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Letter to Neuroscience

NOCICEPTIVE STIMULUS INDUCES RELEASE OF ENDOGENOUS β-ENDORPHIN IN THE RAT BRAIN

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The hypothesis that the naturally occurring analgesic peptide, β-endorphin, is released in the brain in response to pain had never been directly validated. In this study, we applied a brain microdialysis method for monitoring β-endorphin release *in vivo*, to test this hypothesis in the brains of conscious, freely moving rats. Herein we first show that endogenous β-endorphin can be measured *in vivo* in the brain under physiological conditions. Upon induction of a nociceptive stimulus by injection of formalin into the hind-paws of rats, the extracellular levels of β-endorphin in their arcuate nucleus increased by 88%, corresponding to their nociceptive response. This direct evidence for the release of endogenous β-endorphin in the brain in response to nociceptive stimulus indicates a possible mechanism for organisms to cope with pain. © **1998 IBRO. Published by Elsevier Science Ltd.**

β-Endorphin is an endogenous opioid peptide that acts as a neuromodulator and neurotransmitter in the CNS. The cell bodies in the brain that synthesize β-endorphin are found predominantly in the hypothalamic arcuate nucleus, and their axons and terminals occur in abundance along the walls of the third ventricle.[5,19] In humans, rats, and many other species, injection of exogenous β-endorphin into various brain areas (including the arcuate nucleus) or the cerebrospinal fluid exerts an analgesic effect stronger than that of morphine.[6,9,13,17] This leads to the hypothesis that β-endorphin may be spontaneously released in the brain in response to pain thereby producing a natural analgesia. However, this hypothesis has not been validated due to lack of appropriate tools to study the release of brain β-endorphin in awake animals. Induction of nociceptive stimulus in experimental animal models causes a moderate increase in the β-endorphin levels of plasma.[2] In patients suffering from various types of pain, either elevations or no change in the β-endorphin levels of plasma have been noted.[3,7,11,14] However, intravenous infusion of β-endorphin does not produce an analgesic effect in humans and rats.[9,10,15] Therefore, whether β-endorphin can serve as an endogenous modulator of pain should be tested in the brain rather than in the plasma.

Most analytical studies of the release of β-endorphin in the brain have been performed *in vitro* and utilized tissue slice preparations. Although this approach has provided important information on the characteristics of β-endorphin release, further insight into the regional regulation of the release and interaction of β-endorphin with other neurotransmitter systems required development of an appropriate *in vivo* technique. Such *in vivo* measurements would also allow the concomitant assessment of regional release of β-endorphin in the brain corresponding to behavioral effects. The microdialysis technique, which requires the insertion of a small (500 μm diameter) probe into the brain, permits *in vivo* monitoring of substances in the extracellular space of awake animals and exploration of the relationships between neurochemical, physiological, and behavioral parameters.[4] This technique is currently the method of choice for monitoring the release and metabolism of classical neurotransmitters in conscious animals. We established a method by which microdialysis could be used to monitor β-endorphin release *in vivo*, and used this method to study nociception-stimulated β-endorphin release in the brains of conscious, freely moving rats.

The *in vitro* recovery of β-endorphin by the microdialysis probe's membrane[4] was determined by

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Abbreviations: aCSF, artificial cerebrospinal fluid, ECF, extracellular fluid.

