Effects of Isoflurane on Contractile Properties of Diaphragm


Isoflurane has been shown to depress skeletal muscle force in vitro, but data are not available regarding the effects of isoflurane on diaphragmatic muscle function in vivo. To answer this question, 15 rats anesthetized with pentobarbital and mechanically ventilated were studied. They were divided into three groups of five animals each, according to the administered concentration of isoflurane. Diaphragmatic function was assessed by measuring the transdiaphragmatic pressure (Pdi) generated during bilateral supramaximal phrenic nerve stimulation at 0.5 Hz, 20 Hz, 50 Hz, and 100 Hz under quasi-isometric conditions. After a control measurement (C), isoflurane was administered at a constant concentration (0.5, 1, or 1.5 MAC) and Pdi measurements were repeated after 30 min of isoflurane exposure (T1) and 30 min after discontinuing isoflurane (T2). In the group breathing 1.5 MAC isoflurane, the time constant of diaphragmatic relaxation (τ) and integrated electrical activity of the diaphragm (Edi) were also assessed. The Pdi amplitude generated by single twitch (0.5 Hz) was unchanged at the three isoflurane concentrations. A significant increase in Pdi at 20 Hz was observed at T1, which returned to control after 30 min recovery (T2). No change in Pdi during 50 Hz stimulation was noted during 0.5 and 1 MAC isoflurane exposure, whereas it was reduced at T1 during 1.5 MAC. For 100 Hz stimulation, a significant decrease in Pdi was noted for all groups at T1, which returned toward control values at T2. Edi was markedly reduced for 50 and 100 Hz stimulation, but this reduction was also transient, since Edi returned toward control values at T2. After 30 min isoflurane, τ was significantly increased compared with control values (16 ± 2 ms versus 22 ± 4 ms, P < 0.02). These results demonstrate that the effects of isoflurane on diaphragmatic force are related to the frequency of stimulation of the muscle. For 20 Hz stimulation, the enhancement of diaphragmatic pressure generation may be caused by the increased τ, which leads to a tonic contraction for lower frequencies of stimulation. By contrast, depression of Pdi at 50 and 100 Hz was in part due to impaired neuromuscular transmission, as assessed by the decrease in Edi during isoflurane. (Key words: Anesthetics, volatile; isoflurane. Muscle, diaphragm: contractility.)

IT HAS BEEN RECENTLY demonstrated that the contractile properties of the diaphragm may be modified by pharmacologic interventions. For example, digoxin1 and aminophylline2 enhance diaphragmatic contractility, whereas halothane markedly depresses diaphragmatic function.3 While isoflurane has been shown to depress skeletal muscle force of contraction in vitro,4 no data are available regarding the effects of isoflurane on the contractile properties of the diaphragm in vivo. Therefore, the aim of the present study was to assess, in intact rats, the effects of isoflurane on diaphragmatic function.

Materials and Methods

Fifteen rats, weighing 380-420 g, were anesthetized with pentobarbital sodium (5 mg/100 g body wt) intraperitoneally. The experimental protocol was in agreement with the recommendations regarding the animal studies of the French Law (Ministère des Affaires Sociales et de la Solidarité Nationale). Following tracheostomy, the animal lungs were mechanically ventilated using a rodent ventilator throughout the experiment (FIO2 = 0.5). The left carotid artery was cannulated for arterial blood gas measurements and ventilation was adjusted if necessary in order to ensure PAcO2 between 37 and 42 mmHg before the beginning of the experiment. Rectal temperature was continuously monitored and maintained constant at 37°C. All the rats were studied while in the supine position. The 15 rats were divided into three groups of five animals each, according to the end-expired concentration of isoflurane which was maintained: 0.5 MAC in one group, 1 MAC in the second group, and 1.5 MAC in the third group.5 Isoflurane was administered using a continuous flow Cyprane Keighley vaporizer (Isotec, England), and end-expiratory concentration of isoflurane was measured with an infrared analyzer (Cosma 815, France).

