

Title: IRREVERSIBLE OPIOID RECEPTOR BLOCKADE DECREASES THE ANALGESIC EFFECTS OF KETAMINE AND NITROUS OXIDE IN MICE

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Introduction. Published reports suggest that nitrous oxide (N₂O)- and ketamine (K)-induced analgesia may be mediated by the opioid receptor (OR)/endogenous opioid peptide (EOP) system. If such is the case, then "irreversible" OR blockade should decrease the analgesic effects of N₂O and ketamine. Accordingly, we examined the analgesic effects of K and N₂O in mice pretreated with naloxonazine (NXZ), a derivative of naloxone which is a μ -selective irreversible OR antagonist. We also examined the brain OR binding characteristics in mice so pretreated.

Methods. Male Swiss-Webster (CF1) mice (22-26 g) were used. NXZ was synthesized from naloxone according to published methods.¹ Twenty-four h prior to analgesia testing, experimental groups were injected with NXZ, 35 mg/kg, sc, in 0.5 ml volume. Control groups were injected with the vehicle (V) at the same time. Five min before the injection of HAc (15 min before analgesia testing), groups of control and experimental mice were injected, sc, either with normal saline (NS), K, 30 mg/kg, or morphine (M), 1.0 mg/kg, all in a volume of 0.01 ml/g body weight. When N₂O analgesia was studied, mice in wire cages were placed in a clear plastic box (14 liters volume) after ip injection of HAc. 10 l/min of either 80% N₂O/20% oxygen mixture or compressed air flowed through the box. For analgesia testing, mice, in groups of 5, were injected, ip, with 1% acetic acid (HAc), 0.01 ml/g, and placed in individual wire cages for ease of identification. Results are based on the number of abdominal constrictions (writhes) per mouse observed during the 5 min interval from 10-15 min after HAc injection. Each mouse was used only once. The observer was uninformed as to drug pretreatment and treatment. Other groups of mice were pretreated with NXZ or V, and analysis of OR binding in each brain (minus cerebellum) was performed 24 h later using ³H-[D-Ala², MePhe⁴, Gly-ol⁵]-enkephalin (³H-DAGO), a μ -specific ligand in six concentrations (0.25-10 nM) in the presence or absence of 5 x 10⁻⁶ M levorphanol to assess specific binding according to previously described methods.² Specific binding of ³H-DAGO was subjected to Scatchard analysis to give the apparent receptor density (B_{max}) in fmol/mg protein and affinity (K_d) in nM. Student's t test for unpaired data was used to compare NXZ- versus V-pretreated groups. P < 0.05 was considered significant.

Results. The figure shows that NXZ pretreatment did not change the number of writhes in mice treated with NS or given air to breathe, yet it significantly increased the number of writhes in M- and N₂O-treated groups compared to the V-pretreated groups. NXZ pretreatment also reduced the analgesic action of K. V-pretreated

mice injected with NS writhed 12.6 ± 1.7 (SEM) times, not different from the 12.4 ± 1.5 times in NXZ-pretreated mice (n = 10 each). K decreased the number of writhes to 2.95 ± 0.72 times in V-pretreated mice, but only to 6.2 ± 1.43 times in NXZ pretreated mice, a significant increase in the number of writhes compared to V-pretreated mice (n = 20 each). B_{max} was 62.3 ± 4.9 fmol/mg protein and K_d 2.6 ± 0.5 nM in V-pretreated mice (n = 11), whereas the respective values were 80.7 ± 4.2 fmol/mg protein and 3.2 ± 0.6 nM in NXZ-pretreated mice (n = 12), a significant 30% increase in apparent receptor density with no change in receptor affinity.

Discussion. Irreversible OR blockade decreased the analgesic effects of M, as expected, as well as that of N₂O and K, providing further indirect evidence that these drugs act on the OR/EOP system. The increase in the apparent OR density in the face of decreased response to M, K and N₂O is not easily explained.

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References.

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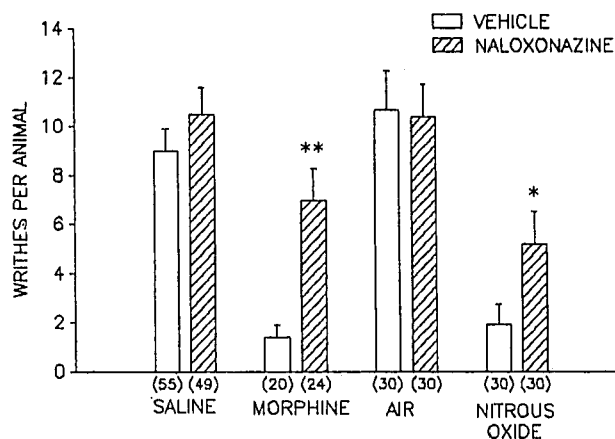


Figure. Effects of four treatments (abscissa) on abdominal constrictions in mice pretreated 24 hours earlier either with vehicle or naloxonazine, 35 mg/kg, sc. Number of animals in each group is shown in parentheses at the bottom of each bar. Error bars are SEM. Asterisks denote a significant increase compared with vehicle pretreated mice (* - P < 0.02; ** - P < 0.001).