DECREASE OF β-ENDORPHIN IN THE BRAIN OF RATS FOLLOWING NITROUS OXIDE WITHDRAWAL

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SUMMARY

β-Endorphin levels in the whole rat brain were not changed during acute (25 min) or chronic (48 h) exposure of rats to N_2O. However, a significant decrease of β-endorphin was found in the whole brain, brain stem and subcortex during the withdrawal from chronic exposure to N_2O. It has been suggested that decrease of β-endorphin levels during N_2O withdrawal could be ascribed to unspecific stress accompanying drug withdrawal.

Decrease of central β-endorphin during N_2O withdrawal might have a significant modulatory effect on transmitter balance, neuronal excitability and corresponding withdrawal behaviour. Furthermore, the decrease of β-endorphin levels in the whole brain during N_2O withdrawal might contribute to the postanaesthesia N_2O-excitatory syndrome in humans. This might explain the known therapeutic effect of the opioid drug, meperidine on the excitatory N_2O withdrawal phenomena during recovery from N_2O anaesthesia in man.

KEY WORDS

behaviour, brain, brain stem, cortex, β-endorphin, nitrous oxide, radioimmunnoassay, subcortex, withdrawal.

INTRODUCTION

Nitrous oxide (N_2O) in subanaesthetic concentrations is an analgesic agent in man and animals. This analgesic effect of N_2O can be reversed by opioid antagonists /1-5/. Further studies in rats have indicated that N_2O may cause elevation of β-endorphin concentrations in the cells of regions associated with antinociception /6/. Moreover, evidence has been brought forward that 60% N_2O, but not 30%, caused a significant increase in release of β-endorphin from cultured basal hypothalamic cells /7/.

It has also been found in the rat that N_2O causes an increase of met-enkephalin in the brain and cerebrospinal fluid (CSF) /8,9/, but not in the brain areas associated with antinociception /10/. Similarly, neither plasma β-endorphin /11/ nor CSF-β-endorphin levels were altered in humans breathing N_2O /12/.

However, there are indications that in heroin and/or cocaine dependent rats β-endorphin concentrations decrease, particularly in the limbic area /13/. The fact that excitatory N_2O withdrawal phenomena during recovery from anaesthesia can be therapeutically treated by the morphine-like drug meperidine prompted us to determine the central β-endorphin levels in the whole brain, brain stem, cortex and subcortex of the rat during acute and chronic exposure to N_2O in order to compare them with normal β-endorphin concentrations.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (weighing 200-250 g) were housed singly in a temperature and light-controlled room and fed *ad libitum*. The experiments were performed between 10.00 and 15.00 h to avoid diurnal influences /14/.

Exposure to N_2O

Five groups of animals (n=9) were used. Four groups of animals, exposed to balanced concentrations of N_2O (70% N_2O and 30% O_2), were placed into a sealed anaesthetic chamber as described previously /15/. A circuit system of gases in the chamber maintained stable temperature and gas mixtures, while the waste gases were eliminated continuously. Two groups of animals were exposed to N_2O for 25 min (acute exposure), while two other

groups were exposed to N_2O for 48 hours (chronic exposure). The animals were decapitated immediately or 30 min after an acute or chronic exposure to N_2O.

Determination of central β-endorphin

β-Endorphin levels were determined in the brain stem, cortex, subcortex and whole brain in various experimental conditions. The experimental conditions of the animals were as follows: 1. breathing air; 2. breathing N_2O; 3. breathing air (30 min) following 25 min exposure to N_2O; 4. breathing N_2O (48 h), and 5. breathing air (30 min) following 48 h exposure to N_2O. The last experimental condition was selected, since the preliminary results indicated that the most pronounced withdrawal symptoms appear after discontinuation of 48 h exposure to N_2O.

β-Endorphin levels were determined in accordance with the standard procedure. Immediately, the brain stem, cortex and subcortex were dissected. Brain stem and cortex were dissected in order to separate the subcortical limbic region in which the alterations of β-endorphin concentrations after chronic treatment with heroin or cocaine /13/ have been observed. The intact tissues were each placed in 2.5 ml 1N acetic acid, which had been warmed to 98°C in a water-bath. After 15 min, the tissues were chilled on ice and homogenized. The supernatant was obtained by centrifugation (1000 *g* for 1 hour), frozen overnight, then thawed and adjusted to pH 7.5 with 1N NaOH and supplemented with 0.2 M NaH_2PO_4. The supernatant was again frozen overnight, thawed and centrifuged (1000 *g* for 1 hour). The clear fraction was processed in the β-endorphin radioimmunoassay (RIA) kit with 100% crossreactivity for the rat (Milab, A-40; Malmö, Sweden). The protein extracts were measured from aliquots of homogenates, as previously described /16/. The sensitivity of the RIA was 8 pg. Crossreactivity studies indicate that the antibody does not bind.

