12} = 1.25 \), \( P = 0.28 \); right HWL in thermal test: \( F_{1;12} = 2.51 \), \( P = 0.14 \)).
compared with the control group.

3.5. Inflammation-induced hyperalgesia was enhanced by intra-ARC administration of the antiserum against beta-endorphin

It is not clear whether endogenous beta-endorphin is involved in the endogenous antinociceptive system. Rats with inflammation received 0.01 n 6, 0.05 n 6 or

0.1 ml of the antiserum against beta-endorphin n 6. One δP

microliter of 0.9% saline was injected into the ARC as a control n 6.

As shown in Fig. 4, the HWL to both thermal and mechanical stimulation decreased significantly after intra-ARC injection of 0.05 ml (left HWL in thermal test: F1;10 28:89, P , 0:001; right HWL in thermal test:

15:77, P , 0:01. Left HWL in mechanical test:

216:82, P , 0:001; right HWL in mechanical test:

33:07, P , 0:001) or 0.1 ml of the antiserum

against beta-endorphin (left HWL in thermal test: F1;10 40:84, P , 0:001; right HWL in thermal test:

42:79, P , 0:001. Left HWL in mechanical test:

68:82, P , 0:001; right HWL in mechanical test:

21:54, P , 0:001), but not 0.01 ml of the anti

serum against beta-endorphin (left HWL in thermal test: F1;10 2:89, P 0:12; right HWL in thermal test:

0:35, P 0:56. Left HWL in mechanical test:

0:17, P 0:69; right HWL in mechanical test:

0:14, P 0:71) compared with the control group.
3.6. Influence of carrageenan-induced inflammation on the concentration of beta-endorphin-like immunoreactivity in the hypothalamus

In order to investigate whether there was an influence of inflammation on the content of beta-endorphin in the hypothalamus, the beta-endorphin-like immunoreactivity in the hypothalamus tissue was measured in intact rats \( n = 10 \) and in rats with inflammation \( n = 10 \) by RIA. There was high concentration of beta-endorphin-like immunoreactivity in the hypothalamus tissue of rats. The concentration of beta-endorphin-like immunoreactivity in the hypothalamus was higher in rats with inflammation than that in intact rats (Student’s t-test: \( t = 22.23, P < 0.05 \)), as shown in Fig. 5.

![Graphs showing effects of intra-ARC injection of antiserum against beta-endorphin on HWLs to thermal (A,B) and mechanical stimulation (C,D) in rats with inflammation.](image)

Fig. 4. Effects of intra-ARC injection of the antiserum against beta-endorphin on HWLs to thermal (A,B) and mechanical stimulation (C,D) in rats with inflammation. (A,C) (HWL of left hindpaw); (B,D) HWL of right hindpaw. Intra-ARC administration of 1 ml of 0.9% saline served as the control group. HWL, hindpaw withdrawal latency; ARC, the arcuate nucleus; beta-END, beta-endorphin. Data are presented as mean ± SEM. The statistical difference between groups was determined by two-way ANOVA.

4. Discussion

4.1. Effects of intra-ARC injection of naloxone in rats with inflammation

The results of the present study showed that intra-ARC administration of naloxone to block all the opioid receptors enhanced the hyperalgesia in rats with carrageenan-induced inflammation. This indicates that opioid receptors in the ARC may be activated by the endogenously released opioid peptides during inflammation. This finding is consistent with a previous report that intra-ARC injection of naloxone blocked analgesia in defeated mice,
which also implied the involvement of endogenous opioid peptides in the endogenous antinociceptive system (Miczek et al., 1985).

After intra-ARC injection of naloxone, there was an observed enhancement of hyperalgesia in rats with inflammation; the effect of naloxone may be due to the antagonism of both a ‘basal’ analgesic action and the noxious-evoked activation of endogenous opioid peptides in the ARC. The second explanation is more reasonable, because the results of the present study demonstrated that naloxone had no effect in intact rats. Moreover, endogenously released opioid peptides in the ARC may be too low to exert an antinociceptive effect in intact rats (Zangen et al., 1998). Therefore, intra-ARC injection of naloxone to block the opioid receptors had no significant influences on the nociceptive response in intact rats. In response to the carrageenan-induced inflammation, the tonic release of endogenous opioid peptides may be elevated. Zangen et al. (1998) showed that peripheral noxious stimulus increased the level of beta-endorphin in the ARC of rats. Thus naloxone administered to the ARC would block the inflammation-induced release of endogenous opioid peptides in the ARC, consequently enhance the hyperalgesia.

