# Enflurane Impairs Canine Diaphragmatic Contractility *in Vivo*

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We examined the effect of enflurane on diaphragmatic contractility in six anesthetized mechanically ventilated dogs. The diaphragmatic force was assessed from transdiaphragmatic pressure (Pdi) developed at functional residual capacity against an occluded airway during cervical phrenic nerve stimulation. Pdi-stimulus frequency relationship was compared at three levels of anesthesia, namely 1, 1.5, and 2 MAC (minimum alveolar concentration) of enflurane. The sequence of changing anesthetic concentration was randomized between animals. Pdi at 50 and 100 Hz stimulation was significantly decreased with increasing MAC while Pdi at 10 Hz stimulation was not affected by the depth of anesthesia. Pdi of 20 Hz stimulation was significantly decreased at 2 MAC as compared to those at 1 and 1.5 MAC. We conclude that enflurane decreases contractility of the diaphragm mainly through impairment of the neuromuscular transmission and/or membrane excitability. Part of its effects is, however, probably related to the impairment of excitation-contraction coupling, as suggested by the depression of Pdi at 2 MAC in response to 20 Hz stimulation. (Key words: contractility, diaphragm, enflurane)

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It is well recognized that volatile anesthetics depress skeletal muscle force *in vitro*[1,2]. This phenomenon could be accounted for by the effects of the drugs on the neuromuscular transmission and/or contractile machinery within the muscle cell. Indeed, Waud and Waud have demonstrated that enflurane depresses indirect twitch response at 1.5–2.5 MAC and the direct response at 6–8 MAC[1].

Recently Aubier et al. have developed a method to assess the contractility of diaphragm *in vivo* and have examined the effects of various pharmacological agents on fatigued diaphragm[3–6]. In this connection, Dureuil et al.[7] and Veber et al.[8] have demonstrated that halothane and isoflurane impair contractile properties of rats diaphragm *in vivo*. Furthermore, Clergue et al.[9] also have demonstrated that halothane impairs contractile properties of canine diaphragm *in vivo*. However, data are not available regarding the effects of enflurane on canine diaphragm. Accordingly, we examined the effects of enflurane on diaphragmatic function in intact dogs.

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## Materials and Methods

### *Animals*

Six mongrel dogs weighing 10 to 15 kg were positioned supine, anesthetized with thiopental 15 $mg \cdot kg^{-1}$ intravenously, and maintained under surgical plain of enflurane anesthesia (1–1.5 MAC in oxygen). The animals were initially intubated orally with a cuffed endotracheal tube, later with a

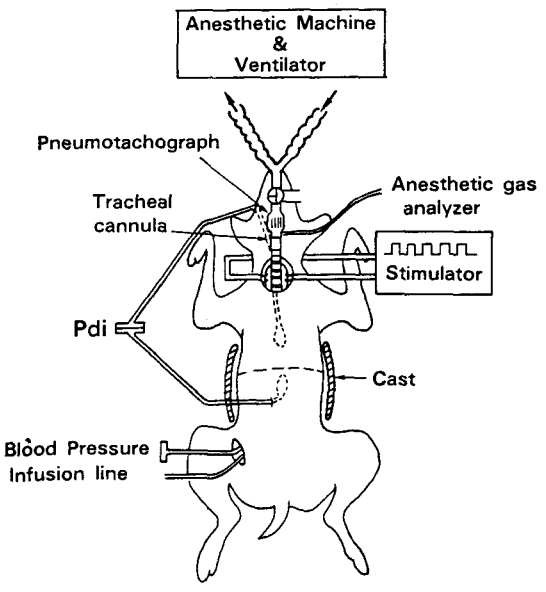


**Fig. 1.** Schematic representation of experimental settings.

Animals were placed in supine position and mechanically ventilated by ventilator through pneumotachograph. Transdiaphragmatic pressure (Pdi) was measured with two balloons as difference between abdominal (Pab) and esophageal pressure (Ppl). Bilateral cervical phrenic nerves were stimulated with stimulator. Catheters were inserted in femoral artery and vein to monitor arterial blood pressure and to infuse fluids, respectively.

