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Cellular Effects of Isoflurane on Bulbar Respiratory Neurons in Decerebrate Cats

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ABSTRACT—Effects of isoflurane on the membrane potential trajectory and synaptic activity in bulbar respiratory neurons were investigated in decerebrate, vagotomized and artificially ventilated cats. A 2-min inhalation of 1.6% end-tidal concentration of isoflurane produced depolarization of the membrane in 10 out of 18 inspiratory, 8 out of 15 post-inspiratory and 5 out of 12 expiratory neurons and hyperpolarization in the rest of the population recorded in the ventral respiratory group. In both depolarized and hyperpolarized cells, periodically occurring excitatory and inhibitory synaptic waves were decreased, and input resistance was increased. Concomitantly, isoflurane reduced the excitatory and inhibitory postsynaptic potentials evoked by electrical stimulation of the vagus nerve, superior laryngeal nerve and cervical spinal cord. The effects of isoflurane on membrane potential and input resistance became negligible when excitatory and inhibitory synaptic potentials were suppressed by iontophoretically applied tetrodotoxin. The present results suggest that the respiratory neuronal responses induced by isoflurane are attributed mainly to the decrease of excitatory and inhibitory synaptic interactions in the bulbar respiratory network of neurons.

Keywords: Isoflurane, Respiratory neuron, Postsynaptic potential, Input resistance, Tetrodotoxin

Anesthetic agents are known to decrease the respiratory motor activities as a part of the general depression of the central nervous system (1-3). It was reported that isoflurane, one of the currently most-used volatile anesthetic, reduced the respiratory-modulated discharges of bulbar neurons and phrenic motoneurons in experimental animals (4, 5). However, since the previous studies used extracellularly recorded action potentials and nerve-bundle discharges as a marker of the neuronal response, information is still lacking about the cellular and synaptic mechanisms underlying the respiratory neuronal depression induced by isoflurane (2, 5). Recently, we demonstrated that the decrease of bulbar respiratory neuronal activities induced by a 90-sec inhalation of 2% halothane was due to generalized lessening of synaptic interactions in the respiratory neuronal network (6, 7). Hence, the present study was undertaken to examine the effects of isoflurane on the membrane potential trajectory and synaptic activity recorded from bulbar respiratory neurons and to compare them with the effects of halothane. To exclude any possible peripheral effects of the inhalational anesthetic (1-3) and to remove a mixed effect of the background anesthesia, we used decerebrate, vagotomized and artificially ventilated cats. In addition, a coaxial multibarrelled microelectrode (8) was used in some experiments, which allowed an intracellular recording combined with an extracellular iontophoresis of drugs. This technique would discriminate possible pre- and postsynaptic effects of isoflurane by selectively eliminating action potentials and postsynaptic potentials in the recorded neuron (8-10).

MATERIALS AND METHODS

Surgical procedures

Experiments were carried out on 26 cats weighing 2.5-3.8 kg. They were initially anesthetized with halothane for tracheal intubation and cannulation into the femoral vein, femoral artery and urethra. The head of the animal was mounted on a stereotaxic frame, and midcollicular decerebration was performed according to the procedures described by Kirsten and St. John (11). The phrenic nerve, vagus nerve, carotid sinus nerve and superior laryngeal nerve were exposed and cut bilaterally. A C2-C3 laminectomy and an occipital craniotomy were carried out to expose the spinal cord and the medulla oblongata, respectively. A wide pneumothorax was performed on both sides to decrease the movement of the brain stem associated with ventilation. Then halothane

anesthesia was discontinued. The animals were paralyzed with pancuronium bromide (0.3 mg/kg initially and 0.1 mg/kg hourly), and the lungs were ventilated mechanically with oxygen-enriched air. Tracheal pressure was kept below 8 cmH_2O at the maximum lung inflation. An expiratory flow resistance of 1–2 cmH_2O was applied to prevent the collapse of the lung. The end-tidal concentrations of O_2 and CO_2 were maintained at 28–32% and 4–5%, respectively, by adjusting the rate of ventilation and oxygen mixture at a fixed tidal volume of 10 ml/kg. Arterial blood pressure was continuously monitored and kept higher than 80 mmHg by infusing lactated Ringer solution as required. Rectal temperature was maintained at 37–38℃ by a heating pad.