Diaphragmatic function was assessed by measuring the transdiaphragmatic pressure (Pdi) generated during bilateral phrenic nerve stimulation. The thorax was opened through a large thoracotomy. Coil stimulation electrodes were positioned around each phrenic nerve above its pericardial course. The thorax was used to form a pool which was filled with mineral oil and maintained at 37°C. The phrenic nerves were stimulated using a Grass S48 stimulator (Grass Instruments, Quincy, MA) that delivered supramaximal (5V) equidistant square-wave pulse of 0.1 ms duration. Single twitch stimulations were performed first, followed by 1-s periods of stimulations applied at frequencies of 20, 50, and 100 Hz. During stimulations, the animals were apneic and the airways were occluded at functional residual capacity (FRC). Peak abdominal pressure (Pab) was measured using a thin-walled latex balloon (1.5 cm in length) containing 0.5 ml of air that was positioned via an abdominal incision beneath the diaphragm. The abdomen was closed in layers and the balloon was connected to one side of a differential pressure transducer by means of a polyethylene catheter. Since the thorax was opened throughout the experiment, pleural pressure (Ppl) was unchanged during phrenic nerve stim-

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ulation. Therefore, Pdi, which is the difference between measured changes in abdominal and pleural pressures, was equal to changes in Pab. Constancy of diaphragmatic geometry and muscle fiber length during contractions was achieved by placing a closely fitted plaster cast around the abdomen and the lower part of the rib cage. In the rats exposed to 1.5 MAC isoflurane, changes in diaphragmatic relaxation were assessed by determining the time constant of the exponential phase of the decay of Pdi generated following 0.5 Hz phrenic nerve stimulations. The time constant (τ) was calculated by regression analysis of data points from 70% peak Pdi. Electromyographic activity of the diaphragm (Edi) was recorded with electrodes directly placed into the costal part of each hemidiaphragm. They were positioned during the midline laparotomy performed for abdominal catheter placement (see above). The recording electrodes were fashioned from no. 12 fish hooks, insulated to nearly 3 mm from the tip. They were connected to a Dia 150G1 preamplifier (Dantec, Denmark), the output signals of which were rectified and integrated through a leaky integrator. A schematic representation of the experimental design is shown in figure 1.

The same protocol was followed in all the animals. After control measurements (C), isoflurane was added to the inspiratory gas. After 30 min of isoflurane exposure, diaphragmatic function was again assessed (T1). Then, isoflurane was discontinued and final measurements were performed after 30 min recovery (T2). In the group exposed to 1.5 MAC isoflurane, Edi was recorded during electrical stimulations of the phrenic nerve at C, T1, and T2 to assess the function of the neuromuscular junction and τ was also calculated at C and T1. All values are given as mean ± SEM. Intragroup and intergroup differences at the different times of the study were assessed using two-way analysis of variance. Changes in τ between C and T1 were analysed using a t test for paired data. The level of significance was set at P < 0.05.

**Results**

The effects of isoflurane on Pdi at the three different isoflurane concentrations are summarized in table 1. No significant difference between groups was noted during control and 30 min after isoflurane discontinuation. By contrast, after 30 min isoflurane, Pdi at 50 Hz was significantly reduced in the group breathing 1.5 MAC when compared with the groups exposed to 0.5 and 1 MAC isoflurane (P < 0.05) and the decrease in Pdi at 100 Hz was more important in groups 1 MAC and 1.5 MAC when compared with the group 0.5 MAC (P < 0.05 and P < 0.01, respectively). Changes in Pdi at 100 Hz were related to isoflurane concentration, since decrease in Pdi was most important in group 1 MAC than in group 1 MAC (P < 0.05). Figure 2 shows the average changes in Pdi for the different frequencies of stimulation during 0.5 MAC, 1 MAC, and 1.5 MAC isoflurane. While Pdi at 0.5 Hz stimulation remained unchanged for the three concentrations of isoflurane, a significant increase in Pdi for 20 Hz stimulation was noted after 30 min isoflurane, which averaged 25% with 0.5 MAC isoflurane (P < 0.001), 22% with 1 MAC (P < 0.001), and 22% with 1.5 MAC (P < 0.05). For 50 Hz stimulation, no change

\[ \text{Table 1. Pdi Values at the Different Times of the Study in the Three Groups of Animals} \]

