Enflurane Supresses Phrenic Nerve-diaphragm Transmission *in vivo*

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We examined the effects of enflurane on the diaphragmatic function in 15 pentobarbital-anesthetized, mechanically ventilated dogs. They were divided into three groups of five animals each, according to the administered concentration of enflurane. The diaphragmatic function was assessed from transdiaphragmatic pressure (Pdi) and integrated diaphragmatic electromyography (Edi) developed at functional residual capacity against an occluded airway during bilateral supramaximal phrenic nerve stimulation at 0.5, 10, 20, 50 and 100 Hz under quasi-isometric condition. After a control measurement, enflurane was administered at a constant end-expired concentration (0, 0.5 and 1 MAC) and the measurement was repeated after 1 hour of exposure. The Pdi amplitude generated by single twitch (0.5 Hz) and during 10, 20 and 50 Hz stimulation was unchanged between the groups. No change in Pdi during 100 Hz stimulation was noted during 0 and 0.5 MAC exposure, while it was reduced by 1 MAC of enflurane. When the values of Pdi were expressed as % of maximum Pdi (%Pdi,max) that developed during control measurement and analyzed in terms of %Pdi,max – stimulus frequency relationship, a significant decrease in %Pdi,max was noted for 100 Hz stimulation in 0.5 and 1 MAC groups compared to the control. Similarly, Edi during 100 Hz stimulation obtained in 0.5 and 1 MAC groups was markedly depressed compared to the control. Edi during 50 Hz stimulation was also decreased at 1 MAC. Relative changes in Edi following enflurane administration were greater than the corresponding changes of Pdi. These results demonstrate that enflurane impairs diaphragmatic function through its inhibitory effects on neuromuscular transmission. (Key words: anesthetics, volatile – enflurane, muscle, skeletal – diaphragm) diaphragm)

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Previously, we demonstrated that increasing enflurane concentrations produce progressive decreases in the force generation of the canine diaphragm in response to high stimulation frequencies[1]. Although selective loss of force at high frequency stimulation is generally considered to indicate failure of neuromuscular transmission and/or membrane excitation[2,3], it may not be entirely valid to apply this notion to the preparation used in the previous experiments since diaphragmatic force was indirectly evaluated by measuring the transdiaphragmatic pressure (Pdi) during phrenic nerve stimulations. Furthermore, previous report did not have control measurements; i.e. data in the absence of enflurane exposure. Thus, in the present study, we determined both

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evoked diaphragmatic electromyographic activity (Edi) and Pdi, and comparisons were made between the changes in Edi and Pdi induced by different levels of enflurane anesthesia in order to elucidate the site of action of enflurane on the diaphragm muscle.

Materials and Methods

Institutional approval for the study was obtained from the Animal Care and Use Committee of Chiba University School of Medicine. Fifteen adult mongrel dogs weighing between 10 and 19 kg were anesthetized with 30 $mg \cdot kg^{-1}$ of pentobarbital sodium at induction and 2 $mg \cdot kg^{-1} \cdot hr^{-1}$ during maintenance. All animals were supine, intubated, and mechanically ventilated with 100% oxygen. The left femoral artery and vein were cannulated for the measurement of arterial blood pressure and blood gas determinations and for fluid administrations. Esophageal temperature was continuously monitored and maintained constant at 37–38°C throughout the experiment. The 15 dogs were divided into three groups of five animals each, according to the end-expiratory concentration of enflurane which was maintained: 0 MAC in one group, 0.5 MAC in the second group, and 1 MAC in the third group. Enflurane was administered using a Cyprane vaporizer and end-expiratory concentration of enflurane was measured with an anesthetic gas analyzer (Datex, Normac).

