Effectiveness of diaphragmatic stimulation with single-channel electrodes in rabbits*

Rodrigo Guellner Ghedini, Julio de Oliveira Espinel, Elaine Aparecida Felix, Artur de Oliveira Paludo, Rodrigo Mariano, Arthur Rodrigo Ronconi Holand, Cristiano Feijó Andrade

Abstract
Every year, a large number of individuals become dependent on mechanical ventilation because of a loss of diaphragm function. The most common causes are cervical spinal trauma and neuromuscular diseases. We have developed an experimental model to evaluate the performance of electrical stimulation of the diaphragm in rabbits using single-channel electrodes implanted directly into the muscle. Various current intensities (10, 16, 20, and 26 mA) produced tidal volumes above the baseline value, showing that this model is effective for the study of diaphragm performance at different levels of electrical stimulation.

Keywords: Rabbits; Diaphragm; Electric stimulation; Models, animal.

Brief Communication

The diaphragm is the muscle that is responsible for the proper functioning of the respiratory system. Upper cervical spine injuries can cause quadriplegia and lead to loss of diaphragm function, resulting in dependency on positive pressure ventilation. Neuromuscular diseases such as amyotrophic lateral sclerosis also lead to progressive and cumulative impairment of diaphragm function, death being due to respiratory failure in most cases.\(^1\) The worldwide prevalence of amyotrophic lateral sclerosis is 3–8 cases per 100,000 population, the annual incidence being 2/100,000 population. Half of all affected patients live for at least three years after diagnosis.\(^2\)

Approximately 20% live five years or more, and up to 10% survive for more than ten years.

Because this is a problem of general interest, there is a need for studies providing diaphragmatic stimulation methods and devices that can benefit this population.

The use of electrical currents in order to produce artificial ventilation has been described since the nineteenth century, generating nerve action potentials and direct contractions of the diaphragm and other respiratory muscles through different techniques.\(^3-5\) An animal study comparing phrenic nerve stimulation and intramuscular stimulation showed similar results in terms of...
transdiaphragmatic pressure generation and latency of nerve conduction velocity.\(^6\)

The first studies used cervical or thoracic phrenic nerve stimulation in order to trigger action potentials. That technique proved to be clinically effective but was found to have limitations and pose a risk of mechanical nerve injury.\(^7,8\) \(^9,10\) \(^11-13\) \(^14-16\) \(^17\) Technological evolution and development provided a major breakthrough in the development of diaphragmatic pacing through electrodes implanted directly into the muscle through laparotomy and, subsequently, laparoscopy.\(^9,10\)

Full activation of the diaphragm is related to electrodes implanted into the diaphragm motor points, where phrenic nerve fibers ramify.\(^11-13\) The sites that are tested for motor points are chosen on the basis of anatomical landmarks on the abdominal surface of the diaphragm, and correct electrode placement is determined after diaphragm mapping and exploration, by observing the point at which muscle response to electrical stimulation is strongest.\(^14-16\)

We have previously described a model of electrical stimulation of the diaphragm in rabbits. Using that model, we tested two types of electrodes: bipolar electrodes, with the two poles of the current generator in the same electrode; and monopolar electrodes, with only one pole, depolarization occurring between the hemidiaphragms, with no dispersive electrode. Through laparotomy, the electrodes were placed at the motor points of both hemidiaphragms. We found that both types of electrodes were able to stimulate the diaphragm with different current intensities, producing tidal volumes that were similar to physiological volumes.\(^17\)

The objective of the present study was to evaluate the performance of electrical stimulation of the diaphragm with single-channel electrodes implanted directly into the diaphragm in rabbits.

Seven healthy, female New Zealand rabbits weighing 2-3 kg were used. The animals were treated in accordance with the World Health Organization ethical code for animal experimentation. The study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (Protocol no. 10,260). The animals received an intramuscular dose of ketamine, midazolam, and atropine, being subsequently shaved and submitted to peripheral venipuncture and orotracheal intubation. They were then placed on mechanical ventilation with a Nikkei ventilator (Takaoka Ind e Com Ltda., São Paulo, Brazil). Anesthesia was maintained with inhaled isoflurane. No neuromuscular blocking agents were used for anesthesia, because the myoneural junction had to remain functional.\(^18\) After hemodynamic stabilization, the expiratory lung volume was measured at baseline for subsequent comparisons.

An exploratory (longitudinal) laparotomy was performed for visualization of the diaphragm, followed by identification of the diaphragm motor points, into which the electrodes were implanted. The electrodes were implanted into both hemidiaphragms, and the wires were placed in the subcutaneous space of the abdominal wall (Figure 1). The animals were kept under observation for 15 days. After that period, the animals were intubated, mechanically ventilated, and anesthetized for electrical stimulation of the diaphragm at various current intensities (10, 16, 20, 26, and 32 mA), each being applied three times for ten respiratory cycles, with a 5-min recovery interval between each cycle. We obtained the mean values for each intensity, as previously described.\(^17\) The means were analyzed by generalized estimating equations, being corrected by the Bonferroni method. To that end, the diaphragmatic electrode wires were connected to a Dualpex 961 Phrenics current generator (Quark Medical, São Paulo, Brazil), producing a depolarized current with a rectangular waveform, a symmetric alternating pulse, a frequency of 25 Hz (cycles/s), and a burst width of 0.07 ms. We evaluated the relationship between the volume of air exhaled and the current intensity applied using a flow sensor (Tracer 5; Intermed Ltda., São Paulo, Brazil) connected to the endotracheal tube. All measurements were performed with the animals in the supine position. All animals survived the procedure. However, one animal showed infection, an extensive area of fibrosis, and adhesions, which precluded the generation of an adequate tidal volume. The animal was therefore excluded from the study.

