Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 3, pp. 699–702, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0026-E

Effect of Opioid Peptide Antisera on Nitrous Oxide Antinociception in Rats[1]

SHUICHI HARA,*† MICHAEL J. GAGNON,* RAYMOND M. QUOCK*[2] AND TAKESHI SHIBUYA*‡

*Department of Biomedical Sciences, University of Illinois College of Medicine at Rockford, 1601 Parkview Avenue, Rockford, IL 61107
Departments of †Forensic Medicine and ‡Pharmacology, Tokyo Medical College, Tokyo 160, Japan

Received 18 June 1993

HARA, S., M. J. GAGNON, R. M. QUOCK AND T. SHIBUYA. *Effect of opioid peptide antisera on nitrous oxide antinociception in rats.* PHARMACOL BIOCHEM BEHAV **48**(3) 699–702, 1994.—This study was performed to examine the effects of ICV injection of antiserum against β-endorphin (β-EP) or methionine-enkephalin (ME) on nitrous oxide-induced antinociception in rats using the hot plate test. The injection of β-EP antiserum reversed the antinociceptive effect of nitrous oxide in a dose-related manner up to 200 μg/rat. However, antagonism of nitrous oxide by 400 μg β-EP antiserum was comparable to that produced by 200 μg. On the other hand, similar amounts of ME antiserum had little effect against nitrous oxide antinociception. These findings suggest that β-EP may play an important role in the antinociceptive effect of nitrous oxide.

Nitrous oxide Antinociception Antiserum β-Endorphin Methionine-enkephalin Rats

THERE is evidence that antinociception induced by nitrous oxide is antagonized by various blockers of opioid receptors in experimental animals (2,16,17,22,23,30) and in human subjects (4,10,29). Exposure of rats to nitrous oxide caused an increase in the endogenous opioid peptide (EOP) β-endorphin (β-EP) in the basal hypothalamus and the periaqueductal gray (31). Exposure to nitrous oxide also stimulated secretion of β-EP from basal hypothalamic cells attached to cytodex beads in an in vitro perfusion system (32). Nitrous oxide increased methionine-enkephalin (ME), but not leucine-enkephalin (LE) or β-EP, in fractions of artificial CSF collected from ventricular-cisternally perfused rats (20). In fact, nitrous oxide increased the ME level in several brain regions in rats (21,24,25). There were also increases in ME and ME-Arg-Phe, but not LE, β-EP, or dynorphin (DYN) A in CSF collected from the third ventricle of dogs exposed to 66–75% nitrous oxide (8). These reports suggest the major role of an increase in central EOPs, namely β-EP and ME, in nitrous oxide antinociception. This is also supported by a recent finding of our laboratory that an endopeptidase 24.11 inhibitor, phosphoramidon, potentiated nitrous oxide antinociception (9).

In the present study, we examined whether ICV pretreatment with antiserum selective for β-EP or ME could attenuate the antinociceptive effect of nitrous oxide in rats.

METHOD

Stereotaxic Implantation of ICV Injection Cannulae

Male Sprague–Dawley rats (300–450 g; Sasco Inc., Omaha, NE) were anesthetized with sodium pentobarbital (60 mg/kg, IP) and mounted in a stereotaxic headholder (David Kopf Instrument, Tujunga, CA). A 26-ga guide cannula (Plastic Products, Roanoke, VA) was stereotaxically implanted into the lateral ventricle of each rat at coordinates 0.0 mm AP, 1.5 mm L, and −3.0 mm DV (19). Cannulae were held in place using stainless steel screws and dental cement. These were plugged and capped with 33-ga dummy cannulae until experimentation. All rats were allowed at least a week for recovery prior to antinociceptive testing.

Hot Plate Testing Under Nitrous Oxide

Rats were exposed to a mixture of 70% nitrous oxide/30% oxygen in an enclosed Plexiglas box (25 cm L × 10 cm W ×

[1] These results were originally presented at the Second Annual International Behavioral Neuroscience Society Conference held in Clearwater Beach, FL, April 22–25, 1993.
[2] To whom requests for reprints should be addressed.