Diaphragmatic function was assessed by measuring the transdiaphragmatic pressure (Pdi) and the electromyographic activity of the diaphragm (Edi) generated during bilateral phrenic nerve stimulation[4,5]. The thorax was widely opened through a sternotomy and the stimulation electrodes were positioned around each phrenic nerve at its entrance to the thorax. The phrenic nerves were stimulated using an electric stimulator (Nihon Koden SEN-3201) that delivered equidistant square-wave pulses of 0.2 ms duration. Supramaximal stimulation was determined by recording diaphragmatic twitches as the stimulation voltage was increased. Maximal response was achieved at 20–25 V. The voltage was then increased 10%–20% to ensure that stimulation remained supramaximal. Single twitch stimulations were performed first, followed by 2–3 s periods of stimulations applied at increasing frequencies of 10, 20, 50, and 100 Hz. Two stimulations were made at each frequency at 4 min intervals, and the average value of the two was used in the data analysis. During stimulations, the animals were apneic and the airways were occluded at functional residual capacity (FRC). Abdominal pressure (Pab) was measured using a thin-walled latex balloon (5 cm length, 1.0 ml air) positioned *via* an abdominal incision beneath the costal part of the diaphragm. The abdomen was closed in layers and the balloon was connected to the differential pressure transducer (Nihon Koden TP-601T). Since the thorax was opened throughout the experiment, pleural pressure was unchanged (i.e. atmospheric pressure) during phrenic nerve stimulation. Therefore, changes in Pdi were equal to the changes in Pab. Constancy of the diaphragmatic geometry and muscle fiber length during contractions was achieved by placing a closely fitted cast around the abdomen and the lower part of the rib cage[6]. Edi was recorded with fish-hook electrodes directly placed into the costal parts of each hemidiaphragm. They were positioned during the midline laparotomy performed for abdominal catheter placement and connected to a preamplifier (Nihon Koden EI-601G), the output signals of which were rectified and integrated through a leaky integrator (Nihon Koden AB-621G). Signals of arterial blood pressure, Pdi, raw and integrated electromyographic activity of both hemidiaphragms were recorded on an eight channel recorder (Nihon Koden WS-682G).

The same protocol was followed in all the animals. After control measurements, enflurane was added to the inspired gas (group 1, 0 MAC; group 2, 0.5 MAC; group 3, 1 MAC). After achieving 60 min of stable end-expiratory enflurane level, diaphragmatic function was again determined. In all animals arterial blood samples were withdrawn before starting each run of phrenic nerve stimulations to measure arterial blood

Table 1. Average (± SEM) values of mean arterial blood pressure (MAP), hydrogen ion concentration ($[H^+]$), Pa_{CO_2}, and Pa_{O_2} in the three groups of animals (Group 1:0 MAC, Group 2:0.5 MAC, Group 3:1 MAC)

		Control			Enflurane		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
MAP	(mmHg)	123 ± 10	108 ± 5	119 ± 6	128 ± 10	78 ± 6*	79 ± 3*
$[H^+]$	$(nmol \cdot l^{-1})$	39.2 ± 1.7	38.7 ± 2.9	39.6 ± 1.6	39.3 ± 1.2	36.9 ± 1.8	40.0 ± 0.9
Pa_{CO_2}	(mmHg)	33.4 ± 0.8	33.6 ± 1.4	34.2 ± 0.6	33.1 ± 1.0	32.4 ± 1.6	33.7 ± 0.6
Pa_{O_2}	(mmHg)	350 ± 76	376 ± 37	316 ± 38	345 ± 82	382 ± 20	328 ± 39

(*$P < 0.05$)

gasses and pH (Instrumentation Laboratories, IL1302). All values are given as mean ± SEM. Intragroup and intergroup differences were assessed using two-way analysis of variance for repeated measurements and the Tukey's test. The level of statistical significance was set at $P < 0.05$.

Results

Table 1 illustrates the average ± SEM values of mean arterial blood pressure, hydrogen ion concentration ($[H^+]$), Pa_{CO_2} and Pa_{O_2} in three groups under each experimental condition. Blood pressure was not statisticaly different between groups during the control period, but it decreased significantly with increasing enflurane concentration. Intragroup and intergroup differences in $[H^+]$, Pa_{CO_2} and Pa_{O_2} were not statisticaly significant.

