Anesthesiology
61:151–155, 1984

Anesthetics and Excitatory/Inhibitory Responses of Midbrain Reticular Neurons

Koki Shimoji, M.D., Ph.D., Hitoshi Fujioka, M.D.,† Toshiya Fukazawa, B.S.,‡ Masao Hashiba, M.D.,‡ Yoichi Maruyama, M.D.†*

The effects of nitrous oxide, halothane, ether, isoflurane, thiopental, and thiamylal on the excitatory as well as inhibitory responses of single neurons in the midbrain reticular formation (MRF), believed to be one of the most important sites for the regulation of wakefulness, were studied by long-term, extracellular microelectrode recording in cats and rats. All anesthetics except nitrous oxide suppressed the excitatory responses of MRF neurons evoked by somatosensory stimulation. The inhibitory responses markedly were potentiated by both barbiturates but variously affected by other inhalation anesthetics. Blockade of the inhibitory responses (disinhibition) was observed more frequently with the inhalation agents during the light state of anesthesia. Thus, suppression of excitatory responses is likely to be a general feature of the anesthetic state in terms of the behavior of MRF neurons. Further, potentiation of the inhibitory responses might be characteristic of barbiturate anesthesia. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, intravenous: thiamylal; thiopental. Anesthetics, volatile: ether; halothane; isoflurane. Brain: midbrain reticular formation.

IN THE MIDBRAIN RETICULAR FORMATION (MRF), believed to be one of the most important sites for anesthetic action,[1,2] a single neuron shows the characteristic feature of excitatory as well as inhibitory responses to various stimuli.[3,4] Thus, using long-term, extracellular microelectrode recording, the effects of different anesthetics on both excitatory and inhibitory responses of an MRF neuron can be observed simultaneously.[5,6] Our preliminary communication[7] has shown that the two inhalation anesthetics (halothane and isoflurane) always suppress the excitatory responses with various effects on the inhibitory responses of the MRF neurons. This prompted us to study the effects of other inhalational and intravenous anesthetic agents on these excitatory and inhibitory activities of the MRF neurons. We have found that barbiturates always potentiate the inhibitory responses with concomitant suppression of the excitatory responses, while inhalation anesthetics generally have depressant effects on the excitatory responses with simultaneous blocking, augmenting, or insignificant actions on the inhibitory responses of the MRF neurons evoked by somatosensory stimulation.

* Professor.
† Clinical Assistant.
‡ Research Assistant.

Received from the Department of Anesthesiology, Niigata University School of Medicine, 1-757 Asahi-machi, Niigata 951, Japan. Accepted for publication January 17, 1984. Presented in part at the 30th Annual Meeting of the Japan Society of Anesthesiologists, 1983.

Address reprint requests to Dr. Shimoji.

Methods

Fifteen cats, 2.5–3.5 kg, and 12 rats, 230–260 g, were studied. Animals were anesthetized with 75% nitrous oxide and 1.0% halothane in an air-tight plastic box. An intratracheal tube was inserted and the respiration controlled via a nonrebreathing system (Harvard® pump; An Ealing Company, Natick, Massachusetts). The animals were paralyzed with gallamine, 5 mg/kg, or pancuronium, 0.1 mg/kg, and end-tidal CO_2 concentration was maintained at 4.5%. The femoral artery and vein were cannulated for monitoring the blood pressure and for the administration of drugs or Ringer's lactate solution, respectively. A heating blanket was applied to maintain body temperature constant at 37.5–38.0°C (rectal temperature). The skull was exposed, and a small hole was made at the anteroposterior plane (AP) + 2.0 mm and at the mediolateral plane (ML) 2.0 mm in the cats according to the Horsley–Clark stereotaxic coordinates or at AP + 1.6 mm and ML 2.0 mm in the rats by the atlas of Pellegrino *et al.*[8] Two screw electrodes were fixed to the skull in the areas of the anterior sigmoid and middle suprasylvian gyri, respectively, for recording the electrocorticogram and evoked potential. At the termination of the surgical procedures, 2% lidocaine (about 2 ml in cats and 0.2 ml in rats) was injected around the surgical wound and the pressure points. The activity of the MRF neurons was recorded extracellularly with a KCl (3M)-filled micropipette (5–10 MΩ) in the rats or a tungsten microelectrode (50–100 MΩ) in the cats.[5,6] Microelectrodes were inserted vertically through the trephinized small hole and several layers of the brain up to the region where neurons vigorously responded to flash. This indicated that the electrode tips had reached the colliculus superior.[3,4] After attainment of this characteristic response from the neurons, the electrode was advanced more ventrally by a microdriver to near the level of the aqueductus cerebri. To minimize respiratory and pulsatory movements, bilateral pneumothorax and high-frequency ventilation were induced in four rats. After observing the stability of the activities and several characteristics of the MRF neurons under 75% nitrous oxide with local anesthesia for more than 1 h, an inhalation or intravenous anesthetic was administered. The effects of several anesthetics on the same MRF neurons were studied by long-term recording (4–26 h) (14.2 ± 6, mean ± SE). The interval between administration of two different