Recording procedures

The central end of the phrenic nerve was desheathed and placed on bipolar recording electrodes immersed in warmed mineral oil. The efferent phrenic activities were amplified, rectified and integrated by a leaky integrator with a 0.1 sec time constant. Respiratory neurons were sought in the ventral respiratory group (VRG) region (12), extending 2.0–4.3 mm lateral to the midline, 4.0 mm caudal to 2.0 mm rostral to the obex, and 1.6–4.7 mm below the dorsal surface. Membrane potentials were recorded with a single glass micropipette or with the center pipette of a coaxial multibarrelled electrode, either of which was filled with 2 M potassium-citrate and had a resistance of 20–40 MΩ. Neurons were identified by their patterns of membrane potential fluctuations in relation to phrenic activity (13). Axonal projections of these neurons were examined by antidromic stimulation. Stimulation pulses were applied ipsilaterally to the vagus nerve or superior laryngeal nerve through a bipolar silver wire electrode using a 0.2-msec pulse of 1.5–2.0 V intensity and were applied bilaterally to the ventrolateral parts of C2–C3 spinal cord through an array of 5 concentric stimulating electrodes with 0.2-msec pulses at 10–15 V. For current clamping and measurement of input resistance, intracellular currents were applied through a recording electrode by a high-frequency current injection and voltage-sampling method (model 8100-1, Dagan Corp., Minneapolis, MN, USA). Intracellular injection of Cl^- was achieved by passing continuous currents of between −5 and −7 nA for 10–30 min through a low resistance electrode (8–10 MΩ) filled with 3 M KCl solution.

Drug administration

Tests were started at least 4 hr after discontinuation of halothane anesthesia. Isoflurane (Forane, Abbott Labs., Abbott Park, IL, USA) was vaporized in the inspired oxygen-enriched air by a precalibrated evaporator and administered through the endotracheal tube. The end-tidal concentration of isoflurane was continuously monitored with an anesthetic gas analyzer (Capnomac, Datex Corp., Helsinki, Finland). Since repeated tests had to be performed with the same neuron to keep a stable recording, we selected only one dose acting for a relatively short period. Preliminary experiments revealed that a 2-min inhalation of 1.6% end-tidal concentration of isoflurane produced a consistent effect on phrenic discharge, which was comparable to the effect observed with a 90-sec inhalation of 2.0% halothane (6, 7). Thus, this dose appeared to be suitable for the present study (see Results). The end-tidal concentration of 1.6% was achieved within 30 sec when isoflurane was introduced at the inspired concentration twice as large as that concentration (3.2%). This concentration was maintained steadily in the following 2 min, with the inspired concentration being held at 1.8%. The peak depressant effect in phrenic discharge was obtained at around 2.5 min after the predetermined end-tidal concentration was attained (30 sec after the end of inhalation) and continued 2–3 min thereafter. When the inhalation was discontinued, the end-tidal concentration of isoflurane decreased to near zero within 3 min, and the phrenic activity returned to the control level 20–30 min after the end of isoflurane inhalation.

Tetrodotoxin (TTX) was dissolved at 0.5 mM in 165 mM NaCl solution (pH 6.5), contained in a peripheral drug pipette of the coaxial multibarrelled electrode (8), and ejected by positive pulses of between 25 and 50 nA. A retaining current of −3 nA was applied between test periods. A saline pipette (165 mM NaCl) was used both as a current balance and a drug control.

Data analysis

Values of the membrane potential fluctuations were measured at the most hyperpolarized point during the non-spiking or inactive phase and at the most depolarized point during the spiking or active phase in each respiratory cycle. The effect of isoflurane was evaluated at the peak effect after inhalation. These values were averaged for each group of the respiratory neuron. Data were presented as means ± S.E. Since all values were paired data, differences between the mean values before and after the drug application were analyzed by a paired *t*-test (two-sided). Statistical significance was assumed at $P < 0.05$.

RESULTS

Effects of inhalation of isoflurane on phrenic discharge

A 2-min inhalation of 1.6% end-tidal concentration of isoflurane produced a consistent depressant effect on phrenic nerve discharge. It decreased the peak amplitude of the integrated phrenic neurogram to 64.1 ± 5.9%

(n=11) of the control value and shortened both the inspiratory time (Ti) and expiratory time (Te), with the mean values of percent change being 80.6±4.3% and 84.5±7.2% (n=11), respectively (Figs. 1–3 and 5). Concomitantly, this dose of isoflurane produced a slight fall in blood pressure ranging from 6 to 11 mmHg. Higher and longer doses of isoflurane caused a severe fall of blood pressure which often made a stable intracellular recording impossible, and therefore, one single dose was selected at 1.6% for 2 min for the detailed studies on membrane potentials in bulbar respiratory neurons.

