Effects of Anesthetics on Neuromuscular Transmission and Somatic Reflexes

S. H. Ngai, M.D., Edgar C. Hanks, M.D. and S. E. Farhie, M.D.

Effects of various anesthetics on neuromuscular transmission and somatic reflexes were studied in unanesthetized, midcollicular decerebrate and spinal cats respectively. Diethyl ether was the only agent which significantly decreased the tibialis twitch response to indirect stimulation but an inspired concentration much higher than that required to produce areflexia was necessary. Cyclopropane, and to a lesser extent nitrous oxide and chloroform, increased the tibialis twitch response. It appears that, at least with cyclopropane, this response is a postsynaptic phenomenon distinct from its depressant action on the nervous system. All of the potent anesthetics tested, with the exception of methoxyflurane, abolished spinal (polysynaptic) and cephalic reflexes simultaneously. Methoxyflurane depressed the spinal reflex earlier and at a lower inspired concentration.

It has long been recognized that general anesthetics, in appropriate concentrations, produce skeletal muscle relaxation through their depressant action on the central nervous system. Spontaneous activity as well as reflex response to afferent stimuli diminish and finally disappear. Electrophysiological evidence of synaptic blockade in the spinal cord by pentobarbital, thiopental, urethane and diethyl ether has been reported.\textsuperscript{1–5} On the other hand, it is also well established that diethyl ether interferes with neuromuscular transmission. Recent studies of Sabawala and Dillon\textsuperscript{6,7} with human intercostal preparations \textit{in vitro} showed that a number of anesthetic gases and vapors also reduced the twitch response to indirect stimulation. These findings differ from the results obtained by Watland et al.\textsuperscript{8} in nerve-muscle preparation in situ. In the present study the effects of a number of anesthetics on the neuromuscular transmission and somatic reflexes were examined in the decerebrate or spinal cat. Anesthetic concentrations required to abolish the spinal (polysynaptic), corneal, and masseter reflexes, and to cause electroencephalographic (EEG) changes were compared in cats after transection of the cervical spinal cord.

Methods

Forty-eight cats weighing from 2.0 to 4.2 kg. were used. Under diethyl ether anesthesia a tracheostomy was performed. For the study of neuromuscular transmission, midcollicular decerebration was carried out through a left temporal craniotomy. The ether was then discontinued and the animal allowed to breathe room air for at least 90 minutes before measurement was begun. The peroneal nerve was isolated and ligated; other branches to the sciatic nerve were divided. Supramaximal stimuli were applied to the peroneal nerve peripheral to ligation. Stimulating currents were obtained from a Grass stimulator (Model S4A) through a stimulus isolation unit, consisting of square wave pulses 0.1 millisecond duration, 3–4 volts intensity and 0.1 cycle per second frequency. Twitch response of the tibialis anterior was registered with a Grass force displacement transducer (Model FT-03, loaded with 300 g. springs). Arterial pressure was measured through a catheter placed in a femoral artery with a Statham strain gauge (Model P23ID). For twitch response of denervated muscle to direct stimulation (with square wave pulses of 2 msec. duration and 15 volts intensity applied to the muscle with two steel electrodes) the peroneal nerve on one side of an etherized cat was divided two
weeks prior to the experiment. During the experiment twitch response of the innervated tibialis muscle of the other side to indirect stimulation was also measured. In these animals, the arterial pressure was measured through a common carotid artery.

For the study of reflex activity, instead of decerebration, the spinal cord was transected at the level of the first cervical segment, also during diethyl ether anesthesia. A Takaoka respirator driven by oxygen provided ventilation. The sciatic nerve was isolated and ligated. Electrical stimuli were delivered to the nerve with a bipolar electrode central to the ligation. The stimuli consisted of square-wave pulses of 2 msec. duration, 4–5 volts intensity and 0.5 cycle per second frequency. Reflex contraction of the ipsilateral or contralateral quadriceps femoris was recorded either electromyographically with two silver electrodes placed in the muscles approximately 2–3 mm. apart or with a Grass force displacement transducer attached to the patellar tendon detached from its insertion. As neural structures rostral to the transection were viable (encephalà isolé), corneal and masseter reflexes (a forceful closing of mouth in response to depression of the mandible) were elicited periodically and the results noted. EEG was recorded from biparietal leads. All recordings were made on a Grass polygraph (Model 5).