Conflicting results have been obtained by systemic or intracerebroventricular injection of naloxone in formalin test (North, 1978; Sugimoto et al., 1986; Kocher, 1988). These may be due to the broad effects of these approaches. Here, we focused on the influence of naloxone on nociception in the ARC only.

4.2. Involvement of the mu-opioid receptor in the endogenous antinociceptive system

The present study showed that intra-ARC administration of beta-FNA, the selective antagonist against the mu-opioid receptor, induced enhancement of hyperalgesia in rats with inflammation, while the delta- and kappa-opioid receptor antagonists had no significant effect. The results indicate that there may be a tonic release of endogenous opioid peptides that activate the mu-opioid receptor in the ARC, thereby exerting an antinociceptive effect in rats with inflammation. Similarly, Wu et al. (2002) reported that intracerebroventricular administration of the selective mu-opioid receptor antagonist d-Phe-Cys-Tyr-Orn-Thr-Pen-Thr-NH(2) (CTOP) dose-dependently enhanced the nocifensive response in rats with formalin-induced inflammation, indicating a modulating effect of mu-opioid receptors on the transmission of nociceptive information at the supraspinal sites. It is known that mu-opioid receptors are involved in the antinociceptive modulation in many brain regions. Some regions in the central nervous system are specialized in primarily regulating sensory or affective component of the pain experience (Treede et al., 1999; Price, 2000; Harte et al., 2000). However, the activation of mu-opioid receptors in the thalamus appeared to regulate both sensory and affective components (Bushnell and Duncan, 1989; Harte et al., 2000). In the present study, blocking delta- and kappa-opioid receptors by administration of the selective delta-opioid receptor antagonist naltrindole and kappa-receptor antagonist nor-BNI to the ARC did not alter the HWL of rats with
inflammation, indicating that delta- and kappa-opioid receptors in the ARC were not involved in the carrageenan-induced hyperalgesia. The results are supported by the recent finding that delta and kappa-opioid receptors were not involved in formalin-induced nocifension at the supraspinal sites (Wu et al., 2002). Morphological studies also showed that the mu opioid receptor was expressed in the ARC (Petersen and LaFlamme, 1997; Bouret et al., 1999; Abbadie et al., 2000), while the delta-opioid receptor was not (Mansour et al., 1994). Taken together, the present study demonstrated that the mu-opioid receptor mediates the endogenous antinociceptive effect in the ARC.

4.3. Intra-ARC administration of the antiserum against beta-endorphin enhanced the hyperalgesia induced by experimental inflammation in rats

The present study found that the antiserum against beta-endorphin, injected into the ARC, induced decreases in HWLs in rats with inflammation. It is consistent with the previous reports showing that intracerebroventricular pre-treatment with the antiserum against beta-endorphin enhanced the nocifensive response to formalin in rats and mice (Porro et al., 1991; Wu et al., 2001). These findings indicate an involvement of endogenous beta-endorphin in the endogenous antinociceptive system at supraspinal levels. The effect of the antiserum against beta-endorphin should be due to blocking the effect of endogenously released beta-endorphin (Porro et al., 1991).

The central proopiomelanocortin (POMC) system is prominently implicated in antinociceptive processes (Przewlocki and Przewlocka, 2001). POMC is the precursor of beta-endorphin and several other bioactive peptides (Garcia and Pelletier, 1993). Lesions of the ARC, the main structure to synthesize beta-endorphin in the brain, reduced post-stress analgesia (Kelsey et al., 1986). This is also evidenced in the POMC knockout mice, which lack the opioid (naloxone reversible) analgesia induced by mild swim stress (Rubinstein et al., 1996). Moreover, the extracellular level of beta-endorphin increased in rats with inflammation (Zangen et al., 1998), suggesting the involvement of endogenous beta-endorphin in antinociception. Previous studies have demonstrated significant increases in beta-endorphin immunoreactivity in ventral periaqueductal gray matter and ventromedial hypothalamus in a formalin-induced inflammation model (Porro et al., 1991; Facchinetti et al., 1992). It has been shown that beta-endorphin has a high affinity for mu-opioid receptors, and the antinociceptive effect induced by beta-endorphin micro-injected into the periaqueductal gray matter is also mediated by mu-opioid receptors (Monroe et al., 1996). Thus, the study supports the hypothesis that the elevated levels of betaendorphin activate mu-opioid receptors, subsequently attenuating the inflammation-induced hyperalgesia.