| (Group 1: 0.5 MAC, Group 2: 1 MAC, and Group 3: 1.5 MAC isoflurane) |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | Control                     | 50 Min Isoflurane (T1)     | 50 Min Recovery (T2)       |
|                             | Group 1                     | Group 2                     | Group 3                     | Group 1                     | Group 2                     | Group 3                     |
| Twitch (cm H2O)             | 7.8 ± 0.8                   | 7.8 ± 1.6                   | 7.6 ± 1.5                   | 7.4 ± 1.9                   | 7.2 ± 1.4                   | 7.6 ± 1.0                   | 7.3 ± 1.7                   | 7.7 ± 1.1                   |
| 20 Hz (cm H2O)              | 10.0 ± 2.0                  | 10.5 ± 2.6                  | 8.8 ± 1.6                   | 12.7 ± 5.3                  | 10.3 ± 1.4                  | 10.5 ± 2.0                  | 11.7 ± 3.4                  | 10 ± 0.8                    |
| 50 Hz (cm H2O)              | 42.3 ± 7.7                  | 40.3 ± 8.1                  | 40.8 ± 2.3                  | 41.8 ± 9.7*                 | 24.8 ± 4.2                  | 39.7 ± 8.8                  | 39.3 ± 7.6                  | 42.6 ± 4                    |
| 100 Hz (cm H2O)             | 45 ± 8.4                    | 39.6 ± 8.5                  | 41.0 ± 8.7                  | 39.0 ± 7.5*†                | 18.3 ± 3.9*†                | 41 ± 8.7                    | 41.1 ± 8.3                  | 42.7 ± 5.4                  |

Mean ± SD.

* P < 0.05 vs. group 3.

† P < 0.05 vs. group 1.

‡ P < 0.01 vs. group 1.
in Pdi was noted during isoflurane exposure at 0.5 and 1 MAC, whereas a marked reduction in Pdi which averaged 40% was observed at 1.5 MAC when compared with control \((P < 0.01)\). During 100 Hz stimulation, a significant decrease in Pdi was observed in all groups after 30 min isoflurane exposure. Transdiaphragmatic pressure was decreased by 13\% \((P < 0.05)\) in group 0.5 MAC, by 30\% \((P < 0.001)\) in group 1 MAC, and by 59\% \((P < 0.001)\) in group 1.5 MAC. In all groups, a complete recovery of Pdi was observed 30 min after isoflurane discontinuation for all frequencies of stimulation. Pdi values at T2 were not different from control values. In the group breathing 1.5 MAC, no significant change in Edi was observed after 30 min isoflurane exposure for 0.5 Hz and 20 Hz stimulation (fig. 3). By contrast, Edi during 50 and 100 Hz stimulation was markedly reduced at T1 when compared with control values \((P < 0.05\) and \(P < 0.001\), respectively), indicating a marked impairment in neuromuscular transmission. As shown in figure 4, \(\tau\) was significantly increased after isoflurane exposure when compared with the control period \((16 \pm 2\) ms \(vs.\ 22 \pm 4\) ms, \(P < 0.02\)).

**Discussion**

The main finding of this investigation is that the effects of isoflurane on diaphragmatic function depend upon the end-expiratory concentration of isoflurane and upon the frequency at which the muscle is stimulated. Thus, isoflurane increases the transdiaphragmatic pressure for 20 Hz stimulation. At 50 Hz, which is considered as the physiologic range of intrinsic neural firing rates,7 Pdi is un-
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changed under 0.5 and 1 MAC isoflurane, whereas it decreases during 1.5 MAC. At 100 Hz stimulation, a decrease in diaphragmatic force generation is noted at three isoflurane concentrations.

In this study, diaphragmatic function was assessed by the Pdi measured at different frequencies of stimulation. This method has been extensively used in dogs and more recently in rats. In our rat model, a maximum isolation of the diaphragm in vivo at relatively constant length was achieved by the opening of the thorax. Isoflurane-induced changes in lung volumes and pulmonary compliances could interfere with Pdi measurements by producing alterations in diaphragmatic shape. Therefore, the purpose of the open thorax was to ensure a constant end-tidal diaphragmatic length in order to eliminate the influence of possible alterations in lung mechanics on diaphragmatic function generation. Furthermore, the cast placed around the abdomen minimized outward displacement of the abdominal wall, limiting changes in diaphragmatic length during contraction. It has been shown, in the dog, that, with the cast in place, shortening of diaphragmatic length was <10% at any frequency of stimulation and thus did not affect the tension produced for a given stimulus. Thus, it is possible to ensure that diaphragmatic contractions in our model were "quasi-isometric." In addition, Pdi generated during diaphragmatic stimulation also depends upon the abdominal wall compliance, a parameter which could be influenced by isoflurane. This effect should be minimal in our rats, since the abdominal plaster cast ensured a constant abdominal displacement during stimulation. Tightness of fit of the cast may have also been a factor influencing Pdi generation by interfering with abdominal pressure and diaphragmatic length. This factor, however, does not seem of major importance, since we did not observe important inter-animal differences in Pdi, although tightness of fit of the cast was not controlled (table 1).