Figure 1 shows representative recordings of Pdi, Edi and raw EMG of the diaphragm at various stimulation frequencies obtained during control period (fig. 1A) and during 1 MAC of enflurane exposure (fig. 1B). As illustrated in figure 1A, both Pdi and Edi increased with increasing stimulation frequencies from 0.5 to 100 Hz. There was no noticeable fade phenomenon either in Pdi or Edi even at stimulation frequency of 100 Hz. By contrast, Pdi with 50 and 100 Hz during 1 MAC enflurane showed a gradual decline in pressure during phrenic nerve stimulation (fig. 1B). Similar, and more prominent, fade phenomenon was also observed in Edi at 100 Hz. Pdi and Edi at stimulation frequencies of 0.5–20 Hz were essentially identical between control and enflurane exposure.

Table 2 demonstrates the mean ± SEM values of Pdi at stimulation frequencies of 0.5, 10, 20, 50 and 100 Hz in three groups under each experimental condition. There were no statistical differences among the control values of Pdi at each stimulation frequency between groups. In group 1 (0 MAC) and group 2 (0.5 MAC), Pdi during control and during enflurane administration were not different at any stimulation frequencies, while in group 3 (1 MAC) Pdi at 100 Hz stimulation was significantly reduced during enflurane exposure.

Figure 2 depicts force-frequency characteristics of the diaphragm during control period and enflurane exposure in three groups. Values of Pdi were expressed as the percentage of the maximum Pdi obtained in each animal during control period (%Pdi,max). As shown in figure 2A, average force-frequency relationship of the group 1 (0 MAC) animals was not altered during the course of the experiment. Similarly, 0.5 MAC of enflurane did not exert any significant changes in the force-frequency curve (fig. 2B). Whereas 1 MAC of enflurane significantly decreased %Pdi,max at stimulation frequencies of 50 and 100 Hz as compared to control which were determined in the absence of enflurane (fig. 2C).

Edi expressed as percentage of the control amplitude (Edi %control) was illustrated in figure 3 for three groups at each frequency of phrenic nerve stimulation. There