After a steady level of responses was obtained during the control period, the anesthetic mixture was administered to decerebrated animals through a Sierra pediatric Y breathing valve. The Takaoka respirator was used for spinal animals. An Ohio 3000 series anesthesia machine with calibrated flow meters and a Vermitrol vaporizer provided the anesthetic mixture. Vapor concentration was calculated from the liquid temperature, vapor pressure and total oxygen flow. When more than one anesthetic was administered in one animal, an interval of at least 60 minutes between administrations was allowed.

Rectal temperature was monitored with a mercury thermometer and maintained in the range of 38–40° C. with a heating pad placed under the animal. When inhalation of anesthetics resulted in a fall in systolic arterial pressure below 50 mm. of mercury, methoxy-

<table>
<thead>
<tr>
<th>Agent</th>
<th>No. of Experiments</th>
<th>Inspired Concentration (%)</th>
<th>No. of Experiments Shown</th>
<th>Decrease (1% of control)</th>
<th>No Effect</th>
<th>Increase (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl Ether</td>
<td>6</td>
<td>10–25</td>
<td>6</td>
<td>0 (50–90)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Halothane</td>
<td>4</td>
<td>1–2</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichlorethylene</td>
<td>4</td>
<td>0.4–1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>4</td>
<td>0.5–1</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>4</td>
<td>0.4–1</td>
<td>4</td>
<td>(110–135)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrous Oxide</td>
<td>5</td>
<td>50–80</td>
<td>5</td>
<td>(110–115)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclopropane</td>
<td>9</td>
<td>10–30</td>
<td>9</td>
<td>(110–190)</td>
<td></td>
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</tr>
</tbody>
</table>

Amine HCl in single doses of 0.5 mg. was given intravenously to restore the arterial pressure.

Anesthetics studied were: nitrous oxide, cyclopropane, diethyl ether, chloroform, halothane, trichlorethylene and methoxyflurane. Only cyclopropane was tested for its effect on the direct twitch response of denervated muscle.

Results

Neuromuscular Transmission. The effect of various anesthetics on the twitch response of the tibialis anticus to stimulation of the peroneal nerve was studied in 21 decerebrate cats. The results are shown in table 1. The duration of exposure to nitrous oxide and cyclopropane was 10–20 minutes and that to all the anesthetic vapors, 30 minutes or longer. Diethyl ether was the only anesthetic which consistently decreased the twitch response to indirect stimulation. With 10 per cent vapor in the inspired mixture the response was approximately 90 per cent of the control, and with 25 per cent vapor, the response was approximately 50 per cent of the control. Halothane, trichlorethylene and methoxyflurane did not change the response significantly. Nitrous oxide, cyclopropane and chloroform increased the response. Of the latter three agents, cyclopropane appeared to be the most effective in potentiating this response. In one experiment
the twitch height increased to 190 per cent of control (fig. 1).

In 4 of these 21 animals the peroneal nerve on one side was sectioned 2 weeks before the experiment to allow complete motor nerve degeneration. Twitch response of the denervated tibialis was elicited by direct electrical stimulation. Administration of cyclopropane in concentrations of 20–30 per cent also increased this response (fig. 1). The extent of potentiation, however, was not as marked as with indirect stimulation. The greatest increase observed under the condition of the experiment was 30 per cent.

**Spinal (Polysynaptic), Corneal and Masseter Reflexes.** The effect of anesthetics on these reflexes was studied in 27 cats after the spinal cord was transected at the first cervical segment. Upon recovery from the initial ether anesthesia these reflexes were regularly elicited. EEG showed a low amplitude, high frequency pattern. Anesthetic gas or vapor and oxygen mixture was administered in a manner whereby the anesthetic concentration was increased in steps and held constant at each step for a period of 10 minutes for the gases or 20 minutes or more for the vapors. The lowest concentration required to abolish the spinal reflex

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**Table 2. Effect of Anesthetics on Somatic Reflexes**

