Anesthesiology
V 75, No 3A, Sep 1991

A663

Title: CONTRASTING ANALGESIC ACTION OF THE INTRATHECAL KAPPA AGONIST (U50-488H) IN VISCERAL PAIN PROCESSING AS COMPARED TO MORPHINE AND CLONIDINE

Authors: Y. Harada, M.D., K. Nishioka, M.D., L.M. Kitahata, M.D., Ph.D., J.G. Collins, Ph.D.

Affiliation: Yale University School of Medicine, Department of Anesthesiology, New Haven, CT 06510

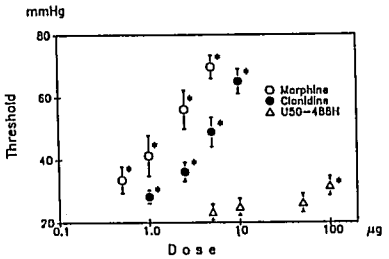
Introduction: Clinically, there is a need for a better understanding of pharmacologic control of visceral pain. It has been reported that kappa opioid agonists exert antinociceptive effects at the spinal level in visceral pain processing.[1] That conclusion was based on the writhing test. Recently, a more appropriate test for visceral pain, the colorectal distension technique, was developed.[2] The purpose of this study was to examine the visceral antinociceptive effect of a kappa opioid agonist as compared to a mu opioid agonist and an alpha-2 adrenergic agonist using the colorectal distension test.

Methods: This protocol was approved by the Yale Animal Care and Use Committee. Male Sprague-Dawley rats (280-360 g) were used. Each animal received intrathecal morphine (M, n=44) clonidine (CL, n=46) or U50-488H (U50, n=26) in 5 µl through a chronically implanted catheter whose tip was located near the lumbar enlargement of the spinal cord. Doses tested were as follows: 0.5, 1.0, 2.5, 5 µg of M (n=10,11,11,12 respectively); 1.0, 2.5, 5, 10 µg of CL (n=10,11,12,13 respectively); 5, 10, 50, 100 µg of U50 (n=6,7,7,6 respectively). In this study, a modified colorectal distension technique was used. The minimal distension pressure which caused abdominal constrictions was defined as the responding threshold. A cut-off pressure was set at 80 mmHg to avoid tissue damage. Thresholds were measured before drug injection for control and repeatedly measured at 5, 10, 15, 20, 30 and 45 min after injection. At 18 min. after M (5 µg) or CL (10 µg) administration, naloxone (N, 5 µg / 5 µl, n=6) or yohimbine (Y, 20 µg / 10 µl, n=7) was administered via the catheter. Student's t test was employed for statistical analysis. Significant differences were considered at $p < 0.05$.

Results: The range of mean of control thresholds (mmHg) was 20.6 ± 1.0 to 23.1 ± 0.8. Fig.1 shows dose-response relationships for each drug at the time of peak effect. All doses of M and CL increased thresholds significantly and dose-dependently, whereas U50 produced a significant increase in threshold (31.7 ± 3.0) at only 100 µg, a maximum dose that could be dissolved in 5 µl of saline. N or Y decreased thresholds significantly.

Discussion: Schmauss and Yaksh reported that U50 was more potent in the writhing test than in the tail-flick or hot-plate test.[1] Those results have been interpreted to mean that kappa agonists are good for visceral pain. Their study, however, reported that morphine was more potent than U50 in the writhing test (ED50 in nM 1.8, U50 17.0). In our present study, using what may be a more physiologic visceral stimulus, the kappa agonist is even less potent than morphine or clonidine in blocking a response to colorectal distension. Our results suggest that combinations of morphine and clonidine, rather than a kappa opioid may be beneficial for the treatment of visceral pain.

This study was supported by NIH NS-09871



*:P<0.05, compared with control threshold.

References:
1. J Pharmacol Exp Ther 1984;228:1-12
2. Brain Res 1988;450:153-169

A664

TITLE: PRELIMINARY CHARACTERIZATION OF α_2-ADRENOCEPTORS IN THE SHEEP SPINAL CORD BY AUTORADIOGRAPHY

AUTHORS: B Bucklin MD, JC Eisenach MD, R Booze PhD

AFFILIATION: Section on Obstetric Anesthesia and Department of Physiology and Pharmacology, Wake Forest University Medical Center, Winston-Salem, NC 27157-1009

Introduction. α_2-Adrenergic receptors have been shown to play an important role in nociceptive processing. Several α_2-receptor subtypes have been characterized by pharmacologic, biochemical, and genetic analyses. Although the presence of α_2-adrenergic receptors in the spinal cord can be demonstrated functionally and autoradiographically, these studies have been unable to quantify and specifically localize these receptors. Quantification and localization of these receptors and their subtypes will be important in further characterization of nociceptive processing and drug development. Therefore, we characterized the α_2-binding conditions of p-[^{125}I] iodoclonidine with sheep spinal cord tissue sections by high-resolution autoradiographic techniques.

Methods. Following approval by the Animal Care and Use Committee, 4 adult sheep were anesthetized. Thoracic spinal cord was removed and stored at -80°C. Serial sections of 25 microns thickness were thaw-mounted onto chrome-alum/gelatin-subbed slides, incubated in 50mM Tris-HCl/10mM $MgCl_2$ (pH7.5) for 30 min at room temperature and then incubated at varying concentrations of p-[^{125}I] iodoclonidine (.05nM to 1.5nM) 90 min at room temperature. Non-specific binding was defined as binding which was not inhibited by 100 μM phentolamine. Analysis of the saturation experiments was performed using EBDA, and saturability of binding was determined by LIGAND. Localization of α_2-receptors in the sheep spinal cord was performed using p-[^{125}I] iodoclonidine (.5nM) as the ligand. Non-specific binding was defined as mentioned previously. Films were developed 3 days after exposure.

Results. p-[^{125}I] iodoclonidine bound to a single population of binding sites over the concentration range studied. Non-regression linear analysis determined the binding to be saturable and of high affinity (K_d=.5nM). The amount of non-specific binding was low. Images showed localization of α_2-adrenoceptors to the superficial dorsal horn and intermediolateral cell column of the spinal cord.

Discussion. These preliminary studies define the binding conditions necessary for localization of α_2-adrenergic receptors and demonstrate a high density of specific binding sites in the dorsal horn and intermediolateral cell column of the thoracic spinal cord. Future studies will define α_2-adrenoceptor subtypes at sites of hemodynamic control and analgesia.

Supported in part by grant GM35523 from the N.I.H.