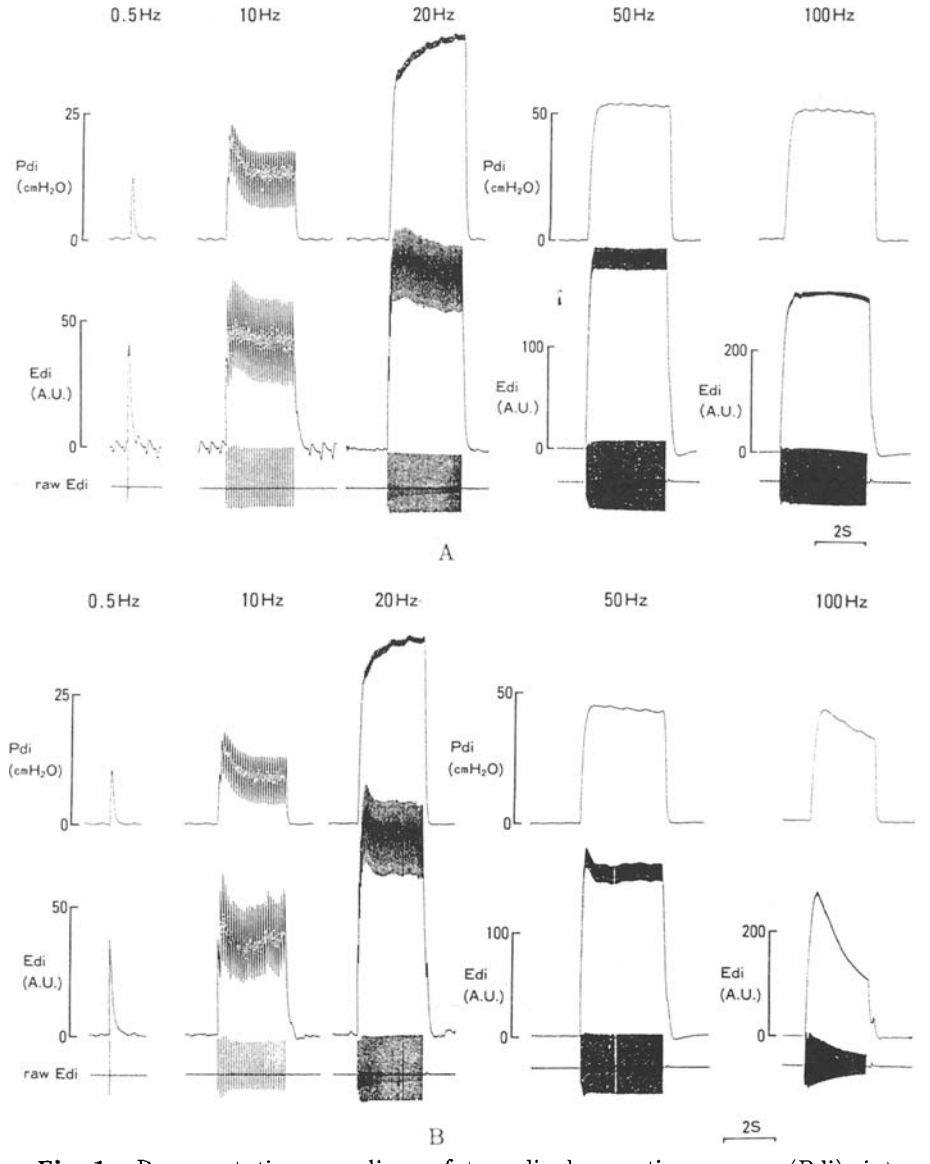

Fig. 1. Representative recordings of transdiaphragmatic pressure (Pdi), integrated electrical activity of diaphragm (Edi) and raw electromyographic activity at various frequencies of phrenic nerve stimulation in a dog. A:control, B:during 1 MAC of enflurane exposure.

were no differences in Edi between groups at stimulation frequencies of 0.5, 10 and 20 Hz. However, Edi at 50 Hz stimulation in group 3 (1 MAC) was significantly less than those obtained in group 1 (0 MAC) and group 2 (0.5 MAC). Similarly, Edi at 100 Hz stimulation in group 2 and 3 was significantly reduced compared to the value found in group 1. In order to quantify the difference between mechanical and electrical components of the depressant effects of enflurane on the diaphragmatic contraction, Pdi was also expressed as the percent of the control value in similar fashion to Edi. Figure 4 demonstrates relationship between Pdi %control and Edi %control. Since apparent changes in Edi were found only at 50 and 100 Hz of stimulations, data corresponding

Table 2. Pdi values at the control period and during enflurane exposure in the three groups of animals (Group 1:0 MAC, Group 2:0.5 MAC, Group 3:1 MAC) values are mean ± SEM

		Control			Enflurane		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Twitch	(cmH_2O)	11.1 ± 1.4	10.7 ± 1.0	11.9 ± 1.4	10.4 ± 1.3	10.7 ± 1.5	10.9 ± 2.0
10 Hz	(cmH_2O)	9.8 ± 1.6	10.5 ± 1.0	14.8 ± 3.0	8.7 ± 1.1	11.3 ± 2.1	14.9 ± 4.0
20 Hz	(cmH_2O)	37.8 ± 4.2	48.6 ± 6.4	48.0 ± 6.4	36.6 ± 4.1	49.5 ± 6.9	45.3 ± 6.3
50 Hz	(cmH_2O)	59.1 ± 7.7	70.2 ± 6.3	69.5 ± 5.7	59.1 ± 7.1	68.1 ± 6.5	62.2 ± 7.1
100 Hz	(cmH_2O)	61.0 ± 7.6	70.0 ± 6.0	70.0 ± 5.3	61.3 ± 6.8	62.9 ± 9.3	52.2 ± 8.5*

(*$P < 0.05$)

