Anesthesiology
58:524-526, 1983

The Involvement of the Central Cholinergic and Endorphinergic Systems in the Nitrous Oxide Withdrawal Syndrome in Mice

J. Rupreht, M.D.,* B. Dworacek, M.D.,* R. Ducardus,† P. I. M. Schmitz, Ph.D.,‡ M. R. Dzoljic, M.D., Ph.D.§

The nitrous oxide withdrawal syndrome in mice was used as an experimental model to examine some of the factors which may play a role in postanesthetic excitation. Predisposition to nitrous oxide withdrawal convulsions as judged by duration of susceptibility was decreased significantly after pretreatment with the cholinesterase inhibitors, physostigmine and galanthamine, or with the opiate receptor blocking agent naloxone. Results are discussed in relation to the central anticholinergic syndrome, endorphin release, and disturbances which follow nitrous oxide anesthesia in humans and animals. (Key words: Anesthetics, gases: nitrous oxide. Antagonists, miscellaneous: galanthamine; physostigmine. Antagonists, narcotic: naloxone. Brain: acetylcholine; endorphins. Enzymes: cholinesterase. Recovery: delirium.)

PATIENTS DEMONSTRATING EMERGENCE DELIRIUM after general anesthesia have been treated successfully with physostigmine salicylate.[1] This type of delirium is a part of the very complex clinical picture described by Longo[2] as the central anticholinergic syndrome (CAS).

Considering the possible factors promoting this syndrome in our patients, we suspected that nitrous oxide might have a significant facilitatory role in the etiology of CAS.[3,4] An animal model for study of the factors involved in CAS is not yet established. However, an excitatory syndrome in mice after exposure to nitrous oxide (and other anesthetics) has been described.[5,6] This syndrome is characterized by signs of excitation such as grimacing, violent jerking, twirling, convulsions, etc., and is elicited by gently lifting the mouse by the tip of the tail. Smith *et al.*,[7] suggested that the same pathophysiologic mechanisms involved in this withdrawal syndrome might contribute to the occurrence of emergence delirium following anesthesia in humans.

In this study, we used the nitrous oxide withdrawal syndrome in mice as an experimental model to examine certain factors which may play a role in postanesthetic excitation. Specifically, we examined the role of the cholinergic and endorphinergic systems. These transmitter/modulator systems were considered mainly because of the therapeutic value of physostigmine in the treatment of CAS, and because of recent evidence suggesting a role for enkephalins in certain postanesthetic phenomena.[8]

Methods

Male mice, F_1 R_y breed, 25 ± 4 g, were used. Mice of this breed were chosen because we could reliably provoke nitrous oxide withdrawal convulsions in them. The experiment involved exposure of mice to a mixture of 1.4 atm nitrous oxide, and 0.6 atm oxygen for a period of 60 min. Each mouse was used only once. None displayed convulsions prior to the anesthetic exposure. The pressure chamber used, was a 500-ml glass bell with an attachment for fresh gases, a manometer, and an overflow valve. Soda lime was placed in the bell to ensure the elimination of CO_2 (monitored by an infrared analyzer). The gas temperature in the bell was held at 22° C with the aid of a waterbath in which the bell was immersed. The mice were placed one at a time into the chamber for exposure to the gas mixture. After 60 min, the pressure was reduced to ambient pressure over a period of one minute. Then, each mouse was lifted gently from the bell and tested for withdrawal convulsions every five minutes until no convulsions were observed for two consecutive tests. The convulsive movements consisted of twirling and jerking with extension and flexion of the legs. During testing, the mice were lifted and held by the tail for up to ten seconds. The test was considered negative if convulsions did not occur within this time. The rectal temperature of mice at the end of the exposure was always above 34° C (34.5 ± 0.4) and was not influenced by the drugs administered. Mice in the control group showed the same decrease in rectal temperature without detectable changes in behavior. All experiments were performed between 0930 and 1200 h by a technician who was unaware of the drug treatment. Each experimental group consisted of 12 mice.

DRUGS

The following commercially available drugs were used: physostigmine salicylate, galanthamine hydrobro-

* Staff Anesthesiologist, Institute of Anesthesiology, University Hospital Dijkzigt, Erasmus University Rotterdam.

† Research Assistant, Institute of Anesthesiology, University Hospital Dijkzigt, Erasmus University Rotterdam.

‡ Biostatistician, Institute of Biostatistics, Erasmus University.

§ Neuropharmacologist, Department of Pharmacology, Medical School, Erasmus University.

Received from the Institute of Anesthesiology, University Hospital Dijkzigt, Erasmus Hospital Rotterdam, The Netherlands, and the Institute of Biostatistics and the Department of Pharmacology, Medical School, Erasmus University, The Netherlands. Accepted for publication November 23, 1982.

Address reprint requests; to Dr. Dzoljic: Department of Pharmacology, Erasmus University Rotterdam, Medical School, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

0003-3022/83/0600/0524 $00.95

mide, and naloxone hydrochloride. Drugs were dissolved in 0.9% NaCl and were injected intramuscularly either 1 min before, or 1 min after exposure of mice to the gas mixture. The drugs dosages were expressed in the terms of their salts, and were administered in a volume of 0.05 ml.