Effects on the membrane potential trajectory

Intracellular recordings were achieved in 45 neurons consisting of 18 augmenting inspiratory (IN), 15 decrementing expiratory or post-inspiratory (PI) and 12 augmenting expiratory (EX) neurons (13). All neurons maintained stable membrane potentials of more than −50 mV during the control period and were successfully tested with isoflurane. Of these respiratory neurons, 12 cells were identified as laryngeal motor (LM) neurons, 7 cells as bulbospinal (BS) neurons, and 26 cells as non-antidromically-activated (NAA) neurons that were not antidromically activated by vagal and spinal cord stimulation (13). Their responses to isoflurane did not differ between LM, BS and NAA neuron groups, so that the subdivision of neuron type based upon the axonal projection was not made hereafter unless specifically stated. As was shown in Table 1, inhalation of isoflurane produced depolarization in nearly half of the neurons examined and hyperpolarization in the rest of the population. Figure 1 illustrates some examples of depolarizing and hyperpolarizing responses in these neurons.

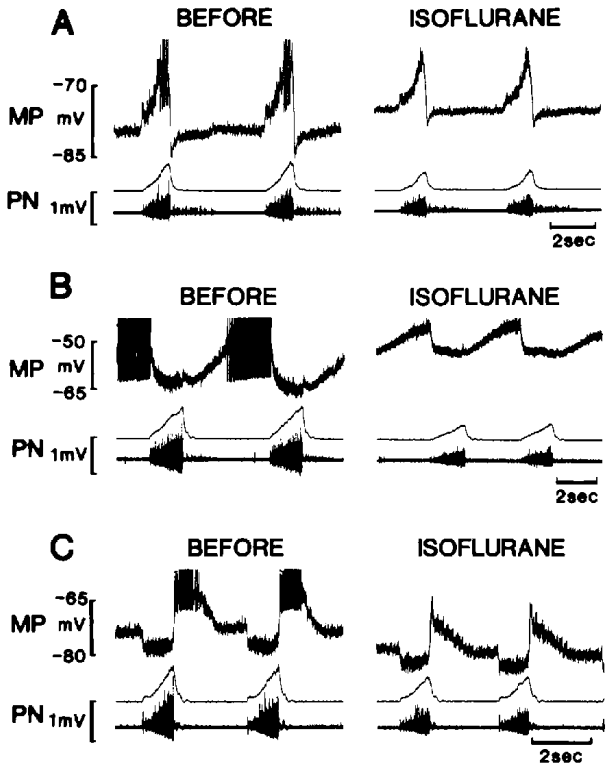

Fig. 1. Effects of isoflurane on membrane potential trajectory in augmenting inspiratory (A), augmenting expiratory (B) and post-inspiratory neurons (C). MP, membrane potentials; PN, integrated (upper trace) and raw (lower trace) activities of phrenic nerve. Records were taken before (left) and 1–2 min after the end of a 2-min inhalation of 1.6% isoflurane (right). Top parts of the action potentials are truncated.

The IN neuron displayed a ramp-like depolarization during inspiration and a sustained hyperpolarization during expiration (Fig. 1A). The initial, rapid hyperpolarization gradually attenuated as the phrenic post-inspiratory discharge subsided (corresponding to stage I expiration or post-inspiration) (13, 14). During stage II expiration when no phrenic activity was observed, the membrane remained hyperpolarized at a relatively steady level. The firing of action potential occurred during late inspiration (Fig. 1A). Isoflurane produced a depolarizing shift of membrane potential during a whole respiratory cycle in this IN neuron. Furthermore, it decreased the amplitudes of inspiratory ramp-depolarization and post-inspiratory rapid hyperpolarization, leading to a substantial decrease in the respiratory fluctuations of membrane potential. The spontaneous firing ceased completely (Fig. 1A).

The EX neuron showed a progressively increasing hyperpolarization during inspiration. A brief depolarization occurred at the transition to post-inspiration, and then an initially increasing and late decreasing hyperpolarizing wave ensued during post-inspiration. During stage II expiration, a steadily augmenting depolarization occurred together with spiking. Isoflurane depolarized this EX neuron and reduced the respiratory fluctuations of membrane potential. The firing of action potentials increased transiently in the first 10 sec after the start of isoflurane inhalation and arrested thereafter (Fig. 1B).

The PI neuron displayed a plateau-phase of depolarization during post-inspiration and an intermediate hyperpolarization during stage II expiration. A prominent hyperpolarizing wave appeared during inspiration. Spike discharge occurred only during post-inspiration. Isoflurane caused hyperpolarization, arrest of spiking and reduction of the rhythmic fluctuations in membrane potential of this PI neuron (Fig. 1C).