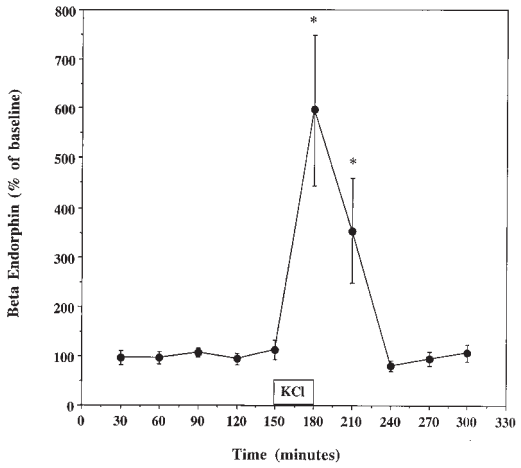

Fig. 1. Effect of KCl on the extracellular levels of β-endorphin in the arcuate nucleus. Microdialysis was performed as previously described.[18] All animal procedures were approved by the Bar-Ilan University Animal Care Committee, in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Briefly Male Sprague–Dawley rats from the breeding colony, Bar-Ilan University, Israel, (230–250 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.). A microdialysis probe (2 mm length, 20,000 mol. wt cutoff value, CMA/10, Carnegie Medicine) was surgically implanted into the arcuate nucleus (2.2 mm posterior and 1.0 mm lateral to bregma and 10.0 mm ventral to dura, at an angle of 3.5°) of each rat using a stereotactic device (David-Kopf Instruments, Tujunga, CA) and cemented to the skull. aCSF was pumped continuously (1 µl/min) through the dialysis probe using a microinjection pump (CMA 100, Carnegie Medicine). The dialysates were collected at 30-min intervals into polyethylene tubes, immediately frozen on dry ice and thawed before assaying for β-endorphin using a commercially available ELISA kit (Peninsula; Belmont, CA). After five baseline collections, aCSF-KCl was perfused for 30 min (indicated by bar). The mean of the 5 dialysates obtained before addition of KCl (42.8 ± 5.8 ng/ml; mean ± S.E.M., n=8 rats) was used as the baseline β-endorphin level. Values statistically different from baseline were determined with ANOVA followed by Student–Newman–Keuls *post hoc* test. *P<0.001.

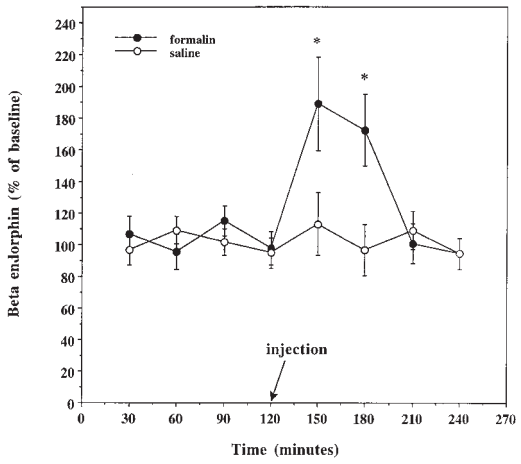

Fig. 2. Effect of formalin-induced nociception on the extracellular levels of β-endorphin in the arcuate nucleus. Microdialysis was performed as described in the legend to Fig. 1. The time of injection of 50 µl of saline or formalin (10% solution) into the plantar side of the rat's hind paws is indicated by an arrow. The mean of the four dialysates obtained before the injection was used as the baseline β-endorphin level. The basal levels were 42.6 ± 6.8 and 45.1 ± 7.6 ng/ml (mean ± S.E.M.; n=6) for the formalin and saline groups, respectively. Differences between saline and formalin treatments were compared by using repeated ANOVA [with a within-group factor of time (fraction number) and a between-group factor of treatment (saline or formalin)] and the Student–Newman–Keuls *post hoc* test. *P<0.001.

placing the probe in artificial cerebrospinal fluid (aCSF; 145 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$, pH 7.4) containing β-endorphin (225 ng/ml), pumping aCSF via the probe at different flow rates (0.5, 1, 2 or 3 µl/min), and determining the β-endorphin content of the microdialysates by an established ELISA assay (Peninsula; Belmont, CA). The linear portion of the standard curve was between 0.04 ng/ml and 5 ng/ml of β-endorphin and the experimental levels of β-endorphin were within the linear portion. The intra-assay variation was 4% and the inter-assay variation was 13%. The recovery of β-endorphin was optimal at a flow rate of 1 µl/min and was equivalent to 0.29 ± 0.02%.

Microdialysis probes were implanted into the arcuate nucleus of anesthetized male Sprague–Dawley rats and 22–24 h after probe implantation experiments were initiated in awake freely moving rats. aCSF was pumped continuously through the dialysis probe and the dialysates were collected at 30-min intervals.[18] In the arcuate nucleus of Sprague–Dawley rats, the basal levels of β-endorphin in the extracellular fluid (ECF) were calculated to be 43.2 ± 6.3 ng/ml (mean ± S.E.M.; n=25), corrected for *in vitro* recovery. When the perfusing aCSF solution was switched to one containing high levels of KCl (aCSF-KCl; 100 mM KCl, 47.7 mM NaCl; 1.2 mM CaCl2, 1.0 mM $MgCl_2$, pH 7.4) for an interval of 30 min, β-endorphin levels in the ECF of the arcuate nucleus increased by 596 ± 153% (Fig. 1), indicating that β-endorphin release in the arcuate nucleus, like the release of other neurotransmitters in the CNS is stimulated in response to depolarization.