tracheal cannula through tracheotomy, and mechanically ventilated with a ventilator (AIKA EVA). The femoral artery was cannulated to monitor blood pressure and to draw blood samples for arterial blood gas analysis using a blood gas analyzer (Instrumentation Laboratories, model 1302). The femoral vein was also cannulated for the administration of fluids and bicarbonate to correct acidosis. Rectal temperature was continuously monitored by a thermistor and was maintained at 37–38°C throughout the experiments by heating lamp. Airflow ($\dot{V}$) was measured by a pneumotachograph (Nihon Koden TV-122T) and a differential pressure transducer (Nihon Koden TP-602T) at the connection between three-way stopcock and the connector to the tracheal cannula. The changes in lung volume ($\Delta V$) were determined by an electrical integration of the $\dot{V}$ signal. End-tidal concentration of enflurane was continuously monitored by an anesthetic gas analyzer (Datex Normac). The experimental design is schematically illustrated in figure 1.

*Transdiaphragmatic pressure*

A catheter with a thin-walled latex balloon (5 cm length, 1.0 ml air) was positioned in the abdominal cavity beneath the costal part of the diaphragm through a small midline abdominal incision and was connected to one side of the differential pressure transducer (Nihon Koden TP-601T). The surgical incision was then closed tightly in layers. An another catheter with a latex balloon (0.4 m*l* air) was also positioned in the middle third of the esophagus, and connected to the other side of the transducer. Thus transdiaphragmatic pressure (Pdi) was determined by the difference of the pressure given by these two balloon-catheter systems. For a given study period, Pdi ($cmH_2O$) was recorded during phrenic nerve stimulation at different frequencies while the airway occluded at end-expiratory lung volume by turning the three-way stopcock. Constancy of diaphragm geometry and muscle length during contraction was achieved by placing a closely fitted plaster cast around the abdomen and lower one third of the rib cage.

*Phrenic nerve stimulation*

Bilateral phrenic nerves were identified in the lower neck, and isolated from the surrounding tissue. A stimulating electrode was positioned on each phrenic nerve. The phrenic nerves were stimulated using an electric stimulator (Nihon Koden SEN-3201), which delivered trains of supramaximal equidistant square-wave pulses. Supramaximal stimulation was determined by recording diaphragmatic twitches while the stimulation voltage was increased. Maximal response was estimated by twitch amplitude, maximal stimulation being achieved at approximately 25V. The voltage was then increased 10 to 20% to ensure that stimulation remained supramaximal. Pulse duration was set to 0.2 ms. Stimulation trains were applied for 2–3 s at frequencies of 10, 20, 50, and 100 Hz. Two stimulations were made at each frequency in

**Table 1.** Average ± SEM values of mean blood pressure (mBP), hydrogen ion concentration ($[H^+]$), $Pa_{CO_2}$ and $Pa_{O_2}$ under three levels of enflurane anesthesia

| | mBP (mmHg) | $[H^+]$ (nmole · $l^{-1}$) | $Pa_{CO_2}$ (mmHg) | $Pa_{O_2}$ (mmHg) |
|---|---|---|---|---|
| 1 MAC | 109.7 ± 9.1 | 41.7 ± 1.1 | 37.6 ± 0.9 | 508.8 ± 8.2 |
| 1.5 MAC | 90.0 ± 9.6* | 39.7 ± 1.4 | 35.6 ± 1.6 | 460.3 ± 8.2 |
| 2 MAC | 63.5 ± 8.3** | 40.4 ± 1.5 | 35.5 ± 1.6 | 458.5 ± 21.0 |

(*$P < 0.05$ vs 1 MAC, **$P < 0.01$ vs 1 and 1.5 MAC)