Behaviour

The behaviour of the animals was monitored during the last 30 min of chronic (48 h) exposure to gas and 30 min after withdrawal of N_2O. The incidence of the following signs was counted: grooming, "wet-dog shakes", scratching and rearing (leaping onto the edge of the box with two feet). The presence of diarrhoea was checked.

Statistics

The evaluation of data was performed with analysis of variance (ANOVA) and the two tailed Student's t-test in order to compare the two means. P-values are significant when $p < 0.05$.

RESULTS

β-Endorphin concentrations

β-Endorphin levels remained unchanged or with no significant oscillations in the whole brain, brain stem, cortex and subcortex during acute (25 min) or chronic (48 h) exposure of rats to N_2O. However, a significant decrease of β-endorphin levels was found during the withdrawal from this anaesthetic gas. A decrease of β-endorphin levels was found in the brain stem, subcortex and the whole brain, while in the cortex of the rat brain no changes of β-endorphin levels were detected during the withdrawal from N_2O (Table 1).

TABLE 1

Amount of immunoreactive β-endorphin-like materials (expressed in ng/mg protein) in various parts of the rat brain and various experimental conditions

Brain area	A	B	C	D	E
Brain stem	0.62 ± 0.03	0.67 ± 0.07	0.63 ± 0.04	0.61 ± 0.04	0.57 ± 0.05*
Cortex	0.31 ± 0.04	0.35 ± 0.02	0.34 ± 0.05	0.35 ± 0.02	0.34 ± 0.02
Subcortex	0.54 ± 0.02	0.52 ± 0.03	0.53 ± 0.02	0.51 ± 0.02	0.43 ± 0.06*
Whole brain	0.45 ± 0.02	0.45 ± 0.02	0.46 ± 0.06	0.43 ± 0.02	0.40 ± 0.01*

A. Breathing of air without previous exposure to N_2O (control).
B. Breathing of N_2O (25 min).
C. Breathing of air (30 min) following 25 min exposure to N_2O.
D. Breathing of N_2O (48 h).
E. Breathing of air (30 min) following 48 h exposure to N_2O.

The amount of immunoreactive β-endorphin-like material was determined immediately at the end of the exposure to N_2O (B and D) or 30 min after N_2O withdrawal (C and E). Results shown are mean ± SEM. All groups: n=9. *Significant difference compared to the control ($p < 0.05$).

Behaviour

Behaviourally, the rats appeared over-excited, manifested by increased motility, during the first 25 min of exposure to N_2O (acute exposure). For the five to seven following hours, they appeared anaesthetized, sporadically attempting a sluggish movement. After twelve hours of exposure to N_2O, all rats behaved normally, fed themselves and resumed their sleep pattern (tolerance to N_2O). However, after N_2O withdrawal following chronic exposure to N_2O (48 h), the rats became over-excited and difficult to manage. Wet-dog shakes and other withdrawal signs, such as grooming, scratching, diarrhoea, but not rearing, were significantly increased (Table 2). Such withdrawal reactions were absent or mild (statistically non-significant) following a shorter exposure to N_2O (25 min, 6 h or 12 h [not shown]).

TABLE 2

The behavioural N_2O withdrawal signs in rats

Withdrawal signs	A	B	C
Grooming	3.8 ± 0.5	3.4 ± 0.3	7.3 ± 0.6*
Wet-dog shakes	0.9 ± 0.1	0.4 ± 0.3	4.6 ± 0.8*
Scratching	0.3 ± 0.1	0.6 ± 0.2	2.9 ± 0.6*
Rearing	7.2 ± 1.4	8.6 ± 1.2	9.1 ± 0.9
Diarrhoea	0 from 11	0 from 9	8 from 12*

A. Breathing of air without previous exposure to N_2O (control, n=11).
B. Breathing of N_2O during the last 30 min of chronic (48 h) exposure to N_2O (n=9).
C. Breathing of air (30 min) after a withdrawal of chronic (48 h) exposure to N_2O (n=12).

The behavioural withdrawal signs in rats (mean ± SEM) were observed during the last 30 min of chronic exposure to gas (48 h, B) and the first 30 min immediately after N_2O withdrawal (48 h, C). These symptoms were compared with similar signs, occurring during 30 min in the normal rats, not exposed to N_2O (A).
*Significant difference compared to the control ($p < 0.05$).
The presence of diarrhoea was checked, while the incidence of other withdrawal signs was counted.