TABLE 1. Effects of Anesthetics on the Excitatory and Inhibitory Responses of the MRF Neurons Evoked by Somatosensory Stimulation in Cats

Anesthetic with Concentration or Dose*	Excitatory Responses		Inhibitory Responses			Number of Neurons Tested
	Suppressed	Potentiated	Blocked	Potentiated	No change	
N_2O (75%)	3	2	3	0	2	5
Halothane 1.0–1.5%	4	0	3	0	1	4
2.0%	3	0	1	2	0	3
Ether 3–5%	9	0	6†	3	0	9
8–15%	8	0	1	1	6	8
Thiopental 5 mg/kg	4	0	0	4 ‡	0	4
15 mg/kg	3	0	0	3 ‡	0	3

* Ten to twenty minutes after the inhalation or 2–5 min after the intravenous administration. Among anesthetics, $P < 0.005$ (expected value, less than 1).

† Significantly higher occurrences *versus* those during the deeper stage (8–15%) of ether anesthesia ($0.005 < P < 0.01$) and to those during thiopental anesthesia ($P < 0.005$).

‡ Significantly higher occurrences of potentiation of inhibitory responses with thiopental, 5 mg/kg + 15 mg/kg, *versus* those with ether, 3–5% + 8–15% ($P < 0.05$).

agents was usually 1.5–2 hs. The effects of nitrous oxide, halothane, ether, and thiopental were studied in cats (table 1), while those of halothane, isoflurane, thiopental, and thiamylal were tested in rats (table 2). When studying the effects of nitrous oxide, the quiet resting state of the animals gently fixed by the stereotaxic apparatus was checked constantly by vital signs (stable blood pressure and heart rate, size of pupils), the frequent appearance of spindle waves in the electrocorticogram, and no or minimum changes in these signs during repetitive electrical stimuli. If some changes in these variables occurred, local infiltrations of lidocaine around the wound and pressure points frequently were carried out.

Vital signs, such as arterial blood pressure, heart rate, body temperature, and end-tidal carbon dioxide, were monitored continuously and adjusted. Unit activities and pulses triggered by each spike were monitored with an oscilloscope (Nihonkohden VC-9®; Nihonkohden Kogyo Inc., 1-31-4, Nishiochiai, Shinjuku-ku, Tokyo 161, Japan) and, at the same time, were stored on FM magnetic tape (TEAC XR-30®; TEAC Corporation, 3-7-3, Naka-cho, Musashino, Tokyo 180, Japan). The taped records of behavior of the MRF neurons, the field potentials detected by the microelectrode, and the electrocorticogram were analyzed by a digital computer (Nihonkohden ATAC 1200®). Electrical stimulation was applied at 0.5–4 Hz through a pair of hypodermic needles inserted subcutaneously into an area where the most effective responses of the MRF neurons could be obtained.