10 cm H) with a sliding lid. A mixture of nitrous oxide/oxygen was delivered into the box from a standard nitrous oxide/oxygen dental sedation system (Porter, Hatfield, PA). The rate of inflow was 10 l/min (7.0 l/min nitrous oxide and 3.0 l/min oxygen). Gas entered the box through an inflow port at one end, circulated through the box, and exited through an outflow port at the other end. The concentration of nitrous oxide and oxygen was monitored using a POET II anesthetic monitoring system (Criticare, Milwaukee, WI). After a 5-min exposure period, the animal was quickly removed from the box and dropped onto a hot plate analgesiometer (IITC, Woodland Hills, CA) with a Plexiglas enclosure (30 cm L × 30 cm W × 45 cm H) through which flowed the same concentration of nitrous oxide in oxygen. The temperature of the surface of the hot plate was 52.0 ± 0.1°C. The response time to paw licking or escape behavior was recorded. A maximum cutoff time of 60 s was used.

Protocol

Baseline response times were determined for all rats in room air prior to ICV injection of antiserum against β-EP or ME. Control animals were pretreated with vehicle (sterile physiological saline) in lieu of antiserum. One hour after the injection, rats were exposed to nitrous oxide/oxygen as described above.

Drugs

Nitrous oxide, U.S.P. and oxygen, U.S.P. were purchased from Rockford Industrial Welding (Rockford, IL). Lyophilized antisera against β-EP and ME were provided by Dr. L.-F. Tseng (Medical College of Wisconsin, Milwaukee, WI). The lyophilized antisera were dissolved in sterile saline vehicle before use. The antisera were injected ICV using a 10-μl microsyringe (Hamilton, Reno, NV) mounted in a model 341A syringe pump (Sage Instruments, Cambridge, MA). The microinjection volume of the antisera or vehicle was no more than 6 μl delivered at a rate of 1.0 μl/min.

The antisera were produced by repeated injection of rabbits with β-EP or ME coupled to bovine thyroglobulin (21). The specificities of these antisera have been previously characterized by Dr. Tseng. The β-EP antiserum did not cross-react with ME, LE, various DYNs, β-melanotropin, or ACTH. There was, however, a 100% cross-reactivity on a molar basis with β-lipotropin. The ME antiserum did not cross-react with oxidized ME, DYN_{1-13}, DYN_{1-17}, or neurotensin. There were low degrees of cross-reactivity (<1%) with ME-Arg-Gly-Leu, LE, LE-Arg, DYN_{1-8}, DYN_{1-10}, and β-EP. There were also varying degrees of cross-reactivity with ME-Arg-Phe (12.7%) and ME-Lys (30%).

Statistical Analysis of Data

The degree of antinociception for each rat was calculated as:

$$\%\ \text{antinociception} = 100 \times \frac{\text{test response time} - \text{baseline response time}}{\text{60-s cutoff time} - \text{baseline response time}}.$$

Statistical analyses were carried out using analysis of variance (ANOVA) and Dunnett's *t*-test after arc-sin transformation of each % antinociceptive datum.

RESULTS

The baseline response time was 20.8 ± 0.7 s. Exposure of rats to 70% nitrous oxide produced a significant prolongation in the latency for hindpaw lick or escape attempt. Following ICV pretreatment with vehicle, control rats showed 80–90% antinociception (Figs. 1 and 2).

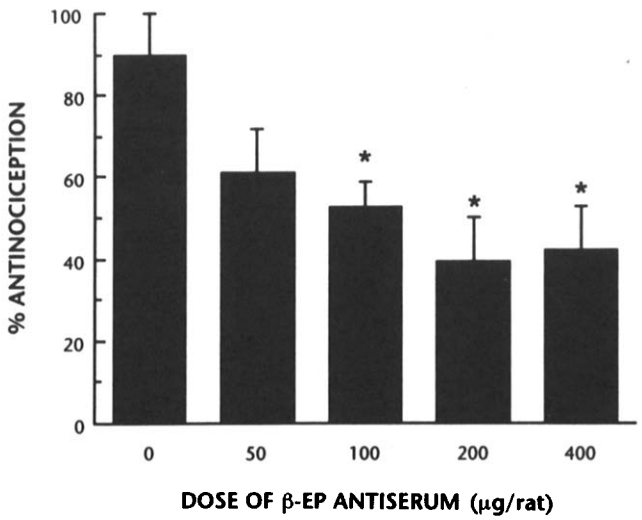

FIG. 1. Influence of ICV pretreatment with β-EP antiserum on the antinociceptive response to 70% nitrous oxide. Data are expressed as mean ± SEM obtained from seven rats per group. Significance of difference: $*p < 0.05$, compared to the vehicle control group.