Statistical Evaluation

In order to test whether the mean duration of predisposition to convulsions in the four pretreated groups and that in the non-pretreated group were equal, a one-way analysis of variance (ANOVA) was performed. Comparisons between each of the four pretreated groups separately with the non-pretreated group are based on Tukey's 95% confidence intervals for contrasts. The same analysis was performed for the four groups in which the agent was administered after exposure to N_2O and for the group which was not treated after the exposure.

Results

Overall Comparisons

Among the groups which received saline, physostigmine, galanthamine, naloxone, or no agent, before exposure to N_2O, a highly significant difference in duration of predisposition to convulsions was seen ($F\ 4, 55 = 38.9, P < 0.0001$). The analogous overall comparison among the groups which received an agent after exposure to N_2O and the one which received none, also reveals a highly significant difference in duration of predisposition to convulsions ($F\ 4, 55 = 42.8, P < 0.0001$).

Control

None of the mice displayed a convulsion prior to the anesthetic exposure if lifted and held by the tail for up to ten seconds. However, all mice subjected to this procedure demonstrated a convulsive pattern during the withdrawal period which followed an exposure to nitrous oxide for 60 min. The average time period during withdrawal in which the convulsions could be elicited in the nontreated mice was 64.6 min (table 1). For saline-treated mice given either before exposure to nitrous oxide or after it was discontinued, the periods of susceptibility were 52.1 and 61.2 min, respectively. These times did not differ significantly from that observed in untreated mice.

Physostigmine

In the group of mice pretreated with physostigmine (0.4 μg/g) the period of susceptibility during withdrawal was decreased significantly (17.5 min). A similar significant decrease was observed when the same dose of physostigmine was given in the first minute after nitrous oxide was discontinued (23.7 min).

TABLE 1. The Influence of Drugs on the Duration of Predisposition to N_2O-Withdrawal Convulsions (min; Mean ± SD)

Agent	Agent Administered before Exposure to N_2O	Agent Administered after Exposure to N_2O
None	64.6 ± 16.8	64.6 ± 16.8
Saline (50 μl/g)	52.1 ± 8.9	61.2 ± 6.8
Physostigmine (0.04 μg/g)	17.5 ± 6.2*	23.7 ± 3.1*
Galanthamine (50 μg/g)	33.3 ± 4.4*	67.5 ± 12.5
Naloxone (0.8 μg/g)	23.4 ± 13.4*	72.9 ± 6.6

* Denotes significant difference compared with the nontreated group, as follows from Tukey's 95% confidence intervals (n = 12, all groups). All drugs were administered intramuscularly.

Galanthamine

This alkaloid (5 μg/g), which has anticholinesterase properties and which easily penetrates the blood-brain barrier, significantly decreased the period of time in which the seizure phenomena could be elicited (33.3 min) when administered before the animals were exposed to the gas mixture. The same dose of galanthamine administered in the first minute of the withdrawal period failed to affect significantly the time period in which the animals were predisposed to convulsions (67.5 min).

Naloxone

Naloxone (0.8 μg/g) administered to mice before exposure to nitrous oxide, decreased significantly the time of predisposition to convulsions. One mouse did not convulse at all during a period of 100 min, while others started to convulse 5 min after the exposure to the gas was discontinued. The average time during the withdrawal period in which the animals retained the tendency to convulse was significantly less than in the control group (23.4 min). However, the same dose of naloxone, administered in the first minute after N_2O was discontinued, failed to influence the duration of time in which the convulsant phenomena could be elicited.

Discussion

In these experiments the time of susceptibility to withdrawal convulsions in mice following nitrous oxide was decreased significantly after pretreatment with the cholinesterase inhibitors, physostigmine and galanthamine, or the opiate receptor blocking agent naloxone.

The cause of such postanesthetic excitation (the withdrawal syndrome) is not known. Presumably, these phenomena are a reflection of the complex interaction of anesthetics on excitatory and inhibitory synaptic trans-

mission.[9-11] Specific information concerned with the functional activity of the transmitter systems during the withdrawal period following nitrous oxide is limited. Recent data indicate that some anesthetics such as halothane, enflurane, and ketamine reduce the turnover rate of acetylcholine in all regions of the rat brain examined.[11] However, nitrous oxide (75%) had no effect on acetylcholine turnover in the rat brain.[12]

That the cholinesterase inhibitors used in the present study decreased the time of predisposition to convulsions following exposure to nitrous oxide suggests a possible important role for central cholinergic transmission in the modulation of the neuronal activity responsible for the postanesthetic excitatory withdrawal phenomena. The anticonvulsant effect of the cholinesterase inhibitors during a withdrawal period might be a direct one, via normalization of the inhibited cholinergic system, or an indirect one, by acting on other transmitter/modulator systems via a hyperactive cholinergic system.

The explanation for the differences in action between physostigmine and galanthamine observed in this study is not known, but the possibility that each drug possesses specific and different pharmacodynamic characteristics is not excluded.