At the termination of the experiment, identification of the entire track of the microelectrode was achieved by fixing the brain with formalin and cutting it along the microelectrode in place.

Poststimulus time histogram analysis was made of 120–480 summated responses at each anesthetic state. Poststimulus time histogram was plotted by an X-Y plotter and at the same time typed in digital number. Responses of neurons to somatosensory stimuli were considered to be excitatory when the responses were increased more than 20% as compared with those summated without stimulation using the same duration of analysis. Similarly, the responses of neurons to stimuli were regarded as

TABLE 2. Effects of Anesthetics on the Excitatory and Inhibitory Responses of the MRF Neurons Evoked by Somatosensory Stimulation in Rats

Anesthetic with Concentration or Dose*	Excitatory Responses		Inhibitory Responses			Number of Neurons Tested
	Suppressed	Potentiated	Blocked	Potentiated	No Change	
Halothane 1.0–1.5%	12	0	8†	1	3	12
2.0%	6	0	2	6	1	9
Isoflurane 1.25–1.5%	9	0	8	0	1	9
2.0–3.0%	9	0	5	3	1	9
Thiopental 15 mg/kg	3	0	0	3 ‡	0	3
Thiamylal 15 mg/kg	3	0	0	3 ‡	0	3

* Ten to twenty minutes after the inhalation or 2–5 min after the intravenous administration. Among anesthetics, $0.005 < P < 0.01$ (expected value, less than 1).

† Significantly higher occurrences of blockade of inhibitory responses *versus* those during 2.0% halothane ($P < 0.05$), thiopental, and thiamylal anesthesia ($P < 0.005$).

‡ Significantly higher occurrences of potentiation of inhibitory responses with thiopental + thiamylal *versus* those during halothane, 1.0–1.5%, and isoflurane anesthesia ($P < 0.005$).

inhibitory when the responses were decreased more than 20% with comparison to those summated without stimulation using the same duration of analysis.

Two-by-three chi-square tests were used for analysis of distribution of number of neurons in inhibitory responses among seven (table 1) or six (table 2) anesthetic groups considered together. For halothane and thiopental, which were administered to both cats and rats, data on inhibitory responses also were analyzed statistically using 2 by 4 chi-square tests to detect differences between the two animal groups. Further, partitioned chi-square tests were performed for statistically independent comparisons within an orthogonal set, such as thiopental groups (5 mg/kg + 15 mg/kg) *versus* halothane groups (1.0–1.5% + 2.0%). Severity scores were ranked, and nonparametric correlation coefficients were calculated. For all statistical comparisons, P values less than 0.05 were considered significant.

Results

When the electrode tip reached the MRF, a variety of spike appearances were recorded, but the analysis was made only on initially negative spikes recorded stably without changes in wave form during the observation period.[3,4] As previously observed, the same units could be fired by an afferent volley from any of the four paws and also by auditory and/or visual stimulations.[3–5] The patterns of evoked firing included a characteristic sequence of early excitation followed by an inhibition (or a transient decrease in firing between excitatory responses) and late excitation; high- or low-frequency sustained discharge with an inhibitory phase. Those sequences of excitatory and inhibitory responses could be demonstrated more clearly in poststimulus time histograms (fig. 1). The observations were made on these characteristic behaviors of the MRF neurons in response to somatosensory stimulation.

All inhalation anesthetics tested suppressed the early as well as the late excitatory responses of the MRF neurons (except for two neurons during nitrous oxide anesthesia) evoked by somatosensory stimulation (table 1). The late excitatory responses generally were more sensitive to anesthetics than the early ones. With inhalation anesthetics, concomitant changes in the inhibitory responses were variable among the neurons: blockade (disinhibition), potentiation (or prolongation), or no significant changes (tables 1 and 2). Disinhibition frequently was observed during the light stage of ether ($P < 0.01$) (table 1, fig. 1) and halothane ($P < 0.01$) (table 2) anesthesia.

