Effect of Beta-Endorphin on Pain Threshold in Hypophysectomized Rats

Hipofizektomili Ratlarda Beta-Endorfinin Ağrı Eşiği Üzerine Etkisi

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Abstract: Beta-endorphin (ß-EP) is a 31 amino acid polypeptide secreted by the pituitary, brain, and various other tissues. It has potent opiate-like activity, can produce analgesia, and may play a physiological role in adapting the immune system to stress by affecting lymphocyte and complement binding activity. To investigate the effect of ß-EP on pain threshold, we analyzed cerebrospinal fluid (CSF) and plasma concentrations of ß-EP in 12 intact and 12 hypophysectomized (HYP) rats. Plasma and CSF samples were obtained from both intact and HYP rats 24 hours postsurgery. Pain threshold values were then assessed using the tail-flick test. Results showed a decrease in the plasma concentration of ß-EP ($p < 0.001$), but no significant difference in the level in CSF.

Key Words: Beta-endorphin, hypophysectomy, pain threshold, tail-flick test

Özet: Beta endorfin 31 aminoasitli polipeptid olup pituiter beyin ve diğer değişik dokulardan salgılanır. Potent opiat benzeri aktiviteyle analjezi sağlayıp lenfosit ve kompleman bağlama aktivitesi üzerine olan etkisiyle immün sistemin strese uyumunu fizyolojik olarak oluşturur. Bu çalışmada 24 rat kullanılmıştır. İntakt ve hipofizektomili ratlarda beyin omurilik sıvısı ve plazma b-endorfin konsantrasyonları çalışılmış ve b-endorfinin ağrı eşiğine etkisi araştırılmıştır. Bu nedenle intakt ve hipofizektomili ratlardan cerrahiden 24 saat sonra Beyin omurilik sıvısı ve plazma örnekleri alınmış ve daha sonra tail-flick testiyle ağrı eşiği değerleri tespit edilmiştir. Sonuçlar plazma b-endorfin konsantrasyonunda azalmayı göstermiştir ($p<0.001$). Beyin omurilik sıvısı b-endorfin değerlerinde belirgin farklılık olmamıştır.

Anahtar Kelimeler: Ağrı eşiği, beta-endorfin, hipofizektomi, tail-flick testi

INTRODUCTION

Pain, that "unpleasant sensory and emotional experience," has been defined in various ways: epicritic and protopathic, acute and chronic, somatic and psychogenic. Traditionally, "pain pathways" have been discussed using the most restricted definition of pain, namely nociception (21). Functional changes occur when the nociceptor is activated (16). Tissue damage results in hyperalgesia both at the site of the injury and in the surrounding area. This results in a decrease in the pain threshold, with the potential for spontaneous pain, pain from normally nonnoxious stimuli (allodynia), and an exaggerated response to noxious stimuli (hyperpathia). The principal aim of our study was to investigate the possible role of ß-EP on pain threshold.

MATERIALS and METHODS

We studied two groups of 12 albino rats weighing 240-310 G. Each group had an equal number of males and females. Group I was anesthetized with 2 mg/100 g body weight Ketamine Hydrochloride (Ketalar, Parke-Davis, England) given intramuscularly, and underwent hypophysectomy via the transcervical route, as described below. Group II was sham operated, and underwent the same procedures as Group I except for removal of the pituitary. We confirmed the completeness of each hypophysectomy by macroscopically examining the sella after each procedure. Animals were considered hypophysectomized when the pituitary gland and the stalk were absent. Results were gathered only from animals that met this criterion.