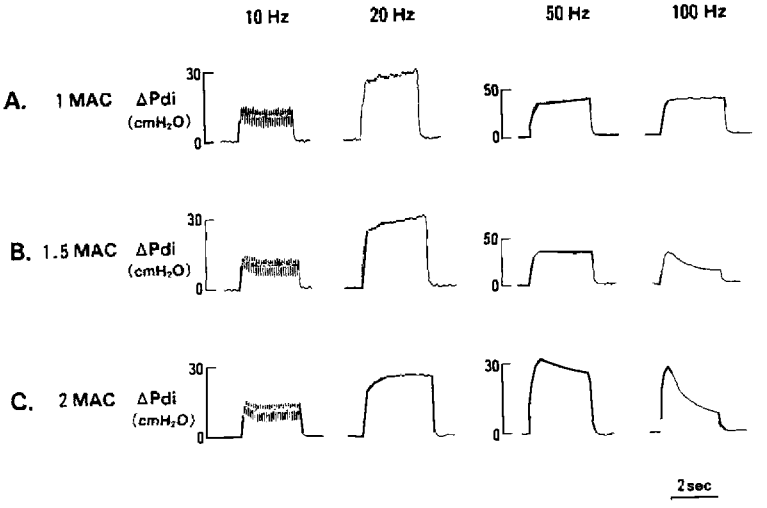


**Fig. 2.** Experimental records of transdiaphragmatic pressure (Pdi) obtained in a representative animal at stimulation frequencies of 10, 20, 50, and 100 Hz. A, B, and C represents the tracings under 1, 1.5, and 2 MAC of enflurane anesthesia, respectively.

two minute interval and the average value of the two was used in the data analysis.

*Experimental Protocol*

Diaphragmatic contractility was assessed under three levels of anesthesia in each animal, namely 1, 1.5, and 2 MAC of enflurane, following 1 hour of stable condition. The sequence of changing anesthetic level was randomized between animals. Arterial blood gases, blood pressure were measured in each depth of anesthesia at the end of each run. Signals of arterial blood pressure, Pdi, $\dot{V}$, and $\Delta V$ were recorded on a four channel recorder (Nihon Koden Recticorder). All reported values are given as mean ± SEM. Statistical analysis was performed using two way analysis of variance and the Tukey's test.

## Results

Table 1 illustrates the average ± SEM values of mean arterial blood pressure, hydrogen ion concentration ($[H^+]$), $Pa_{CO_2}$, and $Pa_{O_2}$ obtained in 6 animals under 1, 1.5, and 2 MAC of enflurane anesthesia. Blood pressure significantly decreased with increasing the depth of enflurane anesthesia, while $[H^+]$ and blood gas tensions were essentially constant regardless of the level of anesthesia.

Representative recordings of Pdi obtained in an animal during phrenic nerve stimulation of various frequencies are shown in figure 2. As illustrated in figure 2A, Pdi increased with increasing stimulation frequencies from 10 to 20 and 50 Hz. Pdi of 100 Hz was slightly smaller than that of 50 Hz in this animal. Figure 2B and C corresponds to the tracings obtained at 1.5

Table 2. Values of Pdi at various stimulation frequencies of phrenic nerve under three levels of enflurane anesthesia

| | Pdi ($cmH_2O$) | | | |
|---|---|---|---|---|
| | 10 Hz | 20 Hz | 50 Hz | 100 Hz |
| 1 MAC | 12.5 ± 2.5 | 27.3 ± 3.0 | 37.4 ± 4.7 | 34.0 ± 4.4 |
| 1.5 MAC | 12.7 ± 2.5 | 28.6 ± 3.0 | 32.7 ± 3.3 | 21.0 ± 5.0* |
| 2 MAC | 11.0 ± 2.1 | 22.8 ± 3.3** | 25.3 ± 4.1*,** | 14.1 ± 3.8*,** |