Since the rats were initially anesthetized with pentobarbital sodium, we actually examined the combined effects of isoflurane and pentobarbital. This agent might also affect diaphragmatic function. However, it has been previously reported that pentobarbital, at pharmacologic concentrations, does not affect peripheral muscle contractility. Although no data are available regarding the effects of pentobarbital on diaphragmatic function, the absence of significant changes in Pdi between control and T1 period strongly suggests that the effects reported during isoflurane are mainly related to this agent.

The search for mechanisms underlying the changes in muscle tension development during isoflurane administration may be helped by the analysis of the Pdi-frequency curves. The changes in Pdi during high-frequency stimulation (50–100 Hz) are related to alterations in membrane excitability, whereas changes during low frequency (20 Hz) stimulation are attributed to alterations in the excitation-contraction coupling process. In the present study, Pdi measured during 100 Hz stimulation was decreased. This change was associated with a parallel decrease in Edi when 1.5 MAC isoflurane was administered. As Edi reflects diaphragmatic fiber activation, our findings suggest that the decrease in Pdi for 100 Hz stimulation is related to an impaired neuromuscular transmission during isoflurane administration. The extent of the Pdi reduction appears directly related to the isoflurane concentration. Similar results have been reported in humans when the ulnar nerve was stimulated under isoflurane anesthesia. Waud and Waud have shown that isoflurane blocks the capacity of carbachol to depolarize the nerve membrane but does not alter the affinity of acetylcholine receptors for d-tubocurarine, suggesting a pre-junctional action of this drug. By contrast, in our study, an enhancement of diaphragmatic force generation was noted during 20 Hz stimulation during isoflurane administration. This increase in Pdi when phrenic nerves are stimulated supramaximally at 20 Hz may be secondary to an increased diaphragmatic contractility. However, if improved diaphragmatic contractility was responsible for this
effect, greater Pdi generation for other frequencies of stimulations should also have been observed. In fact, during 0.5 Hz stimulation, no change in peak Pdi was noted in spite of a constant diaphragmatic electrical activity. Consequently, increased diaphragmatic contractility is unlikely under isoflurane. On the other hand, the prolonged diaphragmatic relaxation induced by isoflurane may contribute to the increase in Pdi for 20 Hz stimulation. Indeed, when diaphragmatic relaxation rate is prolonged, greater fusion of the single twitch Pdi contractions occurs, especially at low frequencies of stimulation. The consequence of the increased mechanical fusion of Pdi complexes is to enhance diaphragmatic strength generation and produce a shift to the left of the Pdi-frequency curve for low frequencies of stimulation (i.e., 20 Hz). Changes in Pdi at 50 Hz depend on isoflurane concentration, since Pdi remains unchanged under 0.5 and 1 MAC isoflurane, whereas it decreases under 1.5 MAC isoflurane. These findings indicate that, at 50 Hz for low isoflurane concentrations, the mechanical enhancement in Pdi observed at 20 Hz did not occur or was masked by the depressive effect of isoflurane on the neuromuscular junction. At 1.5 MAC isoflurane, the reduction in Pdi is mainly due to the drug-induced neuromuscular junction depression as assessed by the concomitant decrease in Edi at 50 Hz stimulation.

Our findings demonstrate further that isoflurane-induced changes in diaphragmatic function differ from those observed during halothane. In rats breathing halothane, impairment of diaphragmatic function was present at all frequencies of stimulation and was attributed to a direct depressive effect of halothane on the muscle fibers. The exact mechanisms underlying the differing effects of isoflurane and halothane on diaphragmatic function in vivo remain unclear. Further studies are needed to evaluate the role of respiratory pump impairment on the respiratory depression produced by the halogenated anesthetics in clinical practice.

References