Figure 1 also shows the effects of ICV pretreatment with β-EP antiserum on the antinociceptive effect of 70% nitrous oxide. Increasing doses of β-EP antiserum reduced the antinociceptive effect of nitrous oxide in a dose-dependent manner up to a dose of 200 μg/rat. However, further increasing the dose of the antiserum to 400 μg/rat failed to produce any greater antagonism of nitrous oxide antinociception. On the other hand, ICV injection of ME antiserum at comparable

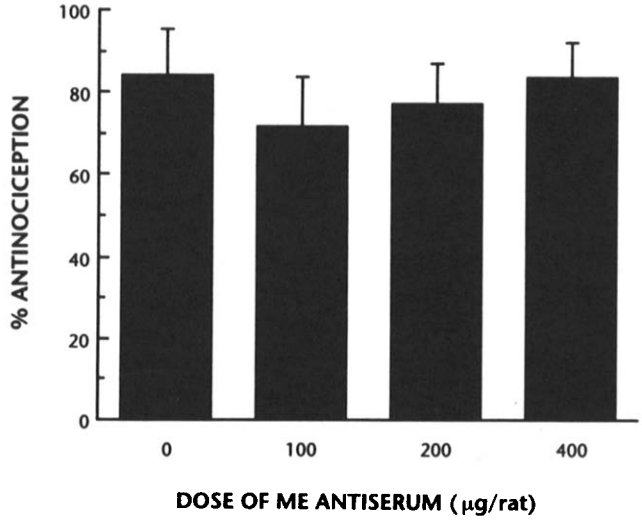

FIG. 2. Influence of ICV pretreatment with ME antiserum on the antinociceptive response to 70% nitrous oxide. Data are expressed as mean ± SEM obtained from eight to nine rats per group. There were no significant differences between the vehicle control group and any of the pretreatment groups.

doses (100–400 μg/rat) caused no significant change in the antinociception induced by 70% nitrous oxide (Fig. 2). One hour after ICV injection of β-EP or ME antiserum alone, rats exhibited minimal antinociceptive responsiveness, comparable to saline-treated control rats (data not shown).

DISCUSSION

The rat hot plate test has long been used as a method for determining opioid antinociceptive activity (6). The use of hindpaw lick or escape attempt as behavioral end points in the rat hot plate paradigm has been demonstrated to minimize interference by nonanalgesic drug activities such as motor impairment (3). Exposure to nitrous oxide produced prominent antinociception as evidenced by a significant prolongation in the latency to hindpaw lick or escape attempt. Nitrous oxide has low anesthetic potency, and unless administered under hyperbaric conditions, nitrous oxide cannot cause gross motor impairment that might perturb detection of antinociception in the hot plate test. Previously, we demonstrated that nitrous oxide-induced antinociception is mediated by central μ- and ε-opioid receptors because it was antagonized by D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 and β-EP_{1-27}, respectively, but not by the δ-opioid antagonist naltrindole or the κ-opioid antagonist norbinaltorphimine (16).

The pharmacological application of antisera takes advantage of their high selectivity for target substances. It has been demonstrated that EOP antisera injected intrathecally or into the periaqueductal gray can modify opioid peptide-induced as well as acupuncture-induced antinociception in rodents (5,12, 13,27). In the present study, we demonstrated that ICV injection of β-EP antiserum antagonized nitrous oxide antinociception in a dose-related manner in rats. It is unlikely that the antagonism of nitrous oxide antinociception by β-EP antiserum is due to nonspecific effects of the antiserum (e.g., nonspecific binding to substances other than β-EP) because ME antiserum, which was prepared by the same method as the β-EP antiserum and given at the same doses, did not exhibit significant effects on the antinociception. In addition, it seemed that neither ICV injection of β-EP nor ME antiserum changed the pain threshold, although injection of DYN A_{1-13} antiserum per se into the rat brain stem caused analgesia (11). Thus, our findings suggest an important role of β-EP in nitrous oxide antinociception. These findings are consistent with the earlier observations that exposure of rats to nitrous oxide increased the β-EP level in several brain regions (24,31). Recently, we have also reported that β-EP_{1-27}, a putative competitive inhibitor of β-EP, partially antagonized nitrous oxide antinociception (16). However, exposure of dogs (8) and human subjects (28) to nitrous oxide did not increase the β-EP level in CSF. This discrepancy might be due to species differences. Another possible explanation may be that the concentration of β-EP released from neural sites into the CSF was so low that it was undetectable.