In contrast, the inhibitory responses always were potentiated (270–830% of control) and/or prolonged (220–410% of control) by the administration of thiopental or thiamylal ($P < 0.005$) (tables 1 and 2).

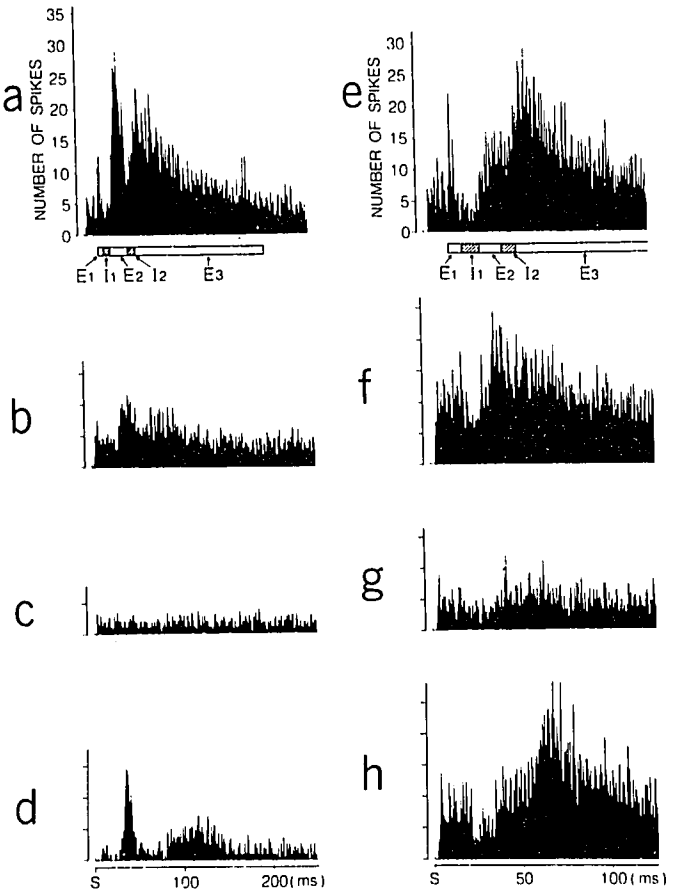

FIG. 1. Specimen records of poststimulus time histograms of an MRF neuron affected by several anesthetics. Electrical stimulation was delivered to the contralateral forepaw at 2 Hz (*A–D*) and 4 Hz (*E–H*). This MRF neuron responded to touch at all four limbs and to light. Contralateral forepaw stimulation evoked the initial weak excitation (E_1) followed by a transient inhibitory phase (I_1). The next prominent excitation (E_2) also was followed by a relative inhibition (I_2) and subsequent late facilitation phase (E_3) during control (*A*) (75% nitrous oxide). Halothane (1.0%, 10 min) suppressed all excitatory responses (E_1, 46%; E_2, 44%; E_3, 28%, compared with the control values) without significant effects on inhibitory phases (*B*). Ether (8%, 20 min), which was administered after recovery from halothane anesthesia, also suppressed all excitatory responses (E_1, 19%; E_2, 4%; E_3, 7%), and obscured the inhibitory phases (*C*). With intravenous administration of thiopental (15 mg/kg, 5 min) after recovery from ether anesthesia, excitatory responses also were suppressed (E_1, 0.4%; E_2, 33%; E_3, 14%) with concomitant potentiation (I_1, 620%; I_2, 450%) and prolongation (I_2, 380%) of the inhibitory response phases (*D*). The early inhibitory phase (I_1) can be demonstrated more clearly at a higher frequency stimulation (4 Hz) in poststimulus time histogram (*E*), which was recorded during a quiet resting state under local anesthesia 1.5 h after the administration of thiopental. By inhalation of nitrous oxide (75%, 8 min) the inhibitory responses were blocked (I_1, 23%; I_2, 78%) with variable effects on the excitatory responses (E_1, 92%; E_2, 158%; E_3, 81%) (*F*). Readministration of halothane (1%, 8 min) predominantly suppressed the excitatory responses (E_1, 32%; E_2, 29%; E_3, 22%) with minimum effects on the inhibitory responses (*G*). Both excitatory and inhibitory responses were recovered partially 30 min after the termination of halothane inhalation (*H*).