Hypophysectomy Technique:

The cervical area of the rat was shaved after getting anesthesia with ketamine hydrochloride. After intubation with a common serum set, the animal was laid in the supine position. Neck dissection was done with the aid of an operating microscope (Zeiss, Opmi-6, Germany). We exposed the cervical vertebral column by retracting the trachea and larynx to the left. The clivus was approached transcervically by following the longus colli muscle. We opened the anterior part of the junction (blue point) of the clivus and sphenoid sinus using a diamond-tipped drill. The pituitary stalk was cut, and the pituitary gland, which was visible as a dark gray-red nodule, was removed entirely using an aspirator and jewelry forceps. We filled the area with sponges, and the anatomical layers were then sutured consecutively. The pituitary glands were subjected to histopathological examination under the light microscope (Figure 1). All the rats had been fed a standard diet, and they tolerated the procedures well.

Twenty-four hours after surgery, and with the rats under ketamine hydrochloride anesthesia, we exposed the right common carotid artery (CCA). A polyethylene catheter was inserted through a small arteriotomy with the aid of an operating microscope, and blood samples were collected through a CCA cannula. Also, a metal cannula was introduced into the cisterna magna through a site on the sagittal midline rostral to the interparietal sutura occipitalis, and cerebrospinal fluid (CSF) samples were collected for ß-EP determination.

The blood samples were collected into plastic tubes containing 0.6 ml of 10% EDTA and 0.5-mg Bacitracin. Plasma was separated off by centrifugation and stored at -70 °C until it was assayed for ß-EP. Serum and CSF levels of ß-EP were measured using a solid-phase two-site immunoradiometric assay (Nichols Institute Diagnostic, The Netherlands). Nociceptive latency was assessed by the tail-flick method. Rats were placed in a cylindrical plexiglass enclosure on top of the tail-flick test device (Columbus, Type 812, Ohio, USA) and their response was recorded. In this test, control latency was derived from the mean of two latency measurements taken from each animal before surgery. The latency was then measured again after the operation. The cut-off time was 8 seconds.

RESULTS

The concentration of ß-EP in the CSF of hypophysectomized (HYP) rats did not differ significantly from that in intact rats. However, the plasma ß-EP concentration was significantly reduced after hypophysectomy. Main tail-flick test results for Groups I and II were 4.2 ± 0.04 s. preoperatively but after hypophysectomy the mean for Group I was 2.7 ± 0.02 s. ($p<0.001$). The findings are summarized in Table I.

Table I. Concentration of ß-EP in the CSF and plasma of hypophysectomized and intact rats.

	Intact Rats	Hypophysectomized Rats
ß-EP concentration in plasma	892±174(pg/ml)	208±27(pg/ml)
ß-EP concentration in CSF	1162±355(pg/ml)	1096±230(pg/ml)
Tail-flick test	4.2 ± 0.04(s)	2.7 ± 0.02(s)

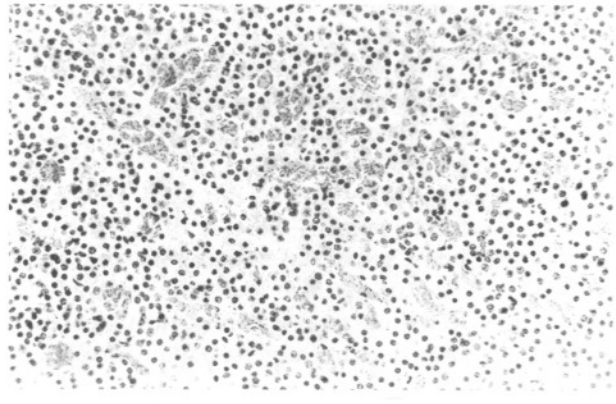

Figure 1: Uniform neuroendocrine cells with centrally placed nuclei and abundant cytoplasm form nests and cords (Hematoxylin-Eosin X 50).

DISCUSSION

ß-EP is an untriakontapeptide found in the pituitary gland and the brain. It is known to have potent opiate-like analgesic effect when injected into the cerebral ventricles of mice, rats, cats, and man (7,8,15,18), which indicates that the pharmacological actions of ß-EP closely resemble those of morphine.