DISCUSSION

In this study, no changes of β-endorphin levels were found in the whole brain during acute or chronic exposure of rats to N_2O. This is in accordance with data that breathing of 70% N_2O in oxygen had no significant effect on whole rat brain β-endorphin concentrations /17/. Similarly, no alteration of β-endorphin levels has been found in the CSF in humans breathing N_2O /12/. These results might support the idea that the analgesic effect of N_2O /1,2/ is due to its interactions with the opioid receptors rather than to the release of endogenous opioid peptides /3,18/. However, the question still remains, since the local changes of β-endorphin and enkephalins in different brain areas during exposure to N_2O, particularly those involved in pain transmission, have not been extensively investigated.

A decrease of β-endorphin was found in this study during the withdrawal of N_2O. A possibility that prolonged exposure to N_2O and oxygen may produce various nitrous and nitric oxide derivatives which may react with different proteins and interfere with the radioimmunoassay seems unlikely. There were no differences in the β-endorphin concentrations between control animals (breathing air) and those exposed to N_2O for 25 min. In addition, the animals decapitated immediately after 48 hours exposure also showed no alterations in β-endorphin concentrations. Therefore a decrease of β-endorphin concentrations is not an artefact but a phenomenon associated with N_2O withdrawal. However, it could be a specific phenomenon of N_2O drug dependence or unspecific common factors accompanying other drug withdrawals as well. The fact that a similar decrease of β-endorphin levels in the rat brain and human CSF has been observed during alcohol withdrawal /19, 20/ and naloxone precipitated withdrawal in morphine dependent rats /21/ might indicate that this is a rather non-specific phenomenon. In addition, several authors have observed a release of brain endorphin with various stressors /22,23/ which increased β-endorphin in rat blood, but not in the brain /23/. Drug withdrawal is also a stress factor which might be associated with increased release of β-endorphin followed by an exhaustion of the β-endorphin pool due to insufficient time for a compensatory increase in synthesis of opioids in order to replace the acute deficiency. All these might indicate that decrease of β-endorphin during N_2O withdrawal is a general reflection of unspecific (stress) factor(s) accompanying various withdrawals, rather than a phenomenon specifically related to the N_2O withdrawal.

One of the additional questions is whether a decrease of β-endorphin during N_2O withdrawal has any correlation to the withdrawal symptoms, particularly withdrawal excitability. It is known that abrupt termination of chronically used sedative drugs, such as alcohol, barbiturates, benzodiazepines /24/ and opiates /25/, may induce a seizure in man. Similarly, withdrawal of some chronically used drugs, such as ethanol /19/ or morphine /26/, may induce in animals spontaneous convulsions or epileptic electrocorticographic activity. Handling-induced withdrawal convulsions were observed in mice following withdrawal of alcohol /27/, diazepam /28/ or N_2O /29,30/. The cause of withdrawal excitability after exposure to N_2O or other drugs is not quite clear, but might be due, at least partially, to both the release of β-endorphin or to the decrease of central pools of β-endorphin. Specifically, it has been found that central administration of β-endorphin in rats can induce a generalized convulsion /31/. Similarly, a decrease of β-endorphin may cause an imbalance in other transmitter systems by facilitating the release of various excitatory neurotransmitters. It is known that opioid peptides exert a tonic inhibitory control on the release of various neurotransmitters /32/. Thus, a deficit of β-endorphin in the brain during withdrawal may profoundly increase neural excitability by affecting transmitter release. In this respect, it is of importance to note that administration of peptidase inhibitors or other enkephalinase inhibitors which inhibit the biodegradation of endogenous opioid peptides, may attenuate the EEG seizure phenomena in the rat /26/ or handling-induced N_2O withdrawal convulsions /33/.

Similarly, it is known that some excitatory post-anaesthesia phenomena, such as behavioural and motor restlessness, very much alike to N_2O withdrawal /34/, as well as cardiovascular hyperactivity /35/, occur during recovery from N_2O anaesthesia in man. The notion that maladaptation of the organism to the withdrawal of N_2O is due to the decreased β-endorphin can be substantiated by the fact that morphine-like meperidine can be used effectively for the treatment of the postanaesthetic excitation phenomena in man /36,37/. It seems that treatment of excitatory N_2O-withdrawal phenomena during recovery from anaesthesia with meperidine would now appear to have a rational biochemical background.