For all three types of neurons, isoflurane decreased synaptic noise and the membrane potential fluctuations during a whole respiratory cycle. Most EX and PI neurons displayed symmetrical synaptic waves during inspiration (Fig. 2A). Each wave oscillated at a period of 8–15 msec characteristic of the high-frequency-oscillations (HFOs)

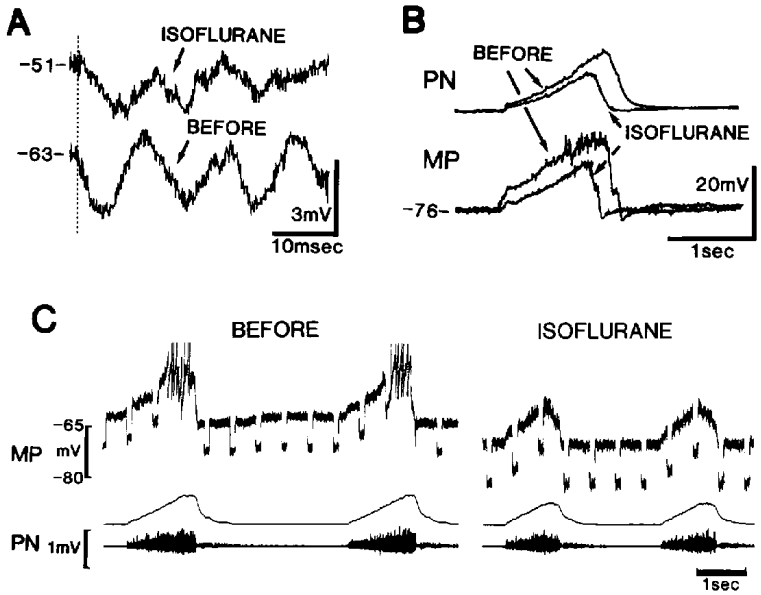

Fig. 2. Effects of isoflurane (1.6% for 2 min) on inhibitory synaptic noise (A), membrane potential trajectory (B) and input resistance (C) of three different respiratory neurons. A: synaptic noise recorded from a post-inspiratory neuron during inspiration or non-spiking phase of the respiratory cycle before (lower trace) and after isoflurane (upper trace). A dotted line denotes the start of a sweep triggered 100 msec after the beginning of the phrenic inspiratory discharge. Reference membrane potentials are shown on the left of each trace. B: superimposed traces of membrane potential (MP) of an inspiratory neuron and integrated phrenic neurogram (PN) taken before and after isoflurane (indicated by arrows). Each trace was made by cycle-triggered averaging in 5 consecutive respiratory cycles. A depolarizing current (0.7 nA) was injected into the cell after isoflurane to adjust the membrane potential to the control level. C: changes in input resistance in an inspiratory neuron measured with an intracellular current of a constant intensity (−1 nA, 100 msec, 2 Hz). Records were taken before (left) and 2 min after the end of a 2-min inhalation of 1.6% isoflurane (right).

Table 1. Effects of isoflurane on membrane potential in bulbar respiratory neurons

Response type:	Depolarization		Hyperpolarization	
	before	isoflurane	before	isoflurane
Neuron type				
IN	(n=10)		(n=8)	
MP	−68.7±2.0	−64.1±2.3*	−66.5±1.3	−68.5±1.2*
dMP	13.9±2.4	7.1±1.8*	13.3±2.3	8.5±1.2*
PI	(n=8)		(n=7)	
MP	−72.0±2.1	−65.5±2.7*	−73.8±1.1	−76.5±2.4*
dMP	10.5±1.9	4.8±1.4**	9.7±1.1	5.7±1.3*
EX	(n=5)		(n=7)	
MP	−66.0±3.3	−60.1±3.6*	−67.8±1.4	−71.2±2.2*
dMP	11.3±1.7	6.0±0.9*	9.6±1.2	7.3±2.0*

Neuron type: IN, PI and EX indicate inspiratory, post-inspiratory and expiratory neurons, respectively. MP: mean membrane potentials±S.E. (mV) measured at the most hyperpolarized point in each respiratory cycle. dMP: mean amplitudes of MP fluctuations presented as the difference between the most depolarized point and the most hyperpolarized point in the respiratory cycle. Data were obtained before and during the peak effect 1–3 min after the end of a 2-min inhalation of 1.6% isoflurane. The number of cells is indicated in parentheses. All values obtained after isoflurane are significantly different from the corresponding before values (**$P<0.01$, *$P<0.05$, paired t-test).

of membrane potential (6, 7, 15). Isoflurane decreased the amplitude of HFO waves, but had little effect on their oscillation rate (Fig. 2A). Figure 2B illustrates cycle-triggered averages of the membrane potential in an IN neuron and shows the integrated phrenic neurogram. Since isoflurane hyperpolarized this neuron, the membrane potential was adjusted to the control potential by a depolarizing current injection. This procedure could lessen the voltage-dependent effect on postsynaptic potentials. Superimposed traces of average membrane potential clearly revealed that isoflurane decreased the inspiratory ramp-depolarization in the IN neuron (Fig. 2B). In addition, the effect of isoflurane was consistent in a given cell when it was repeatedly tested with an interval of 20–30 min (n=15).