<table>
<thead>
<tr>
<th>Agent</th>
<th>No. of Experiments</th>
<th>Minimal Inhaled Concentration (per cent) to Abolish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spinal Reflex</td>
</tr>
<tr>
<td>Nitrous Oxide</td>
<td>14</td>
<td>not abolished (50–80)</td>
</tr>
<tr>
<td>Cyclopropane</td>
<td>12</td>
<td>10 (7.5–15)</td>
</tr>
<tr>
<td>Diethyl Ether</td>
<td>6</td>
<td>6 (4–8)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>6</td>
<td>0.7 (0.4–1.0)</td>
</tr>
<tr>
<td>Halothane</td>
<td>9</td>
<td>0.6 (0.4–1.0)</td>
</tr>
<tr>
<td>Trichlorethylene</td>
<td>5</td>
<td>0.8 (0.6–1.0)</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>12</td>
<td>0.3 (0.2–0.5)</td>
</tr>
</tbody>
</table>

*Note: Numbers in parentheses indicate range.*
Fig. 2. Effect of nitrous oxide on cross extensor reflex and EEG. Cat 8, 2.5 kg. Spinal cord transected at first cervical segment. Upper trace: EEG, biparietal leads. Middle trace: femoral arterial pressure. Lower trace: reflex response of quadriiceps femoris to stimulation of contralateral sciatic nerve at 2 second intervals. With 80 per cent nitrous oxide spinal reflexes were reduced. Cephalic reflexes remained active.

or to reduce it to less than 10 per cent of the control was noted. If the corneal and mas- ter reflexes could still be elicited at this time, the anesthetic concentration was further in- creased until these reflexes were abolished.

The results are shown in table 2. Nitrous oxide in concentrations up to 80 per cent re- duced but did not abolish the spinal reflex (fig. 2). Corneal and masster reflexes re- mained active. With cyclopropane, diethyl ether, chloroform, halothane and trichlorethy- ene, spinal reflex, corneal and masster reflexes were abolished at approximately the same time. A high amplitude, low frequency EEG pattern was usually observed. One notable exception was that with methoxyflurane. In 12 animals the lowest inspired vapor concentration required to abolish the spinal reflex averaged 0.3 per cent, ranging from 0.2 to 0.5 per cent (fig. 3). At the time when the spinal reflex was completely or almost com- pletely abolished, the corneal and masster reflexes remained active. The EEG pattern was not significantly altered. Further increase in vapor concentration resulted in complete areflexia.

Recovery of reflex activity took place in 10–20 minutes with nitrous oxide, 15–30 minutes with cyclopropane and 30–60 minutes with all the anesthetic vapors.

Discussion

Results from the present study reconfirmed the classical concept that skeletal muscular relaxation during general anesthesia results primarily from the depressant action of anes-

Fig. 3. Effect of methoxyflurane on flexion reflex. Cat 10, 2.7 kg. Spinal cord transected at first cervical segment. Upper trace: EEG high amplitude artifacts represent blinking. Middle trace: femoral arterial pressure. Methoxamine HCI 0.5 mg. was given intravenously between panels 2, 3 and panels 3, 4. Bottom trace: response of quadriiceps femoris to stimulation of ipsilateral sciatic nerve at 2 second intervals. After 20 minutes of inhalation of 0.3 per cent methoxyflurane, spinal reflex was abolished. Cephalic reflexes remained active at this time.
thetics on the central nervous system. Neuromuscular transmission, as measured with nerve-muscle preparation in situ, was not impaired except with diethyl ether. Even with diethyl ether, an inspired concentration of more than 10 per cent was required to reduce the twitch response of tibialis muscle to indirect stimulation significantly; whereas somatic reflexes were abolished with inspired concentrations much less than 10 per cent. Anesthetic depression of motor neurones and synaptic transmission along the reflex pathways ² ³ ⁴ appears to account for the loss of muscle tone during deeper levels of surgical anesthesia.

Although the twitch response of a muscle to indirect stimulation was maintained during the inhalation of halothane, trichlorethylene or methoxyflurane, this does not necessarily mean that these agents do not modify neuromuscular transmission in any way. It has been demonstrated in nerve-muscle preparations in dogs in situ that halothane, as well as other anesthetics, potentiate the effect of curare. ⁸ The basis for this interaction has not been and cannot be elucidated from gross measurement of twitch response. Detailed study of the effects of these anesthetics on the neuromuscular synaptic potential, endplate action potential as well as resting membrane potential would be necessary.

