Title: THE INFLUENCE OF NITROUS OXIDE AND NOCIOCEPTIVE STIMULI ON RAT PLASMA AND BRAIN ENDORPHIN CONCENTRATIONS

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Introduction: The endogenous opioid peptides (endorphins) appear to play an important role in the modulation of pain and perhaps anesthesia as well. Numerous investigators have proposed that inhaled anesthetics, including nitrous oxide, act in part by stimulating the release of endorphins which interact with opiate receptors in the pain pathways to produce the analgesic component of anesthesia. This proposal is based on the observation that naloxone produces a partial reversal or inhibition of the analgesia. However, the results are conflicting and there is to date no quantitative evidence for a general anesthetic-induced release of endorphin. The object of this study was to measure rat plasma and brain endorphin concentrations with and without pain-induced stress and also to determine the influence of nitrous oxide on these responses.
Methods: Sixteen male Sprague-Dawley rats (200-320 gm) were randomly divided into 4 equal groups. Each animal was placed in a plastic chamber and allowed to breathe room air (groups I and II) or 75% nitrous oxide in oxygen (groups III and IV). After a 15 minute stabilization period, group II and IV animals had a ring-type tail clamp applied. The tail clamp was in place for 30 minutes following which the animals were decapitated. Blood was collected after decapitation for subsequent plasma separation and the whole brain except for the cerebellum was removed. The brain was processed for radioimmunoassay of endorphins according to the method of Rossier et al. The plasma samples were processed to remove B-lipotropin by stripping with a Sepharose anti-B-lipotropin gel, acidified, and purified by extraction on ODS-silica columns. A commercially available radioimmunoassay kit (Immuno Nuclear Corp., Stillwater, Minn.) was used for the B-endorphin assay. This assay has a 50% cross-reactivity with B-lipotropin. All experiments were performed the same time each day. Multivariate analysis of variance was used for data analysis.

Results: Plasma B-endorphin concentrations were significantly higher in all tail-pinch and nitrous oxide groups (II,III,IV) when compared to the room air control group I. Whole brain and plasma B-endorphin concentrations were higher in the tail-pinch group II than in the control group I. Nitrous oxide increased plasma B-endorphin concentrations when compared to room air irrespective of whether the animals had their tails clamped or not. Nitrous oxide had no significant effect on whole brain B-endorphin concentrations.
Discussion: The results of this study demonstrate that painful (tail-clamp) stimulation in the rat increases whole brain and plasma B-endorphin concentrations, and suggests that endorphins are involved in the modulation of pain. Nitrous oxide anesthesia, with or without painful stimulation, produces similar increases in plasma B-endorphin concentrations. These data show that the analgesic and possibly anesthetic action of nitrous oxide does involve changes in plasma B-endorphin concentrations and suggests that nitrous oxide probably acts in part by mechanisms involving the endorphin opiate receptor system.
Reference: Rossier J, Vargo TM, Minick S, Bloom FE, Guillemin R: Regional dissociation of B-endorphin and enkephalin contents in rat brain and pituitary. Proc Natl Acad Sci USA 74:5162-5165, 1977.

	Group I	Group II	Group III	Group IV
Tail Clamp	No	Yes	No	Yes
Anesthesia	No	No	Yes	Yes

B-Endorphin Concentrations (Mean ± SEM)

	Group I	Group II	Group III	Group IV
Whole Brain (ng/gm)	106 ± 4	127 ± 6	117 ± 7	107 ± 7
Plasma* (pmole/liter)	7.6 ± .3	13.8±3.0	15.4±3.5	14.5±2.1

*Groups II,III,IV versus Group I, p=0.23