Table 1 summarizes the effect of isoflurane on membrane potential in IN, PI and EX neurons. Isoflurane depolarized the membrane in 23 neurons and hyperpolarized in the remaining 22 cells. The decrease of membrane potential fluctuations appeared to be consistent in all types of the respiratory neurons. The amplitudes of membrane potential changes induced by isoflurane did not differ between IN, PI and EX neuron groups. Inhalation of isoflurane caused a complete cessation of the spontaneous firing in 18 out of 23 depolarized cells and in 20 out of 22 hyperpolarized cells. Examples are shown in Fig. 1. In the other two hyperpolarized and five depolarized cells, the rhythmic firing continued to occur after isoflurane, but their firing rates were invariably reduced. For the latter five depolarized neurons, the amplitude of action potential was decreased and their duration was prolonged, the effects being similar to those observed in the stimulus-induced spikes (Fig. 4: A and C). Thus, isoflurane consistently decreased the spike discharge in IN, PI and EX neuron groups.

Effects on input resistance

Input resistance was measured in six neurons, of which four cells (two IN and two PI neurons) were hyperpolarized and two cells (one IN and one PI neuron) were depolarized by isoflurane. For all depolarized and hyperpolarized neurons, isoflurane increased input resistance during a whole respiratory cycle. For three IN neurons, mean values of the input resistance change induced by isoflurane were 135±17% of the control during inspiration and 157±21% during post-inspiration. These values for three PI neurons were 151±15% during post-inspiration and 166±18% during inspiration. An example for an IN neuron is shown in Fig. 2C.

Effects on inhibitory synaptic waves after intracellular injection of chloride ions

To examine isoflurane's effects on spontaneous waves of inhibitory postsynaptic potentials (IPSPs), Cl^- ions were accumulated in two IN and two PI neurons. For the PI neurons, injection of Cl^- reversed the hyperpolarizing synaptic waves to depolarizing potentials during inspiration and stage II expiration (Fig. 3A). Isoflurane produced a marked reduction of the reversed IPSP waves in both phases (Fig. 3B), which returned to the pre-application pattern 30 min after the end of inhalation (Fig. 3C). For the IN neurons, injection of Cl^- produced a reversal of hyperpolarizing waves to depolarization during stage I and II expiration. Isoflurane had a similar depressive effect on these reversed potentials (not illustrated).

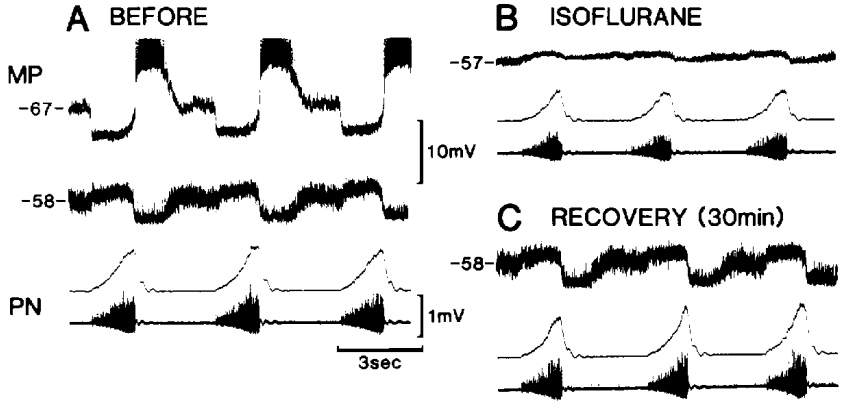

Fig. 3. Effects of isoflurane (1.6% for 2 min) on inhibitory synaptic waves after intracellular injection of Cl^-. A: membrane potential trajectories (MP) before (upper trace) and after intracellular injection of Cl^- with −8 nA iontophoretic current for 30 min through a 3 M KCl filled electrode (lower trace). PN: integrated and raw phrenic neurograms. B, C: membrane potentials after intracellular injection of Cl^-, taken 2 min (B) and 30 min (C) after administration of isoflurane. Records A–C were taken from the same post-inspiratory neuron. Reference membrane potentials are shown on the left of each trace.