The early discovery that stress could increase plasma levels of ß-EP as much as 15-fold led to the hypothesis that physiological function of this circulating opioid peptide is modulation of nociception (11). This investigation examined the role of pituitary ß-EP secretion in mediating analgesia. Physical stressors, however, are nonselective stimuli that bring about a complex spectrum of physiological responses. Many of these responses serve mechanisms that are independent of the neuroendocrine control of ß-EP secretion. Moreover, different types of stressors vary in their ability to evoke pituitary ß-EP release (14), and some have been reported to induce a nonopioid form of antinociception that, by functional definition, could not be mediated by ß-EP (17). Consequently, results derived solely from experiments involving physical stress cannot conclusively define the role, if any, of ß-EP secretion in antinociception.

Recent studies involving immunocytochemical and radioimmunoassay techniques have shown there are at least two opioid peptide systems in the brain (19). The ß-EP system is located mainly in the medial diencephalon and mesencephalon. Two groups of ß-EP-containing neurons located in the basal hypothalamus have varicosities that terminate in the midline structures of the hypothalamus, thalamus, and dorsal raphe (18). Given the number and range of novel environmental stimuli that increase nociceptive thresholds (9). Many studies have established that both operant and reflex pain threshold elevations are induced following acute exposure to such severe stressors as in escapable foot shock, centrifugal rotation, and cold water swims (CWS) (1,2,3). Acute exposure to CWS induces powerful analgesia in normal rats (10). While the analgesic effectiveness of CWS is diminished by hypophysectomy, morphine's analgesic effects are enhanced by the procedure. This make sense with regard to previous experiments indicate that hypophysectomy does not alter basal pain thresholds (5).

Most autonomic and neuroendocrine physiological stress responses are disrupted following hypophysectomy (4). Thus, this procedure could help to determine whether the pituitary is involved in an organism's analgesic response to stress, and in understanding the role of stress-induced analgesia. The latter includes the action of ß-EP, which is contained in a common precursor molecule in cells of the intermediate lobe of the pituitary, and is released in higher concentrations following injury (6,13). The increases in ß-EP levels following stress occur in plasma but not brain tissue (13), and are inhibited by hypophysectomy (6). In our study, we found that the plasma concentration of ß-EP was significantly reduced at 24 hours following hypophysectomy. Since the drop in plasma ß-EP level was considerable, the diminished pain threshold following hypophysectomy might be attributed to the lack of pituitary ß-EP in the circulating plasma. Furthermore, disruption of the pituitary-adrenal axis by hypophysectomy rather than eliminating its effectiveness (20).

However, these data cannot be discussed simply in terms of the rats' motor and sensory capacities, since motivational factors also seem to be involved (19). Research shows that hypophysectomy significantly lowers the threshold levels for various responses to shock. Thus, it follows that removal of the pituitary would increase the rats' sensitivity to electric shock stimuli. In our study, HYP rats had a lower pain threshold than controls.

The peptides in the CSF are generally believed to be of brain neuronal origin (12). Yet, we found no change in the level of ß-EP in the CSF after hypophysectomy. This suggests that the overall release of ß-EP from brain neurons was unchanged by the surgery. Nevertheless, local changes in the secretory activity of ß-EP neurons could have occurred in the brain without altering the levels in the CSF. ß-EP released from pituitary and brain tissue plays an important role in antinociception.

The mechanism involved in the pain threshold increase that occurs after hypophysectomy remains unknown. In our study on rats, hypophysectomy led to a fall in the level of plasma ß-EP, but there was no change in the CSF ß-EP level. Ours is the first study to investigate the relationship between hypophysectomy, antinociception, and levels of ß-EP in the CSF. Our results in the rat show that hypophysectomy causes plasma levels to drop, but does not affect CSF ß-EP levels. These findings make sense in light of the fact that antinociception is blocked by hypophysectomy. Considering all this

information together, we conclude that there is strong support for the hypothesis that pituitary ß-EP release mediates antinociception in rats.

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