It is interesting that nitrous oxide, cyclopropane and chloroform actually increased the twitch response of tibialis muscle to indirect stimulation. This phenomenon has been observed by Sabawala and Dillon ⁹ with cyclopropane and by Watland et al. ⁵ with cyclopropane and chloroform. Since cyclopropane appeared to be the most potent in this respect, it was chosen for further study. As the response of a denervated muscle to direct stimulation also increased during cyclopropane inhalation, it seems most likely that this agent acts at points beyond the neuromuscular synapse to facilitate muscular contraction. The study by Van Posnak ⁹ on the depressant action of cyclopropane on motor nerve terminals (presynaptic) would support this view.

The facilitatory effect of cyclopropane is not limited to skeletal muscles. Price and Price ¹⁰ found that cyclopropane caused contraction of rabbit aortic strips in vitro and potentiated its response to norepinephrine. Preliminary study in this laboratory has confirmed these findings and demonstrated that cyclopropane also accentuated the response of rat gut strip to acetylcholine in vitro. * The intriguing question arises: Does cyclopropane accentuate contractile response to nerve action or transmitter substances through its action on the membrane or its effect on the contractile processes within the cell? The answer must await further investigation.

The inspired concentrations of anesthetics required to abolish various somatic reflexes are not to be taken as comparisons of anesthetic potency. Nor do they approximate the actual partial pressure of anesthetics attained in arterial blood. The wide difference of the solubility coefficients of these agents precludes estimations in this respect. Measurement of anesthetic concentration in the alveolar gas or the blood was not carried out. However, the data do allow comparison of response of various reflexes to a given anesthetic in a given animal. When the data are examined in this light, it is evident that methoxyflurane abolished the spinal reflex at a lower concentration than that required to abolish cephalic reflexes. Recordings of spontaneous muscular activity with the electromyograph in man and in the cat also showed electrical quiescence before significant EEG changes could be detected. The early abolition of spinal reflexes could explain the profound muscular relaxation obtained when methoxyflurane is administered to surgical patients.

Conclusions and Summary

In unanesthetized midcollicular decerebrate and spinal cats the effects of a number of anesthetic gases and vapors on the tibialis twitch response to indirect stimulation, the spinal reflex, corneal reflex and masseter reflex were studied. Ten per cent or more of diethyl ether (inspired) was required to reduce the tibialis twitch response significantly; whereas only 6 per cent vapor of this agent was needed to abolish all other reflexes. Halothane (up to 2 per cent), trichlorethylene (up to 1 per

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* This work was carried out by Mr. Thomas J. Snyder, a postdoctorial trainee (NIOMS, Grant ST1-Gn-56-06) in collaboration with Drs. S. H. Ngai and H. Bartelstone.
cent), and methoxyflurane (up to 4 per cent) did not significantly alter the tibialis twitch response. Chloroform, nitrous oxide and cyclopropane actually increased the twitch response. Nitrous oxide reduced but did not abolish reflex activity. Cyclopropane, halothane, trichlorethylene and chloroform abolished spinal and cephalic reflexes at about the same time. With methoxyflurane the spinal reflex was abolished earlier and at a lower inspired concentration than that required to abolish the cephalic reflexes. These results reaffirmed the classical concept that muscular relaxation during general anesthesia is primarily the result of anesthetic action on the central nervous system.

The potentiating action of cyclopropane on the tibialis twitch response was also observed in denervated muscle on direct stimulation. This result and other evidence in the literature indicate that cyclopropane may have an action on the postsynaptic structures or contractile processes distinct from its depressant action on the nervous functions.

The susceptibility of the spinal reflex to depression by methoxyflurane may be the basis for the muscular relaxation observed during clinical anesthesia with this agent.

Portions of this work were presented before the American Society of Anesthesiologist's during its Annual Meeting, October 1961, at Los Angeles. An abstract appeared in Anesthesiology 23: 158, 1962.

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References


July–August 1965 Symposium

Fetal and Maternal Physiology in the Perinatal Period

Owing to the renewed interest in anesthesia for obstetrics the Editors of the JOURNAL have selected as the subject for the annual symposium—Fetal and Maternal Physiology in the Perinatal Period. Recent investigations call for a compilation of information in this area, and it is hoped that the material presented will provide the stimulus for the better anaesthetic care that should be the lot of the pregnant woman and fetus.