The formalin test for pain, in which scaled intensities of nociception can be induced according to the concentration of formalin injected into a paw, has become a popular model for pain induced by acute injury and is postulated to reflect pain commonly experienced by humans.[8,16] The induction of a nociceptive stimulus by injection of formalin (50 µl of a 10% solution) into the hind-paw of Sprague–Dawley rats caused a significant increase (88 ± 39%, n=6, P<0.05) in the levels of β-endorphin in the ECF of their arcuate nucleus (Fig. 2). These elevated levels returned to baseline within 90 min following the formalin injection. Injections of saline into the hind-paw did not cause any change in β-endorphin levels of ECF in the arcuate nucleus.

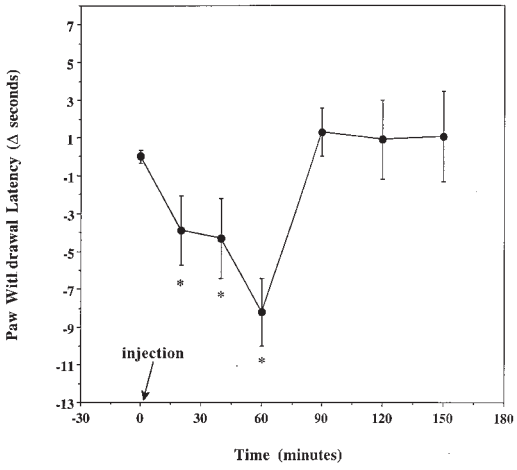

Fig. 3. Effect of formalin injection on nociceptive response. The paw withdrawal latency time (in response to a thermal stimulus induced by a radiant light source) for the formalin injected (50 μl of a 10% solution) leg was subtracted from the latency time of the saline-injected (50 μl) leg. The time of injection is indicated by an arrow. Values significantly different from pre injection (time zero) were determined using ANOVA followed by Student–Newman–Keuls *post hoc* test; $n=6$, $*P<0.01$. Note: paw withdrawals indicative of flinching behavior (quicker, in bursts, and accompanied by intense paw licking), which occurs after formalin injection[8] were not counted as ''withdrawal responses'' to the heat stimulus. The formalin-associated flinching behavior is most prevalent at 0–5 (up to 10 flinches/min) and 45–50 (~12 flinches/min)min following formalin injection. Since both the behavioral manifestations and the kinetics of the paw withdrawal latency differed from those of flinching behavior, the results presented here should indicate latency induced by the thermal stimulus.

Sensitivity to pain (nociception; in this study an increased sensitivity to a noxious heat stimulus after formalin injection) was determined by the paw withdrawal latency test.[12] These behavioral tests showed a nociceptive response for 60 min after the formalin injection (Fig. 3), which paralleled the elevation of ECF β-endorphin in the arcuate nucleus of the brain.

Stress is known to induce antinociception in rats and other species, and this antinociceptive effect is partially reversed by naloxone, an opioid antagonist.[1] Although Rossier *et al.*[15] demonstrated in rats that stress induced by foot-shocks is followed by a five- to six-fold increase in the levels of β-endorphin in plasma and by an antinociceptive response, an intravenous infusion of β-endorphin (even three orders of magnitude higher than that induced by stress) did not cause antinociception. A stressful stimulus did not appear to affect the β-endorphin content in the brains of the rats, except for a slight reduction in this opiate peptide in the hypothalamus.[15] In the present study, a nociceptive stimulus elicited the release of β-endorphin into the ECF of the arcuate nucleus of the hypothalamus *in situ* in freely moving animals. This release might account for the previously observed[15] temporary reduction in tissue β-endorphin stores.

Following the demonstration of β-endorphin as a strong analgesic factor in the brain in previous studies, it is now evident that endogenous β-endorphin is released in the brain in response to nociceptive stimulus. This phenomenon indicates a physiological mechanism by which an organism can cope with pain.

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