It is likely that nitrous oxide antinociception involves some mechanism beyond the neuronal release of β-EP, because β-EP antiserum at 400 μg/rat did not cause a further antagonism of nitrous oxide antinociception. In this respect, we examined the effect of ME antiserum on nitrous oxide antinociception, because previous studies have reported significant increases in immunoreactive ME levels in the CSF of centrally perfused rats (20) as well as in several brain regions of rats exposed to nitrous oxide (21,24,25). In addition, a similar effect of nitrous oxide on ME has been reported in dogs (8). However, in the present study, ICV injection of ME antiserum had no significant effect on nitrous oxide antinociception in rats. Thus, the role of ME in the actions of nitrous oxide remains to be determined.

The interaction of endogenous opioid systems with nitrous oxide may not be unique to this drug. There is convincing evidence that opioid mechanisms mediate the analgesic component of general anesthesia though not the anesthesia itself. Narcotic antagonists can reverse the analgesic effect of nitrous oxide and other general anesthetic agents, such as cyclopropane, halothane, and enflurane (7), but not general anesthesia itself (1,15,17,18,26).

ACKNOWLEDGEMENTS

This study was supported by NIH Grant DE-06894. We thank Dr. L.-F. Tseng (Medical College of Wisconsin, Milwaukee, WI) for his generous gifts of β-EP and ME antisera.

REFERENCES

1. Bennett, P. B. Naloxone fails to antagonize the righting response in rats anesthetized with halothane. Anesthesiology 49:9–11; 1978.
2. Berkowitz, B. A.; Finck, A. D.; Ngai, S. H. Nitrous oxide analgesia: Reversal by naloxone and development of tolerance. J. Pharmacol. Exp. Ther. 203:539–547; 1977.
3. Carter, R. B. Differentiating analgesic and non-analgesic drug activities on rat hot plate: Effect of behavioral endpoint. Pain 47: 211–220; 1991.
4. Chapman, C. R.; Benedetti, C. Nitrous oxide effects on cerebral evoked potential to pain: Partial reversal with a narcotic antagonist. Anesthesiology 51:135–138; 1979.
5. Chengmin, H. E.; Han, J. Attenuation of low- rather than high-frequency electroacupuncture analgesia following microinjection of β-endorphin antiserum into the periaqueductal gray in rats. Acupunct.: Sci. Int. J. 1:94–99; 1990.
6. Fennessy, M. R.; Lee, J. R. The assessment of and the problems involved in the experimental evaluation of narcotic analgesics. In: Ehrenpreis, S.; Neidle, A., eds. Methods in narcotics research. New York: Marcel Dekker, Inc.; 1975:73–99.
7. Finck, A. D.; Ngai, S. H.; Berkowitz, B. A. Antagonism of general anesthesia by naloxone in the rat. Anesthesiology 46:241–245; 1977.
8. Finck, A. D.; Samaniego, E.; Ngai, S. H. Nitrous oxide selectively releases met(5)-enkephalin into canine third ventricular cerebrospinal fluid. Anesth. Analg. 70:S103; 1990.
9. Gillespie, T.; Simonds, J. B.; Quock, R. M. Potentiation of nitrous oxide (N_2O) antinociception by the endopeptidase 24.11-inhibitor phosphoramidon (PHOS) in the rat hot plate paradigm. FASEB J. 7:A488; 1993.
10. Gillman, M. A.; Kok, L.; Lichtigfeld, F. J. Paradoxical effects of naloxone on nitrous oxide analgesia in man. Eur. J. Pharmacol. 61:175–177; 1980.
11. Hamann, S. R.; Martin, W. R. Analgesic actions of dynorphin A (1–13) antiserum in the rat brain stem. Brain Res. Bull. 29:605–607; 1992.
12. Han, J. S.; Xie, G. X. Dynorphin: Important mediator for electroacupuncture analgesia in the spinal cord of the rabbit. Pain 18: 367–376; 1984.
13. Han, J. S.; Xie, G. X.; Zhou, Z. F.; Folkesson, R.; Terenius, L. Enkephalin and β-endorphin as mediators of electroacupuncture analgesia in rabbits: An antiserum microinjection study. In: Costa, E.; Trabucchi, M., eds. Regulatory peptides: From molecular biology to function. New York: Raven Press; 1982: 369–377.
14. Hara, S.; Gagnon, M. J.; Quock, R. M.; Shibuya, T. Effect of