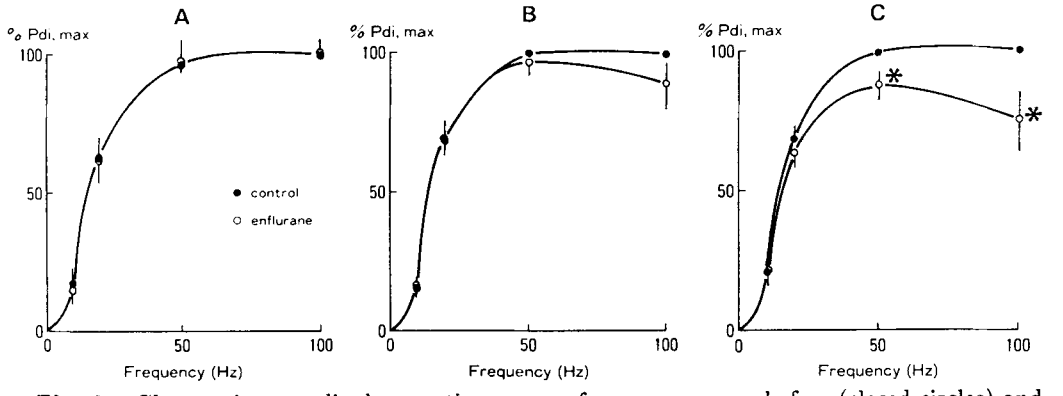

Fig. 2. Changes in transdiaphragmatic pressure-frequency curves before (closed circles) and after (open circles) enflurane administrations. A, B and C represent group 1 (0 MAC), group 2 (0.5 MAC) and group 3 (1 MAC), respectively. Pdi is expressed in percent of maximum Pdi generated during control period (Pdi,max). Values are mean ± SEM. *$P < 0.05$.

to 0.5, 10 and 20 Hz were not included in the identity plot. It can be seen from this figure that the reduction in Pdi was always smaller than the corresponding decrease in Edi.

Discussion

The main findings of this study are that enflurane, at end-expiratory concentration of 1 MAC, reduces the strength of contraction (as assessed by the Pdi-frequency characteristics) of the diaphragm under pentobarbital anesthesia and that such effect of enflurane is primarily mediated through alteration in membrane excitability and/or neuromuscular transmission.

In agreement with our previous observations, Pdi measured during high frequency stimulation (50 and 100 Hz) was selectively decreased while Pdi developed during low frequency stimulation (0.5–20 Hz) was not affected by enflurane administration. Such tetanic fade phenomenon is generally considered to reflect an impaired neuromuscular transmission. In addition, as shown in figure 4, Edi during high frequency stimulation decreased even more greatly than Pdi did. Since Edi reflects diaphragmatic fiber activation, our findings of decreased Edi for a given stimulation strongly suggest that enflurane impaired neuromuscular transmission in phrenic nerve-diaphragm preparation *in vivo*. Although it would be necessary to examine the force-stimulation frequency relations by directly stimulating the muscle to

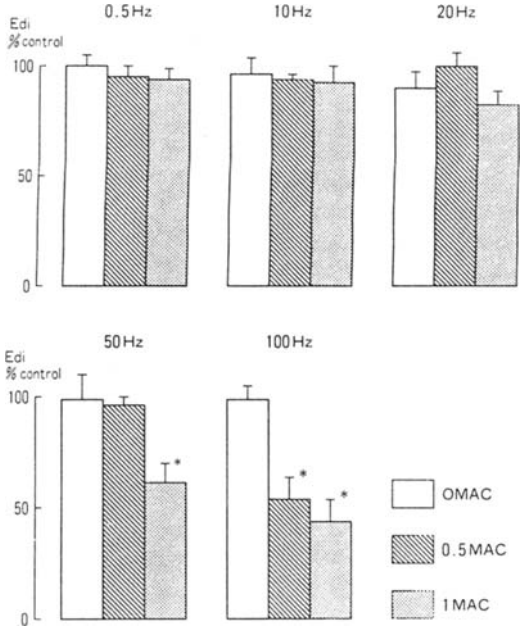

Fig. 3. Mean percent changes in integrated electromyographic activity of diaphragm (Edi) of three groups of animals for phrenic stimulations at 0.5, 10, 20, 50 and 100 Hz after enflurane exposure. Values are mean ± SEM. $*P < 0.05$.