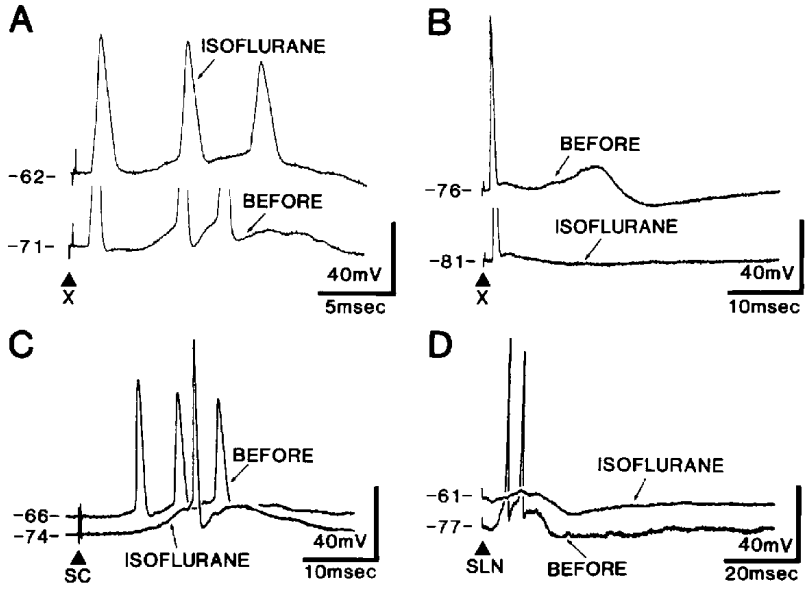

Fig. 4. Effects of isoflurane (1.6% for 2 min) on action potentials and postsynaptic potentials provoked by stimulation of the vagus nerve (x), cervical spinal cord (SC) and superior laryngeal nerve (SLN). A: an antidromic action potential and succeeding excitatory postsynaptic potentials (EPSPs) associated with orthodromic spikes evoked by x stimulation in an inspiratory laryngeal motoneuron. Records were taken before (lower trace) and after isoflurane (upper trace). B: an antidromic action potential and succeeding EPSP and inhibitory postsynaptic potential (IPSP) waves induced by x stimulation in a post-inspiratory laryngeal motoneuron. C: superimposed traces of EPSP waves and associated orthodromic spikes induced by SC stimulation taken before (upper trace) and after isoflurane (lower trace) from an inspiratory non-antidromically-activated (NAA) neuron. D: superimposed traces of EPSPs and associated orthodromic spikes provoked by SLN stimulation in a post-inspiratory NAA neuron.

Effects on evoked action potentials and postsynaptic potentials

Stimulus pulses were applied during the non-spiking phase of the respiratory cycle either to the vagus nerve, superior laryngeal nerve or the cervical cord. Figure 4A illustrates an example of an IN-LM neuron. Stimulation of the vagus nerve provoked an antidromic action potential and a subsequent wave of excitatory postsynaptic potentials (EPSPs) associated with orthodromic spikes. Inhalation of isoflurane produced depolarization of the membrane, which was associated with a decrease in the amplitude and an increase in the duration of evoked action potentials. The firing threshold for the orthodromic spike was elevated, and the occurrence of this spike was delayed. However, the conduction time of the antidromic action potential remained unaltered.

Figure 4B shows a PI-LM neuron in which an antidromic action potential and waves of EPSPs and IPSPs were successively elicited by a stimulus pulse applied to the ipsilateral vagus nerve. After inhalation of isoflurane, the membrane was hyperpolarized, and the stimulus-induced EPSPs and IPSPs were markedly reduced. However, the antidromic action potential was not blocked after isoflurane. Figure 4C illustrates an IN-NAA neuron which displayed an EPSP wave associated with orthodromic action potentials in response to stimulation of the cervical spinal cord. Isoflurane hyperpolarized the membrane and delayed the onset of the first orthodromic spike, of which the firing threshold was elevated from -62 mV to -56 mV. For another example of a PI-NAA neuron, stimulation of the ipsilateral superior laryngeal nerve evoked successive waves of EPSPs and IPSPs (Fig. 4D). Inhalation of isoflurane depolarized the membrane and decreased both EPSP and IPSP waves. Again, isoflurane raised the threshold for the spike generation, resulting in an occasional cessation of orthodromic action potentials. For all evoked EPSPs and IPSPs, isoflurane consistently decreased their amplitudes but had little effect on the start time of these postsynaptic waves after each stimulus pulse.