There were no statistical differences between cats and rats in the behavior of neurons in response to halothane and thiopental, 15 mg/kg, which were administered to both animals (tables 1 and 2).

Thus, the simultaneous occurrences of blockade of excitatory responses and potentiation of inhibitory responses were the characteristic features of barbiturate anesthesia in terms of the behavior of the MRF neurons.

Discussion

The present study has shown that clinical doses of both thiopental and thiamylal markedly potentiate the inhibitory responses of MRF neurons, while other inhalation anesthetics variously affect the inhibitory responses. The excitatory responses of MRF neurons were suppressed by all anesthetics studied, except for nitrous oxide. Therefore, suppression of excitatory responses might be the fundamental basis of the anesthetic state in terms of MRF neurons as suggested in our previous study.[7] The barbiturate-induced suppressive effects on excitatory responses of MRF neurons also were demonstrated (fig. 1, table 1). The characteristic effects of the barbiturates, *i.e.*, suppression of the excitatory responses with marked potentiation of the inhibitory responses of MRF neurons, might account for the clear configuration of the evoked responses during barbiturate anesthesia in poststimulus time histogram (fig. 1). Although the inhibitory responses variously were affected by inhalation anesthetics, the blockade of those responses was noticed more frequently during the lighter stages of anesthesia (tables 1 and 2). These lighter stages might be considered to be similar to the usual depth of clinical anesthesia. Thus, suppression of excitatory responses with simultaneous blockade of inhibitory responses might be a more general feature of clinical anesthesia induced by these inhalation drugs in terms of activities of MRF neurons.

There may be several advantages to the use of the MRF neurons *in situ* in the study of the effects of anesthesia. First, almost all of these neurons are spontaneously active.[3,4] Second, the MRF has been well known as one of the most responsible sites for the regulation of wakefulness, natural sleep, and anesthetic state.[1,2] Third, so many excitatory as well as inhibitory inputs impinge on the single neurons in the MRF[3,4] that both influences can be detected simultaneously and sequentially by the application of long-term recording to provide a tool for studying *in situ* the effects of various anesthetics on both responses at the same time.

The synaptic effects of anesthetics have been variously interpreted: suppression of excitatory synaptic potential,[9–12] potentiation of inhibitory postsynaptic potential,[13–17] or augmentation of presynaptic inhibition.[18,19] These studies, however, have been carried out mainly in the peripheral or spinal cord neurons in *in vitro* preparations, and simultaneous observations on both the excitatory and inhibitory responses have not been made. In *in vitro* studies, the anesthetic concentrations applied have tended to be extremely high[20] as compared with those used clinically. Further, anesthetic effects on neurons might be qualitatively different in various nervous structures, as well as among many animal species.[21,22] On the other hand, the differential effects of anesthetics on neuronal activities were observed in the sympathetic ganglia,[21] the MRF *in situ*,[5,6] the bulbar reticular formation,[22] and in animal behavior.[23] In the MRF, however, it frequently was observed that there were neurons that were so vulnerable to anesthetics that the spontaneous as well as evoked firings became silent even with 75% nitrous oxide in the present study. These neurons were too sensitive to be analyzed by poststimulus time histogram. Such vulnerable neurons also might contribute to wakefulness and the anesthetic state.

In summary, we have found that all anesthetics except nitrous oxide have depressant effects on the excitatory responses of the MRF neurons evoked by somatosensory stimulation. The inhibitory responses always were potentiated by thiopental and thiamylal but variously affected by inhalation anesthetics. The blockade of the inhibitory responses (disinhibition) was observed more frequently during light stages of inhalation anesthesia.

The authors thank Mr. Yukio Sato and Takashi Ebata for their assistances throughout the experiment and also Dr. Shiro Shimosato for his generous donation of isoflurane.