(*$P < 0.01$ vs 1 MAC, **$P < 0.05$ vs 1.5 MAC)

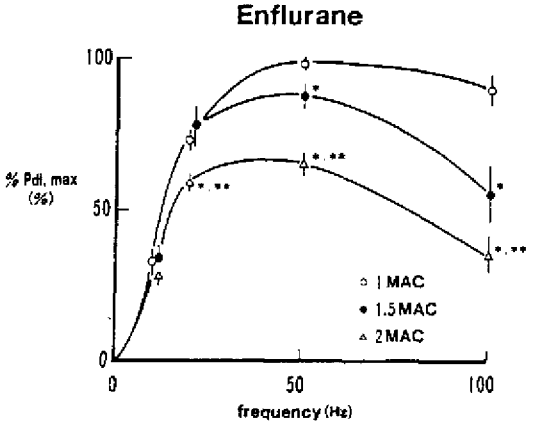


**Fig. 3.** Force-frequency response curves of the diaphragm at three levels of anesthesia obtained in 6 dogs. Values of Pdi were expressed in % of the maximum Pdi determined in each animal during the experimental procedures. ○, ●, and △ denote the values of 1, 1.5 and 2 MAC of enflurane. Each point represents average data (± SEM). Pdi at 50 and 100 Hz was significantly different among the three levels of anesthesia. Pdi at 20 Hz of 2 MAC was also significantly less than those of 1 and 1.5 MAC. * and ** represent statistical difference (*$P < 0.01$ vs 1 MAC, **$P < 0.01$ vs 1.5 MAC).

and 2 MAC of enflurane, respectively. In contrast to the tracings of 1 MAC, Pdi of 50 and 100 Hz at 1.5 and 2 MAC exhibited an initial rapid increase followed by a gradual decline in pressure during phrenic nerve stimulation. Although duration of stimulation greater than 3 s might poduce plateau pressure of Pdi, it may presumably induce muscle fatigue, thus we limited the duration of stimulation to 2–3 seconds and the measurement of Pdi was empirically made at 2 s after starting the stimulation.

Table 2 demonstrates the mean ± SEM values of Pdi at stimulation frequencies of 10, 20, 50, and 100 Hz under three levels of anesthesia. There was no statistical difference among the values of Pdi at 10 Hz under the three depths of anesthesia. At 50 and 100 Hz stimulations, however, Pdi was significantly decreased by increasing enflurane concentration. Although statistically insignificant, Pdi of 20 Hz at 1.5 MAC was slightly greater than that of 1 MAC as observed in isoflurane anesthetized rats[8]. By contrast, Pdi of 2 MAC was significantly decreased from the value of 1.5 MAC at this stimulation frequency.

Figure 3 depicts the force-frequency relationship of three levels of anesthesia obtained in 6 dogs. Values of Pdi were expressed in % of maximum Pdi value obtained in each animal. It is apparent from this figure that enflurane exerts a dose dependent decrease of diaphragmatic force generation at higher stimulation frequencies, namely 50 and 100 Hz, while it does not affect force generating properties at lower stimulation frequency of 10 Hz. Besides, % Pdi, max in response to 20 Hz stimulation at 2 MAC was significantly smaller than those values at 1 and 1.5 MAC.

## Discussion

The main findings of this study are the following. 1) Enflurane decreases the force generation of the diaphragm at higher stimulation frequencies of 50 and 100 Hz while it does not change the contractility at 10 Hz stimulation. 2) The effect of enflurane on the diaphragm is MAC related. This implies that enflurane has a depressant effect on the neuromuscular transmission and/or membrane

excitability of canine diaphragm. Furthermore, significant decrease of Pdi in response to 20 Hz stimulation at 2 MAC suggests the possible impairment of excitation-contraction coupling or of the muscle's intrinsic contractile machinery.