Effects on membrane potential after iontophoresis of tetrodotoxin

Iontophoresis of TTX had a consistent effect on all the respiratory neurons tested (3 IN, 2 PI and 2 EX neurons). It eliminated both spontaneous and antidromically-induced action potentials in the first 10–20 sec of iontophoresis (Fig. 5A). During a subsequent 2-min iontophoresis, TTX progressively decreased the active phase depolarization and the inactive phase hyperpolarization, leading to

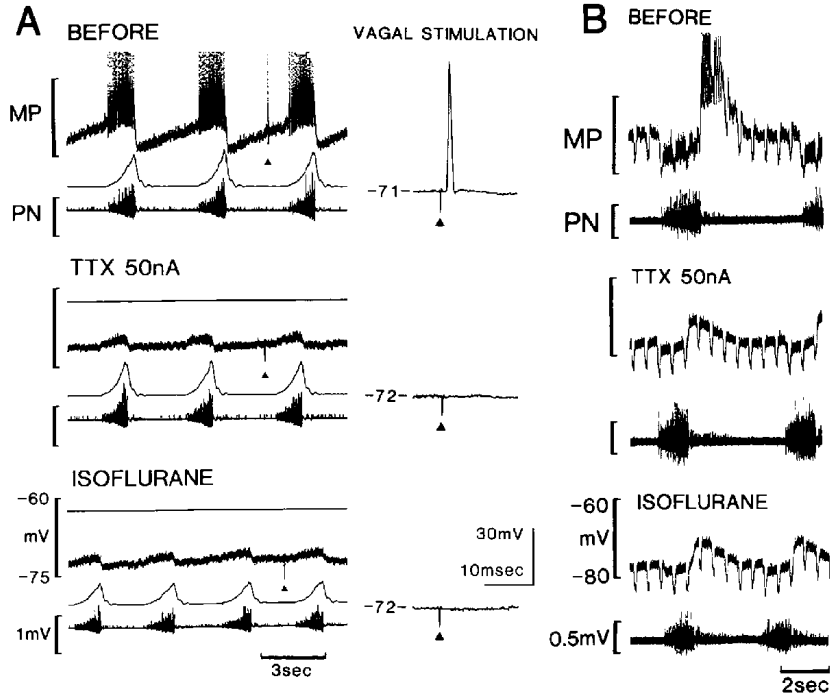

Fig. 5. Effects of isoflurane on membrane potential trajectory (A) and input resistance (B) after iontophoresis of tetrodotoxin (TTX). A: membrane potential trajectories recorded from an inspiratory laryngeal motoneuron before (upper trace) and during iontophoresis of TTX in the absence (middle trace) and presence of isoflurane (lower trace). TTX was iontophoresed during the bar at 50 nA for 5 min. Solid triangles denote the time of vagal stimulation to induce an antidromic action potential, shown at a faster sweep in the right column. B: changes in input resistance in a post-inspiratory neuron treated with iontophoresed TTX (50 nA for 2 min) recorded before (middle trace) and after isoflurane (lower trace).

an important decline of the respiratory fluctuations in membrane potential (Fig. 5A). At the same time, TTX eliminated the stimulus-induced EPSPs and IPSPs. Furthermore, it increased input resistance during a whole respiratory cycle and made the periodical fluctuations of input resistance indistinguishable (Fig. 5B). However, when the effect of TTX on the membrane potential trajectory reached a steady-state after long iontophoretic pulses (usually 2 min), a residual depolarization still occurred during inspiration for the IN neuron (Fig. 5A), during post-inspiration for the PI neuron (Fig. 5B) and during stage II expiration for the EX neuron.

For the IN neuron, isoflurane decreased the duration of the inspiratory residual depolarization observed after TTX. This was due to the decrease of the inspiratory time (Fig. 5A). However, isoflurane caused neither depolarization nor hyperpolarization of the membrane in all the respiratory neurons treated with iontophoresed TTX (Fig. 5: A and B). Also, it did not change the input resistance after TTX (Fig. 5B).

DISCUSSION

A 2-min administration of 1.6% isoflurane produced a decrease in the amplitudes and durations of the respiratory discharges in the phrenic nerve. These effects were essentially the same as those previously observed with 2% halothane administered for 90 sec (6, 7). The concentration of isoflurane may hardly reach a steady-state level in the alveoli when inspired in such a short period (2, 4). However, the end-tidal concentration reached a predetermined value within 30 sec when the inspired concentration was twice as large as that value. Moreover, as the end-tidal concentration was maintained for 2 min, the maximum effect was accomplished and lasted from 0.5 to 3 min after the end of inhalation. Hence, it is presumed that the end-tidal concentration of isoflurane was approaching a peak level around 0.5–3 min after the end of a 2-min inhalation period when a fairly steady-state effect was achieved in the respiratory neural activities.