4.4. Regulation of the POMC gene expression

The present study showed that the beta-endorphin-like immunoreactivity in the hypothalamus tissue was elevated 3 days after carrageenan injection, suggesting that expression of beta-endorphin was elevated in the ARC in response to inflammation. This result is in agreement with the previous study that POMC mRNA level increased in the ARC in rats with lipopolysaccharide-induced inflammation (Sergeyev et al., 2001). The level of POMC mRNA may be regulated by pain information.

Several hormones have been shown to regulate POMC gene expression (Tong et al., 1990; Matera and Wardlaw, 1993; Cheung and Hammer, 1995; Mobbs and Mizuno, 2000). The excitatory and inhibitory amino acids, which mediate the fast synaptic transmission, are also involved in the regulation of POMC gene expression. Glutamate enhanced the adenylyl cyclase-cAMP system-induced beta-endorphin secretion and POMC mRNA expression in cultured hypothalamic neurons of rats (Yang et al., 1995), while GABA receptor mediated a negative regulation of POMC gene expression in the ARC of rats (Garcia and Pelletier, 1994). The positive effect of glutamate, together with the negative effect of GABA, shows the dynamic regulation of POMC gene expression. It is proposed that inflammation-induced up-regulation of POMC is mediated by glutamate or GABA.

4.5. The possible mechanisms of endogenous beta-endorphin-induced antinociception in the ARC

The mechanisms of the analgesic effect of betaendorphin in the ARC are still not clear (Monroe et al., 1996; Przewlocki and Przewlocka, 2001). In the periaqueductal gray matter, it has been proposed that the analgesic effect of opioid peptides works by suppressing the inhibitory influence of neurotransmitters on neurons that form part of a descending antinociceptive pathway (Basbaum and Fields, 1984). Vaughan et al. (1997) showed that the opioid-induced inhibition on GABAergic synaptic currents in the periaqueductal gray matter was controlled by a presynaptic voltage-dependent potassium conductance regulated by mu-opioid receptors. A recent study indicated that opioid peptides negatively regulated the GABA terminals innervating POMC neurons in the ARC (Cowley et
al., 2001). Thus, it is proposed that beta-endorphin may act on GABA-secreting nerve terminals to reduce the release of GABA onto POMC neurons, allowing them to adopt a more depolarized resting potential. It may be one possible way for the endogenous beta-endorphin to affect the endogenous antinociceptive system.

4.6. Clinical significance

In a previous study, the concentration of beta-endorphin in the cerebrospinal fluid was elevated by deep brain stimulation in patients suffering from intractable chronic pain, indicating a direct relationship between the endogenously released beta-endorphin and pain alleviation (Young et al., 1993). In carcinomatous patients, electrical stimulation, which can cause pain relief, led to a marked increase of beta-endorphin-like immunoreactivity, suggesting that beta-endorphin may contribute to the initial pain blockade (Tari et al., 1983). In addition, a recent study showed that sustained pain induced the regional release of endogenous opioid peptides interacting with mu-opioid receptors in a number of human cortical and subcortical brain regions (Zubieta et al., 2001). The endogenous antinociception system is a potential target for the therapeutics of chronic pain.

5. Conclusion

Intra-ARC injection of naloxone, beta-FNA or the antiserum against beta-endorphin enhanced the hyperalgesia induced by inflammation in rats. These results indicate that endogenous beta-endorphin released in the ARC plays an antinociceptive role in rats with inflammation, and that the effect is predominantly mediated by the mu-opioid receptor.

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