Letter to Neuroscience

NOCICEPTIVE STIMULUS INDUCES RELEASE OF ENDOGENOUS β-ENDOPHRIN 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.

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. 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. 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. In patients suffering from various types of pain, either elevations or no change in the β-endorphin levels of plasma have been noted. However, intravenous infusion of β-endorphin does not produce an analgesic effect in humans and rats. 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. 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 was determined by...
placing the probe in artificial cerebrospinal fluid (aCSF; 145 mM NaCl, 1.2 mM CaCl₂, 2.7 mM KCl, 1.0 mM MgCl₂, 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). After five baseline collections, 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). 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 ± 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-K euls post hoc test. *P < 0.001.
formalin injection) was determined by the paw withdrawal latency test. These behavioral tests showed a nociceptive response for 60 min after the formalin injection (Fig. 3), which paralleled the elevation of ECF \( \beta \)-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 Rossi 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 \( \beta \)-endorphin in plasma and by an antinociceptive response, an intravenous infusion of \( \beta \)-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 \( \beta \)-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 \( \beta \)-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 \( \beta \)-endorphin stores.

Following the demonstration of \( \beta \)-endorphin as a strong analgesic factor in the brain in previous studies, it is now evident that endogenous \( \beta \)-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.

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 \( \beta \)-endorphin in the arcuate nucleus of the brain.

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