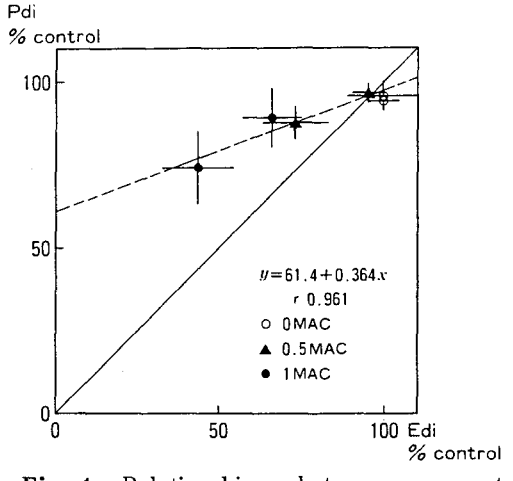

Fig. 4. Relationship between percent changes in transdiaphragmatic pressure (Pdi) and percent changes in electromyographic activity of diaphragm (Edi) of three groups of animals for phrenic nerve stimulations at 50 and 100 Hz.

clearly demonstrate the lack of direct effects of enflurane on the diaphragm muscle itself, it would be practically difficult to perform direct stimulation of the "whole" muscle in

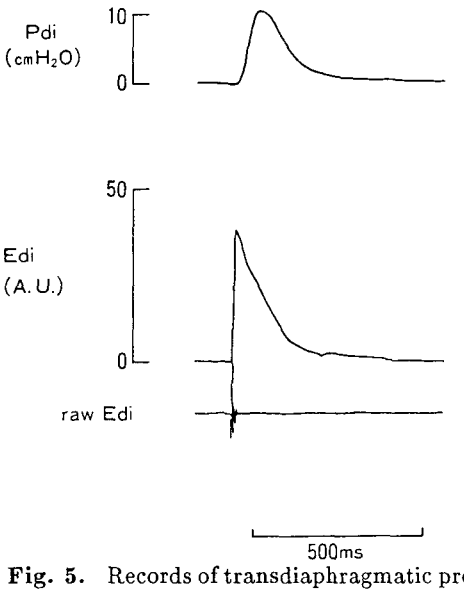

Fig. 5. Records of transdiaphragmatic pressure (Pdi), integrated (Edi) and raw (raw Edi) electromyographic activity of the diaphragm. Note electrical response is fater than mechanical response both during contraction and relaxation.

the present experimental conditions. In this connection, Waud and Waud have demonstrated, using isolated guinea pig nerve-lumbrical muscle preparations, that enflurane at a concentration of 1.5–2.5 MAC depressed twitch tension to nerve stimulation. By contrast, depression of twitch tension to direct stimulation of the muscle was achieved at much higher concentration, namely 6–8 MAC[7]. Thus it would be unlikely that the direct action of enflurane on the diaphragm muscle itself contributed to the present results. Furthermore, if enflurane had exerted a direct effect on the myocytes, decrease of Pdi during high frequency stimulation would have been greater than that of Edi, which is clearly not the case.

The fact that the reduction of Edi at high frequency stimulation was greater than that of Pdi is in marked contrast to the findings obtained during diaphragmatic muscle fatigue[4,8], in which Pdi is predominantly reduced at low frequency stimulations while Edi is relatively unaffected. The greater depression of Edi as compared to Pdi observed in the present experiments probably reflects

greater fusion of the single twitch Pdi than that of Edi since time constant of the electrical relaxation is shorter than the time constant of mechanical relaxation. Although we did not measure the relaxation rate of twitch Pdi and Edi, it is clear from the actual records as in figure 5 that the electrical relaxation is much faster than mechanical relaxation.