The contractile properties of the diaphragm were assessed by its force-frequency characteristics. This method has been extensively used for skeletal muscle[10], and recently in humans and dogs[4–6,9] for the diaphragm. In the latter case the force generated by the diaphragm for a given electrical stimulation was measured in terms of Pdi. As previously demonstrated, the pressure generated by the diaphragm for a given stimulation will be affected by its length and geometry[11,12]. However, in the present experimental condition, the lung volume at which the measurements were made was the functional residual capacity and its constancy was monitored by measuring the end-expiratory transpulmonary pressure. Furthermore, by placing a cast around the lower third of the thorax and abdomen, we could avoid the deformation and the possible changes in compliance of the thoraco-abdominal structures. In this connection it should be noted that, in the dog 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[13].

It has been suggested that selective loss of force at low frequency stimulation is closely related to the impairment of excitation-contraction coupling[14], while selective loss of force at high frequency stimulation indicates failure of neuromuscular transmission and/or impaired membrane excitation[10,15]. Therefore the reduction of Pdi in response to 50 and 100 Hz stimulation at 1.5 and 2 MAC enflurane is presumably due to the impairment of neuromuscular transmission and/or membrane excitation. Similarly, the decrease of Pdi for 20 Hz stimulation at 2 MAC could be due to a failure of excitation-contraction coupling. This alteration of diaphragmatic contractility may be related to a decrease in energy substrate supplies. Indeed, the ability of diaphragm to recieve adequate energy supplies to sustain contraction depends on the diaphragmatic blood flow[16]. Observed decrease of mean arterial blood pressure, to which diaphragmatic blood flow is closely related, with increasing the depth of enflurane anesthesia may have induced energy substrate depletion of the diaphragm and consequently a reduction in its contractile strength. The fact that the diaphragmatic contraction at 50 and 100 Hz, which needs maximal energy supply, was reduced during 1.5 and 2 MAC of enflurane, whereas contraction at 10 Hz stimulation was not affected, presumably indicates the involement of such mechanism. In order to elucidate the underlying mechanisms responsible for the impairment of diaphragmatic contractility by enflurane, further study including the measurements of EMG activity of the diaphragm, diaphragmatic blood flow and the energy balance of the diaphragm[16] would be needed.

Our result of decreased contractility at higher frequencies of stimulation with enflurane is compatible to the observation made by Veber et al., showing the depression of Pdi at 50 and 100 Hz by isoflurane in rats[8]. On the other hand, current result is somewhat different from those reported for halothane[7,9]. Dureuil et al.[7] demonstrated that, in rats, 0.5 to 1.5 MAC of halothane produces a marked decrease in diaphragmatic contractility at stimulation frequencies ranging from 0.5 to 100 Hz whereas it was without effect on hindlimb muscle. Besides, Clergue et al.[9] demonstrated that halothane depresses both Pdi and EMG activities of canine diaphragm at all stimulation frequencies (10–100 Hz). Both of these results suggest that the reduction of contractility of the diaphragm by halothane is mainly caused by an impairment of the excitation-contraction coupling process. The difference of our results to those of Dureuil et al. and Clergue et al. may be related to the differing pharmacological effects of enflurane and halothane on diaphragmatic function. Conversely, since the latter study was conducted on spontaneously breathing animals, hypercapnia and acidemia that de-

veloped with increasing halothane concentration could have induced an impairment of diaphragmatic contractility[13].

Our results suggest that the impairment of the diaphragmatic contractility and, hence, the ventilatory pump dysfunction may be, at least in part, responsible for the ventilatory depressant effect of enflurane. The clinical importance of this phenomenon remains to be examined in humans.

It can be concluded that enflurane produces a marked decrease in force generation of canine diaphragm in response to higher frequencies of stimulation and, to a lesser extent, it impairs diaphragmatic force generation at 20 Hz stimulation. Clearly further studies are needed to elucidate the exact mechanisms underlying the differing effects of various volatile anesthetics on diaphragmatic function *in vivo*.

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