

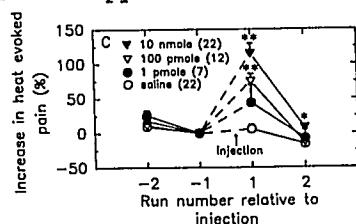
A699

TITLE: INTRADERMAL BRADYKININ INDUCES PAIN AND HYPERALGESIA IN HUMANS**AUTHORS:** D.Manning, M.D., Ph.D., S.Raja, M.D., R.Meyer, M.S., J.Campbell, M.D.**AFFILIATION:** Depts of Anes. & Neurosurg., Johns Hopkins Univ., Balto., MD 21205

Pain and hyperalgesia, the perceptual companions of tissue injury and inflammation, are thought to be mediated, in part, by the endogenous release of chemicals, such as bradykinin (BK). To understand better BK's actions we determined the effects of intradermal (id) BK on cutaneous pain sensations in humans. Trained human subjects (11 F and 12 M ages 18-42) used magnitude estimation techniques to rate their pain to heat and mechanical stimuli before and after id injections of varying doses of BK, and vehicle (neutral normal saline), to the volar forearm. A CO₂ laser stimulator was used to deliver a heat test sequence (HTS) every 10 min. A HTS consisted of 10 1s stimuli starting with 45° C and followed by stimuli that ranged from 41 to 49° C with an inter-stimulus interval of 29s. All subjects gave informed consent and the protocol was approved by the Institutional Clinical Investigational Committee.

BK (0.1 to 10 nmole in 10 μ l) produced several dose-dependent responses including: evoked pain lasting for approx. 2 min., hyperalgesia to heat stimuli lasting for less than 30 min. as well as wheal and flare reactions. BK at 0.1 and 10 nmole doses increased heat evoked pain by 75 ± 16 and 115 ± 15 % respectively over saline control injections (Fig., $p < 0.01$). Cutaneous hyperalgesia to mechanical stimuli was not prominent for any of the BK doses. A second injection of BK (5 or 30 minute intervals) at the same site produced markedly less pain and hyperalgesia to heat stimuli indicating that the algescic and hyperalgesic effects of BK undergo tachyphylaxis.

Rapid development of tachyphylaxis and separation of thermal from mechanical hyperalgesia suggest that BK acting alone cannot account for all aspects of inflammatory hyperalgesia. Inhibition of BK actions however may still have a role in analgesic therapy.



Increase in heat evoked pain after BK injection. The sum of pain ratings to the various temperatures in the HTS is considered an index of overall heat-evoked pain. The change in pain ratings to the HTS after injections are expressed as % of pain ratings immediately prior to injection. Negative and positive run numbers designate pre and post injection runs.

A700

TITLE: POST-TRANSLATIONAL PROCESSING OF B-ENDORPHIN AND ACTH IN THE HUMAN PITUITARY**AUTHORS:** L.H. Bernard, MD, B.M. Chronwall, PhD, W.R. Millington, PhD.**AFFILIATION:** Department of Anesthesiology, Saint Luke's Hospital/School of Basic Life Sciences / University of Missouri-Kansas City, 4400 Wornall, Kansas City, Missouri 64111

Introduction: B-endorphin (BE), an endogenous 31 amino acid peptide, is a potent opioid receptor agonist which also demonstrates cardiovascular activity by causing a decrease in blood pressure. In most species, BE is enzymatically modified at its N and C terminals to form acetylated and shortened (26 and 27 amino acid) peptides. These compounds are structurally similar but produce distinctly different biological effects, including opioid antagonist properties. Likewise, ACTH is cleaved to alpha-melanocyte stimulating hormone (MSH) by a similar post-translational modifying process. The human pituitary, unlike virtually any other species, lacks an intermediate lobe, the site where BE and ACTH are most extensively processed. These C-terminally shortened and N-acetylated (N-Ac) BE peptides have not been previously demonstrated in the human pituitary. The purpose of this study was to determine if the human pituitary contains intermediate lobe cells capable of this post-translational processing.

Methods: Human cadaveric pituitary glands were frozen and sectioned. Histological staining for BE, N-Ac-BE, ACTH, and MSH was performed with standard fluorescent secondary antibody techniques. The antisera to N-Ac-BE recognizes all acetylated forms. BE peptides were quantitated with chromatographic techniques. BE was separated from B-lipotropin (B-LPH), the precursor molecule for BE, by gel filtration chromatography. The individual molecular forms of BE were then separated by ion exchange chromatography. Quantitative amounts were determined by radioimmunoassay which recognizes B-LPH and all BE peptides on an equimolar basis.

Results: A large number of cells labelled by ACTH and BE antisera were found in the anterior lobe, as established previously. A smaller proportion of MSH cells were found along the border of the anterior and posterior lobes and scattered throughout the anterior lobe. Cells staining for N-Ac-BE, which in the rat are only found in the intermediate lobe, exhibited essentially the same cellular distribution as MSH. The quantitative contribution of these processed peptides determined by chromatography revealed an MSH/ACTH ratio of $< 1\%$. BE-1-31 was the principal BE form comprising 85% of total BE. BE-1-27 and BE-1-26 were also present along with very low levels of N-Ac-BE peptides together comprising $< 4\%$ of total BE.

Discussion: These results demonstrate that in the human pituitary, ACTH and BE are post-translationally processed to very small amounts of MSH and BE analogs (N-Ac and shortened forms), respectively. N-Ac-BE and MSH are produced in cells along the border of the anterior and posterior lobes which are most likely remnant cells of the intermediate lobe found in human fetal pituitary. However, unlike the rat and many other species, these peptides are also produced within a sub-population of BE and ACTH immunoreactive cells dispersed throughout the anterior lobe. Despite important differences in primary structure, BE is processed to similar forms in both human and rat pituitary. Opiate active BE-1-31 is the principal form in the human. What role these modified peptides play in the human has yet to be established. The opiate antagonist peptides could impact the central regulation of pain and may be mediators of pain perception as well as pain tolerance. Variations in the amount of these peptides could be a factor in determining individual pain thresholds.