References

1. French JR, Verzeano N, Magoun, HW: A neuronal basis of the anesthetic state. Arch Neurol Psychiatry 69:519–529, 1953
2. Syka J, Popelar J, Radil-Weiss T: Influence of increasing doses of pentobarbital on the mesencephalic reticular formation in rats. Spontaneous firing of neuronal pairs and activity evoked by polarization. Brain Res 88:263–279, 1975
3. Amassian VE, Macy J, Waller HJ: Patterns of activity of simultaneously recorded neurons in midbrain reticular formation. Ann NY Acad Sci 89:883–895, 1961
4. Huttenlocher PR: Evoked and spontaneous activity in single units of medial brain stem during natural sleep and waking. J Neurophysiol 24:451–468, 1961
5. Shimoji K, Bickford RG: Differential effects of anesthetics on mesencephalic reticular neurons: II. Responses to repetitive somatosensory electrical stimulation. ANESTHESIOLOGY 35:76–80, 1971
6. Shimoji K, Matsuki M, Shimizu H, Maruyama Y, Aida S: Dishabituation of mesencephalic reticular neurons by anesthetics. ANESTHESIOLOGY 47:349–352, 1977
7. Shimoji K, Fujioka H, Ebata T: Anaesthetics block excitation with various effects on inhibition in MRF neurones. Brain Res 295:190–193, 1984
8. Pellegrino LS, Pellegrino AS, Cushman AJ: A Stereotaxic Atlas of the Rat Brain. New York, Plenum Press, 1979

9. Nicoll RA: The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. J Physiol 223:803–814, 1972
10. Richards CD: On the mechanism of barbiturate anaesthesia. J Physiol (Lond) 227:749–767, 1972
11. Barker JL, Gainer H: Pentobarbital: selective depression of excitatory postsynaptic potentials. Science 182:720–722, 1973
12. Proctor WR, Weakly JN: A comparison of the presynaptic and post-synaptic actions of pentobarbitone and phenobarbitone in the neuromuscular junction of the frog. J Physiol (Lond) 285:257–268, 1976
13. Larsen MD, Major MA: The effect of hexobarbital on the duration of the recurrent I.P.S.P. in cat motoneurones. Brain Res 21:309–311, 1970
14. Nicoll RA, Eccles JC, Oshima T, Rubia F: Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. Nature 258:625–627, 1975
15. Scholfield CN: A barbiturate induced intensification of the inhibitory potential in slices of guinea-pig olfactory cortex. J Physiol (Lond) 275:559–566, 1978
16. Scholfield CN: Potentiation of inhibition by general anaesthetics in neurones of the olfactory cortex in vitro. Pflügers Arch 383:249–255, 1980
17. Aickin CC, Deizs RA: Pentobarbitone interference with inhibitory synaptic transmission in crayfish stretch receptor neurones. J Physiol (Lond) 315:175–187, 1981
18. Eccles JC, Schmidt R, Willis WD: Pharmacological studies on presynaptic inhibition. J Physiol (Lond) 168:500–530, 1963
19. Higashi H, Nishi S: Effect of barbiturates on the GABA receptor of cat primary afferent neurones. J Physiol (Lond) 332:299–314, 1982
20. Nicoll RA, Madison DV: General anesthetics hyperpolarize neurons in the vertebrate central nervous system. Science 217:1055–1056, 1982
21. Larrabee MG, Posternak JM: Selective action of anesthetics on synapses and axons in mammalian sympathetic ganglia. J Neurophysiol 15:91–114, 1952
22. Cullen KD, Martin RJ: Dissimilar influences of some injectable anaesthetics on the responses of reticulospinal neurones to inhibitory transmitters in the lamprey. Br J Pharmacol 77:493–504, 1982
23. Richards CD, White AE: Additive and non-additive effects of mixtures of short-acting intravenous anaesthetic agents and their significance for theories of anaesthesia. Br J Pharmacol 74:161–170, 1981