Isoflurane produced membrane depolarization in nearly half of the VRG neurons examined and hyperpolarization in the rest of the population. However, each respiratory neuron exerted a consistent response to repeated administration of isoflurane. These results are in good agreement with the results obtained with halothane (6, 7) and thiopental (6). It has been reported that general anesthetic agents exert both excitatory and inhibitory effects in

various types of central neurons (16–19). Moreover, halothane was found to produce two contrasting effects on paired respiratory neurons simultaneously recorded either in both sides of the VRG with two different microelectrodes or in a very restricted area with a single extracellular electrode (7). This means that the different neuronal responses to an anesthetic agent were not due to differences in recording sites, local anesthetic concentrations or general conditions of the animals. Rather, this can be interpreted as indicating that the anesthetic agent produces a specific and selective effect on each respiratory neuron (5–7, 20, 21).

Both depolarizing and hyperpolarizing effects of isoflurane became negligible after iontophoresis of TTX. This specific blocker of the fast Na^+ channel has been shown to block action potentials in pre- and postsynaptic elements and, consequently, eliminate postsynaptic potentials in the recorded neuron (8–10). If isoflurane were acting directly on any ionic conductance in the postsynaptic membrane such as K^+, Cl^-, Ca^{2+}, or persistent Na^+-conductances (10, 16–19, 22, 23), the effects would still occur after application of TTX. However, this was not observed. Depressive effects of isoflurane were clearly seen in Cl^--dependent IPSPs and inhibitory HFO waves as well as in stimulus-induced EPSPs and IPSPs. Furthermore, isoflurane decreased synaptic noise and membrane potential fluctuations and increased input resistance, the effects that have been observed in all IN, PI and EX neurons. Therefore, both depolarizing and hyperpolarizing effects of isoflurane can be ascribed to a decrease in excitatory and inhibitory synaptic drives (16–19). This is in accordance with the results observed in hippocampal (24) and neocortical neurons (25, 26). Moreover, the decrease of synaptic potentials took place without any significant change in the conduction time of antidromic spikes and postsynaptic potentials, indicating that the agent has little effect on axonal conduction in the bulbar respiratory network (1, 6, 17). Hence, the decrease of synaptic drives is attributable to the decrease in the presynaptic neuronal activities and the consequent decrease in the release of the transmitters involved. The membrane potential trajectories of bulbar respiratory neurons are shaped principally by periodically arriving excitatory and inhibitory synaptic drives (8–10, 13, 27). Also, both tonic excitatory and tonic inhibitory synaptic inputs are responsible for the maintenance of membrane potential in each respiratory neuron (6, 8–10). Accordingly, it is assumed that whether a given neuron responds to an anesthetic drug with depolarization or hyperpolarization may reflect the differences in the intensity of either of these excitatory and inhibitory synaptic drives during the drug-induced respiratory depression (6, 24–26).

Isoflurane depressed the firing activity both in hyperpolarized and depolarized neurons. In the depolarized cells, elevation of the firing threshold, decrease in the amplitude and increase in the duration of action potential were commonly observed. These changes seem to be the consequence of excessive depolarization of the cell membrane, which may lead to inactivation of the fast Na^+ conductance and activation of the voltage-dependent K^+-conductances (16–19). However, the increase in K^+-conductance is unlikely a leading mechanism to account for the present results, because a decrease in input resistance was not observed in any case. In addition, while isoflurane invariably decreased the firing in these depolarized cells, a similar dose of halothane produced an increase of spiking in some VRG neurons (6, 7). Therefore, it is assumed that this dose of isoflurane is more depressive on the spike discharge than halothane (4).

Finally, the residual depolarization that was seen after iontophoresis of TTX was shortened by isoflurane. It is postulated that this residual potential is due to rhythmic synaptic potentials generated remotely where a sufficient concentration of TTX is not achieved (8–10). Furthermore, isoflurane decreased the Ti and Te periods, the effect commonly observed with many inhalational anesthetics (1–7, 20, 21, 28–30). One respiratory cycle can be functionally divided into three phases: inspiration and stages I and II of expiration (13, 14). Each sub-phase corresponds to the behavior of IN, PI and EX neurons (13, 14, 27). Isoflurane consistently decreased the firing activity in the three types of respiratory neurons. Hence, the decrease of the respiratory period is attributable to the decrease in the duration of the active phase of each type of neuron. In conclusion, the present results suggest that the respiratory neuronal depression induced by isoflurane are ascribed principally to the decrease in synaptic interactions in the bulbar respiratory network of neurons.

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