Evidently all these data might indicate that decrease of central β-endorphin levels have a significant pro-excitatory effect during N_2O withdrawal and perhaps other abstinential syndromes. However, it is less clear whether some withdrawal symptoms depend

specifically on the decrease of β-endorphin in one particular brain region. It appears that dependence on morphine can develop in many loci within the brain. For example, escape response (rearing) and wet-dog shakes, both associated with movements, depend mainly on the thalamus, diencephalic-mesencephalic connections and periaquaductal grey, but not on frontal cortex /38/. Evidently our experiments cannot clarify the role of one specific brain area in the initiation of the particular withdrawal sign(s).

An additional aspect is a significant increase of diarrhoea during N_2O withdrawal. In the past few decades, it has been established that opioids can affect gastrointestinal transit, both by direct actions on the wall of the bowel and by actions in the CNS. For example, opioid agonists given intracerebroventricularly or intrathecally decreased gastrointestinal transit, motility, secretion and experimentally induced diarrhoea /39/. These data may suggest that decrease of endogenous opioid peptide in the central nervous system may, at least partially, contribute to the morphine withdrawal diarrhoea.

CONCLUSIONS

It can be concluded that acute or chronic exposure to N_2O does not affect the concentration of central β-endorphin in rats. However, when chronic exposure (48 h) to N_2O was ended, a decrease of β-endorphin in the whole brain, brain stem and subcortex occurred, while in the cortex no changes of β-endorphin were detected. It has been suggested that decrease of central β-endorphin during N_2O withdrawal might have a significant modulatory effect on transmitter balance, neuronal excitability in the central nervous system and corresponding withdrawal behaviour.

REFERENCES

1. Berkowitz BA, Ngai SH, Finck AD. Nitrous oxide "analgesia": resemblance to opiate action. Science 1976; 194: 967-968.
2. Berkowitz BA, Finck AD, Ngai SH. Nitrous oxide analgesia: reversal by naloxone and development of tolerance. J Pharmacol Exp Ther 1977; 203: 539-547.
3. Gillman MA, Lichtigfeld FJ. Nitrous oxide interacts with opioid receptors: more evidence. Anesthesiology 1983; 58: 483-484.
4. Lawrence D, Livingstone A. Opiate-like analgesic activity in general

anaesthetics. Br J Pharmacol 1981; 73: 435-442.
5. Yang JC, Clark WC, Ngai SH. Antagonism of nitrous oxide analgesia by naloxone in man. Anesthesiology 1980; 52: 414-417.
6. Zuniga JR, Joseph SA, Knigge KM. The effect of nitrous oxide on the central endogenous pro-opiomelanocortin system in the rat. Brain Res 1987; 420: 57-65.
7. Zuniga JR, Joseph SA, Knigge KM. The effect of nitrous oxide on the secretory activity of pro-opiomelanocortin peptides from basal hypothalamic cells attached to cytodex beads in a perfusion *in vitro* system. Brain Res 1987; 420: 66-72.
8. Quock RM, Kouchich FJ, Tseng LF. Does nitrous oxide induce release of brain opioid peptides? Pharmacology 1985; 30: 95-99.
9. Quock RM, Kouchich FJ, Tseng LF. Influence of nitrous oxide upon regional brain levels of methionine-enkephalin-like immunoreactivity in rats. Brain Res Bull 1986; 16: 321-323.
10. Morris B, Livingstone A. Effects of nitrous oxide exposure on Met-enkephalin levels in discrete areas of rat brain. Neurosci Lett 1984; 45: 11-14.
11. Evans SF, Stringer M, Bukht MDG, Thomas WA, Tomlin SJ. Nitrous oxide inhalation does not influence plasma concentrations of β-endorphin or Met-enkephalin-like immunoreactivity. Br J Anaesth 1985; 57: 624-628.
12. Way WL, Hosobuchi Y, Johnson BH, Eger EII, Bloom FE. Anaesthesia does not increase opioid peptides in cerebrospinal fluid in human. Anesthesiology 1984; 60: 43-45.
13. Sweep CGJ, Wiegant VM, De Vry J, Van Ree JM. β-Endorphin in brain limbic structures as neurochemical correlates of psychic dependence on drug. Life Sci 1989; 44: 1133-1140.
14. Frederickson RCA, Burgis V, Edwards JD. Hyperalgesia induced by naloxone follows diurnal rhythm in responsitivity to painful stimuli. Science 1977; 198: 756-758.
15. Rupreht J, Dzoljic MR. Elimination of irritating gaseous compounds during prolonged exposures to gases. J Pharmacol Methods 1982; 8: 109-114.
16. Lowry OH, Rosenbrough NJ, Farr AL, Randal RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193; 265-275.
17. Williard KF, Gillmor ST, Stanley TH, Meuleman JR, Pace NL. The influence of nitrous oxide and nociceptive stimuli on rat plasma and brain endorphin concentrations. Anesthesiology 1982; 57(3): A 302.
18. Gillman MA, Lichtigfeld FJ. Analgesic nitrous oxide in neuropsychiatry: past, present and future. Int J Neurosci 1989; 49: 75-81.
19. Hutchison WD, Gianoulakis C, Kalant H. Effects of ethanol withdrawal on β-endorphin levels in rat brain and pituitary. Pharmacol Biochem Behav 1988; 30: 933-939.
20. Genazzani AR, Nappi G, Facchinetti F, Mazella GL, Parrini D, Sinforiani E, Petraglia F, Savoldi F. Central deficiency of β-endorphin in alcohol addicts. J Clin Metab 1982; 55: 583-586.
21. Herz A, Hollt V, Przewlocki R. Endogenous opioids and addiction. In: Wuttke W, Weindl VA, Wolt KH, de Dries RR (eds), Brain and Pituitary Peptides. Basel: S. Karger, 1980; 183-189.
22. Millan MJ, Przewlocki R, Jerlicz M, Gramsch C, Hollt V, Herz A. Stress-