intracerebroventricular injection of antisera against β-endorphin and methionine-enkephalin on the antinociceptive effect of nitrous oxide in rats. Abst. Int. Behav. Neurosci. Soc. 2:45; 1993.
15. Harper, M. H.; Winter, P. M.; Johnson, B.; Eger, E. I., II. Naloxone does not antagonize general anesthesia in the rat. Anesthesiology 49:3–5; 1978.
16. Hodges, B. L.; Gillespie, T. R.; Breneisen, J. R.; Quock, R. M. Antagonism of nitrous oxide antinociception in rats by centrally administered D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 and β-endorphin$_{1-27}$. FASEB J. 7:A488; 1993.
17. Lawrence, D.; Livingston, A. Opiate-like analgesic activity in general anesthetics. Br. J. Pharmacol. 73:435–442; 1981.
18. Pace, N. L.; Wong, K. C. Failure of naloxone and naltrexone to antagonize halothane anesthesia in the dog. Anesth. Analg. 58: 36–39; 1981.
19. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
20. Quock, R. M.; Kouchich, F. J.; Tseng, L.-F. Does nitrous oxide induce release of brain opioid peptides? Pharmacology 30:95–99; 1985.
21. Quock, R. M.; Kouchich, F. J.; Tseng, L.-F. Influence of nitrous oxide upon regional brain levels of methionine-enkephalin-like immunoreactivity in rats. Brain Res. Bull. 16:321–323; 1986.
22. Quock, R. M.; Graczak, L. M. Influence of narcotic antagonist drugs upon nitrous oxide analgesia in mice. Brain Res. 440:35–41; 1988.
23. Quock, R. M.; Walczak, C. K.; Henry, R. J.; Chen, D. C. Effect of subtype-selective opioid receptor blockers on nitrous oxide antinociception in rats. Pharmacol. Res. 22:351–357; 1990.
24. Silverstein, W.; Samaniego, E.; Finck, A. D. Nitrous oxide increases opioid peptide concentrations in select rat brain areas. Anesthesiology 77:A731; 1992.
25. Silverstein, W.; Samaniego, E.; Finck, A. D. Nitrous oxide increases Met^5-enkephalin concentrations in rat brain corpus striatum. Anesth. Analg. 74:S294; 1992.
26. Smith, R. A.; Wilson, M.; Miller, K. W. Naloxone has no effect on nitrous oxide anesthesia. Anesthesiology 49:6–8; 1978.
27. Tseng, L. L. F.; Suh, H. H. Intrathecal [Met^5]enkephalin antibody blocks analgesia induced by intracerebroventricular β-endorphin but not morphine in mice. Eur. J. Pharmacol. 173: 171–176; 1989.
28. Way, W. L.; Hosobuchi, Y.; Eger, E. I.; Johnson, B. H. Anesthesia does not increase cerebrospinal fluid opioid peptides in humans. Anesth. Analg. 61:223; 1982.
29. Yang, J. C.; Clark, W. C.; Ngai, S. H. Antagonism of nitrous oxide analgesia by naloxone in man. Anesthesiology 52:414–417; 1980.
30. Zuniga, J. R.; Joseph, S. A.; Knigge, K. M. Nitrous oxide analgesia: Partial antagonism by naloxone and total reversal after periaqueductal gray lesions in the rat. Eur. J. Pharmacol. 142:51–60; 1987.
31. Zuniga, J. R.; Joseph, S. A.; Knigge, K. M. The effects of nitrous oxide on the central endogenous pro-opiomelanocortin system in the rat. Brain Res. 420:57–65; 1987.
32. Zuniga, J. R.; Joseph, S. A.; Knigge, K. M. The effects of nitrous oxide on the secretory activity of pro-opiomelanocortin peptides from basal hypothalamic cells attached to cytodex beads in a superfusion in vitro system. Brain Res. 420:66–72; 1987.