Our findings also indicate that the underlying mechanisms of the depression of diaphragmatic contraction during enflurane anesthesia and during diaphragmatic muscle fatigue are entirely different, namely the former is related to the neuromuscular junction depression while the latter is due essentially to the impairment of the contractile machinery within the myocyte[9]. Our result of neuromuscular transmission failure of the diaphragm during enflurane anesthesia is comparable to the observation made in isoflurane anesthetized rats[10], while it is somewhat different from those observed during halothane anesthesia[11,12]. In halothane anesthetized rats, 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[11]. Similarly, Clergue and his colleagues have shown that, in spontaneously breathing dogs anesthetized with pentobarbital, halothane exerts a dose-dependent reduction of the contractile properties of the diaphragm[12]. They concluded that, besides the direct depression of the central inspiratory drive, impairment of diaphragmatic contractility contributes to the ventilatory depression produced by halothane. Although the exact mechanisms underlying the differing effects of different anesthetics on diaphragmatic function *in vivo* remain unclear, different pharmacological actions of various anesthetics on the neuromuscular transmission, changes in acid-base status and effects on diaphragmatic blood flow explain the differences. In this connection, Howell and her colleagues have clearly shown that hypercapnia and acidemia impair diaphragmatic contractility *in vivo*[6,13]. Thus changes in Pa_{CO_2} and/or pH may have contributed to the results obtained in Clergue's experiments. Possible alteration of the autoregulation of the diaphragmatic blood flow induced by anesthetics may also account for the changes in contractile properties of the diaphragm[14]. Clearly further studies are needed to answer these questions and to examine the role of impairment of diaphragmatic function on the ventilatory depression during clinical anesthesia.

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References

1. Kochi T, Ide T, Isono S, Mizuguchi T, Nishino T: Different effects of halothane and enflurane on diaphragmatic contractility *in vivo*. Anesth Analg 70:362–368, 1990
2. Edwards RHT: Physiological analysis of skeletal muscle weakness and fatigue. Cli Sci Mol Med 54:463–470, 1978
3. Jones DA, Bigland-Ritchie B, Edward RHT: Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contraction. Exp Neurol 64:401–413, 1979
4. Aubier M, Viires N, Murciano D, Medrano G, Lecocguic Y, Pariente R: Effects and mechanism of action of terbutaline on diaphragmatic contractility and fatigue. J Appl Physiol 56:922–929, 1984
5. Aubier M, Viires N, Murciano D, Seta J-P, Pariente R: Effects of digoxin on diaphragmatic strength generation. J Appl Physiol 61:1767–1774, 1986
6. Howell S, Fitzgerald RS, Roussos C: Effects of uncompensated and compensated metabolic acidosis on canine diaphragm. J Appl Physiol 59:1376–1382, 1985
7. Waud RE, Waud DR: Effects of volatile anesthetics on directly and indirectly stimulated skeletal muscle. Anesthesiology 50:103–110, 1979
8. Aubier M, Farkas G, De Troyer A, Mozes R, Roussos C: Detection of diaphragmatic fatigue in man by phrenic stimulation. J Appl Physiol 50:538–544, 1981
9. Jones DA: Muscle fatigue due to changes beyond the neuromuscular junction. *Human Muscle Fatigue: Physiological Mechanisms*, edited by Porter R and Whelan J, London: Pitman, 1981, pp. 178–190
10. Veber B, Dureuil B, Viires N, Aubier M,

Pariente R, Desmonts JM: Effects of isoflurane on contractile properties of diaphragm. Anesthesiology 70:684–688. 1989

11. Dureuil B, Viires N, Nivoche Y, Ficks M, Pariente R, Aubier M, Desmonts JM: Different effects of halothane on diaphragm and hindlimb muscle in rats. J Appl Physiol 63:1757–1762, 1987
12. Clergue F, Viires N, Lemescle P, Aubier M, Viars P, Pariente R: Effects of halothane on diaphragmatic muscle function in pentobarbital-anesthetized dogs. Anesthesiology 64:181–187, 1986
13. Schnader J, Howell S, Fitzgerald RS, Roussos C: Interaction of fatigue and hypercapnia in the canine diaphragm. J Appl Physiol 64:1636–1643, 1988
14. Hussain SNA, Roussos C, Magder S: Autoregulation of diaphragmatic blood flow in dogs. J Appl Physiol 64:329–336, 1988