induced release of brain and pituitary β-endorphin: Major role of endorphins in generation of hyperthermia, not analgesia. Brain Res 1981; 208: 325-338.
23. Rossier I, French E, Rivier C, Ling W, Guillemin R, Bloom F. Foot-shock induced stress increases β-endorphin in rat blood but not brain. Nature 1977; 270: 618-620.
24. Griffiths RR, Sannerud CA. Abuse of and dependence on benzodiazepines and other anxiolytic/sedative drugs. In: Meltzer HJ (ed), Psychopharmacology. New York: Raven Press, 1987; 1535-1541.
25. Herzlinger RA, Randall SA, Vaughan HG. Neonatal seizures with narcotic withdrawal. J Pediatr 1977; 91: 638-641.
26. Pinsky C, Dua AK, La Bella FS. Peptidase inhibitors reduce opiate narcotic withdrawal signs, including seizure activity in the rat. Brain Res 1982; 243: 301-307.
27. Goldstein DB, Pal N. Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. Science 1971; 172: 288-290.
28. Belknap JK, Crabbe JC, Laursen SE. Ethanol and diazepam withdrawal convulsions are extensively codetermined in WSP and WSR mice. Life Sci 1989; 44: 2075-2080.
29. Smith RA, Winter PM, Smith M. Convulsions in mice after anaesthesia. Anesthesiology 1979; 50: 501-504.
30. Harper MH, Winter PM, Johnson BH, Roblin OB, Eger EI. Withdrawal convulsions in mice following nitrous oxide. Anesth Analg 1980; 59: 19-21.
31. Cain DP, Corcoran ME. Intracerebral β-endorphin, met-enkephalin and morphine: kindling of seizures and handling-induced potentiation of epileptiform effects. Life Sci 1984; 34: 2535-2542.
32. North RA, Williams JT. How do opiates inhibit neurotransmitter release? Trends Neurosci 1983; 6: 337-339.
33. Dzoljic MR, Ukponmwan OE, Baas AAG, van den Berg DJ, Rupreht J, Dzoljic MM. Suppressive effects of enkephalins and REM sleep deprivation on seizure. In: Degen R, Rodin EA (eds), Epilepsy: Sleep and Deprivation, 2nd edition. Amsterdam: Elsevier Scientific Publications, 1991; 275-280.
34. Tammisto T, Tigerstedt I. Restlessness and shivering after naloxone reversal of fentanyl supplemented anaesthesia. Acta Anaesthesiol Scand 1979; 23: 51-56.
35. Karliczek GF, Birks RJS, Brenken U. Cessation of nitrous oxide: a possible risk after open heart surgery. Thorac Cardiovasc Surgeon 1980; 28: 120-127.
36. Claybon LE, Hirsh RA. Meperidine arrests postanesthetic shivering. Anesthesiology 1980; 53: S 180.
37. Pauca AL, Savage RT, Simpson S, Roy RC. Effect of pethidine, fentanyl and morphine on post-operative shivering in man. Acta Anaesthesiol Scand 1984; 28: 138-143.
38. Wei E, Loh H. Physical dependence on opiate-like peptides. Science 1976; 193: 1262-1263.
39. Burks TF, Fox DA, Hirning LO, Shook JE, Poreka F. Regulation of gastrointestinal function by multiple opioid receptors. Life Sci 1988; 43: 2177-2181.