In our study sample, the mean expiratory volume at baseline was 14.97 ± 1.75 mL, showing a proportional relationship between expiratory volume and current intensity. When an electrical current of 10 mA was applied, the mean expiratory volume was 15.72 ± 1.17 mL; when a current of 16 mA was applied, the mean expiratory volume was 18.86 ± 3.69 mL; when a current of 20 mA
was applied, the mean expiratory volume was 19.69 ± 3.72 mL; when a current of 26 mA was applied, the mean expiratory volume was 22.01 ± 4.17 mL; and when a current of 32 mA was applied, the mean expiratory volume was 22.36 ± 2.77 mL (Figure 2).

Electrical activation of the diaphragm by intramuscular electrodes is an alternative form of electroventilation for the restoration of respiratory capacity. The ideal electroventilation system is that which is capable of restoring respiratory muscle function and meeting the physiological demands of individuals. One of the challenges to be overcome in the development of an ideal model is the autonomy of current generators. Devices that have high power consumption are larger and heavier, requiring an external source of signal or power. In a previous study, our group examined the relationship between current intensity and expiratory volume using two different types of electrodes, and the responses were found to be similar. In the single-channel electrode model, both hemidiaphragms are depolarized with a single channel of the current generator. This

Figure 1 - In A, electrodes implanted directly into the diaphragm (arrows). In B, the abdominal wall is closed, but the skin and subcutaneous tissue are open and the wires are exteriorized for diaphragmatic stimulation after an observation period of 15 days.

Figure 2 - Mean expiratory volumes at current intensities of 10, 16, 20, 26, and 32 mA.

*Statistically significant differences between baseline volume and current intensities, as analyzed by generalized estimating equations and as corrected by the Bonferroni method. The error bar represents the standard error of the difference among the values obtained over three separate time periods, five minutes apart.
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Full activation of the diaphragm depends on proper electrode implantation into the diaphragm motor points, the entrance area, and the ramification of the phrenic nerves in the muscle; therefore, it is essential to explore the muscle and identify the points at which contraction is more consistent. One of the challenges was to design a specific electrode for diaphragmatic stimulation, given that, in rabbits, the diaphragm is thinner and more delicate than the peripheral muscles.

In the present study, we noted an important difference between the pattern of thoracoabdominal motion during spontaneous breathing and the pattern of thoracoabdominal motion during electrical stimulation of the diaphragm. During spontaneous breathing, the rib cage and the abdominal circumference increased in diameter during inhalation. When the diaphragm was stimulated, the circumference of the base of the rib cage decreased during inhalation, whereas the abdominal circumference increased.

It is assumed that ventilation through the use of electrical currents is more physiological than positive pressure ventilation because patients can use the inspiratory muscles to inflate the lungs. There is currently no ideal model that can fully restore respiratory function, given that such models are not influenced by the respiratory centers and are therefore unable to meet the metabolic demands of individuals. The lack of synchrony between upper airway opening and diaphragmatic contraction leads to the need for tracheostomy.

The animal model described herein proved to be effective for the study of diaphragm performance at different levels of electrical stimulation with a current generator and single-channel electrodes implanted directly into the muscle.

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About the authors

Rodrigo Guellner Ghedini
Physical Therapist. Santa Casa Sisters of Mercy Hospital Complex; and Associate Researcher, Airways and Lung Laboratory, Universidade Federal do Rio Grande do Sul – UFRGS, Federal University of Rio Grande do Sul – Hospital de Clínicas de Porto Alegre – HCPA, Porto Alegre, Brazil.

Julio de Oliveira Espinel
Thoracic Surgeon and Associate Researcher, Airways and Lung Laboratory, Universidade Federal do Rio Grande do Sul – UFRGS, Federal University of Rio Grande do Sul – Hospital de Clínicas de Porto Alegre – HCPA, Porto Alegre, Brazil.

Elaine Aparecida Felix
Associate Professor of Anesthesiology, Department of Surgery, Universidade Federal do Rio Grande do Sul – UFRGS, Federal University of Rio Grande do Sul – Porto Alegre, Brazil.

Artur de Oliveira Paludo
Associate Researcher, Airways and Lung Laboratory, Universidade Federal do Rio Grande do Sul – UFRGS, Federal University of Rio Grande do Sul – Hospital de Clínicas de Porto Alegre – HCPA, Porto Alegre, Brazil.

Rodrigo Mariano
Associate Researcher, Airways and Lung Laboratory, Universidade Federal do Rio Grande do Sul – UFRGS, Federal University of Rio Grande do Sul – Hospital de Clínicas de Porto Alegre – HCPA, Porto Alegre, Brazil.

Arthur Rodrigo Ronconi Holand
Associate Researcher, Airways and Lung Laboratory, Universidade Federal do Rio Grande do Sul – UFRGS, Federal University of Rio Grande do Sul – Hospital de Clínicas de Porto Alegre – HCPA, Porto Alegre, Brazil.

Cristiano Feijó Andrade
Thoracic Surgeon, Santo Antônio Children’s Hospital and Universidade Federal do Rio Grande do Sul – UFRGS, Federal University of Rio Grande do Sul – Hospital de Clínicas de Porto Alegre – HCPA, Porto Alegre, Brazil.