Based upon our clinical experience in the treatment of CAS we concluded that the beneficial effect of physostigmine is related exclusively to cholinesterase inhibition.[13,14] Furthermore, we observed in our patients that nitrous oxide might be a precipitating factor for the postanesthetic excitation as a part of CAS.[3,4] The results of this study indicate that physostigmine and galanthamine decreased susceptibility to nitrous oxide withdrawal convulsions in mice. All of this suggests the possibility of using the nitrous oxide withdrawal syndrome in mice as an animal model for CAS in humans. At present we would like to encourage this idea and a further search for additional relationships between CAS in humans and the proposed animal model.

The anticonvulsant effect of naloxone given to mice before exposure to N_2O also could be explained by a cholinergic dysfunction caused by nitrous oxide. Namely, it has been postulated that nitrous oxide releases endorphins,[8] which may in turn decrease the release of acetylcholine.[15] Therefore, the anticonvulsant effect of naloxone, similarly to physostigmine, might be ascribed to normalization of the cholinergic system. However, other explanations related to the monoaminergic system(s) are also possible. Endorphins and exogenous opiates are reported to inhibit activity of some central noradrenergic nuclei[16] and reduce transmitter release from cerebrocortical noradrenergic nerve endings.[17,18] Functional insufficiency of the noradrenergic system facilitates seizure activity.[19] Thus, an anticonvulsant effect of naloxone might be due to inhibition of the endorphin-induced changes in noradrenergic functional activity. However, since endorphins are modulators of various transmitter systems, the anticonvulsant effect of naloxone might be much more complex than that suggested above. Despite these uncertainties, the anticonvulsant effect of naloxone during withdrawal from nitrous oxide might be of significant value in suggesting further studies of the role of endogenous opioid substances in postanesthetic excitatory phenomena.

References

1. Rupreht J, Dworacek B: Central anticholinergic syndrome in anesthetic practice. Acta Anaesthesiol Belg 27:45–60, 1976
2. Longo VG: Behavioural and electroencephalographic effects of atropine and related compounds. Pharmacol Rev 18:965–996, 1966
3. Rupreht J, Dworacek B: Central anticholinergic syndrome in anaesthetic practice: diagnostic problems. Asian Archives of Anaesthesiology and Resuscitation 7:5–9, 1977
4. Rupreht J: Clinical aspects of the central cholinergic blockade, Toxicological Aspects. Edited by Kovatsis A. Thessaloniki, Thessaloniki Technica Studio, 1980, pp 520–524
5. Goldstein DB, Pal N: Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. Science 172:288–290, 1971
6. Harper MH, Winter PM, Johnson BH, Koblin DD, Eger II EI: Withdrawal convulsions in mice following nitrous oxide. Anesth Analg (Cleve) 59:19–21, 1980
7. Smith RA, Winter PM, Smith M, Eger II EI: Convulsions in mice after anaesthesia. ANESTHESIOLOGY 50:501–504, 1979
8. Berkowitz, BA, Ngai SH, Finck AA: Nitrous oxide 'analgesia' resemblance to opiate action. Science 194:976–968, 1976
9. Nicoll RA: The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. J Physiol (Lond) 223:803–814, 1972
10. Morris ME: Facilitation of synaptic transmission by general anaesthetics. J Physiol (Lond) 284:307–325, 1978
11. Ngai SH, Cheney DL, Finck AD: Acetylcholine concentrations and turnover in rat brain structures during anesthesia with halothane, enflurane and ketamine. ANESTHESIOLOGY 48:4–10, 1978
12. Cheney DL, Ngai SH: Effects of anesthetics and related drugs on the acetylcholine turnover rate in various structures of the rat brain. Molecular Mechanisms of Anesthesia, Progress in Anesthesiology, Vol. 2, Edited by Fink BR. New York, Raven Press, 1980, pp 189–198
13. Dworacek B, Rupreht J: Fysostigmine bij de behandeling van vergiftingen. Ned Tijdschr Geneeskd 123:2154–2155, 1979
14. Rupreht J: Physostigmine reversal of diazepam. ANESTHESIOLOGY 53:180–181, 1980
15. Jhamandas K, Phillis JW, Pinsky C: Effects of narcotic analgesics and antagonists on the *in vivo* release of acetylcholine from the cerebral cortex of the cat. Br J Pharmacol 43:53–66, 1971
16. Gold MS, Byck R, Sweeney DR, Kleber HD: Endorphin-locus coeruleus connection mediates opiate action and withdrawal. Biomedicine 30:1–4, 1979
17. Arbilla S, Langer SZ: Morphine and β-endorphin inhibit release of noradrenaline from cerebral cortex but not of dopamine from rat striatum. Nature 271:559–560, 1978
18. Taube HD, Borowski E, Endo T, Starke K: Enkephalin: a potential modulator of noradrenaline release in rat brain. Eur J Pharmacol 38:377–380, 1976
19. Mason ST, Corcoran M: Catecholamines and convulsions. Brain Res 170:497–507, 1979