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

Efetividade da estimulação diafragmática com eletrodos monocanais em coelhos

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.

Resumo

A cada ano um grande número de pessoas perde a função do diafragma tornando-se dependentes de ventilação mecânica. As principais causas são o trauma raquimedular da região cervical e as doenças neuromusculares. Desenvolvemos um modelo experimental para avaliar o desempenho da estimulação elétrica do diafragma em coelhos com eletrodos monocanais implantados diretamente neste músculo. Foram aplicadas diferentes intensidades de correntes (10, 16, 20 e 26 mA), as quais geraram volumes correntes acima dos valores basais, mostrando que este modelo é eficaz para estudar o desempenho do diafragma sob diferentes tipos de estimulação elétrica.

Descritores: Coelhos; Diafragma; Estimulação elétrica; Modelos animais.

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

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Correspondence to: Cristiano Feijó Andrade. Hospital de Clínicas de Porto Alegre, Serviço de Cirurgia Torácica, Rua Ramiro Barcelos, 2350, CEP 90035-903, Porto Alegre, RS, Brasil.
Tel. 55 51 3359-8684. E-mail: cristianofa@cirurgiatoracica.net
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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] 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

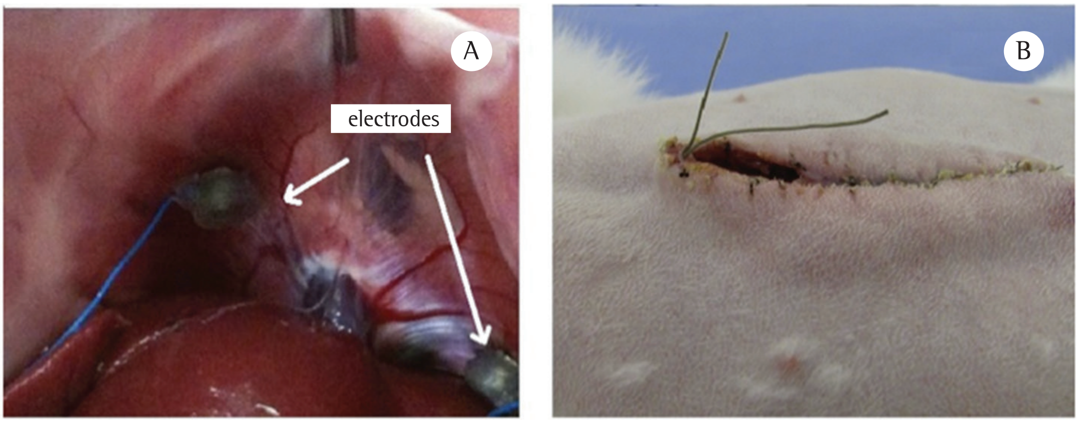

Figure 1 - In A, electrodes implanted directly into the diaphragm (arrows). In B, the abdominal wall is closed, but the skin and subcutaneous tissue are open and the wires are exteriorized for diaphragmatic stimulation after an observation period of 15 days.

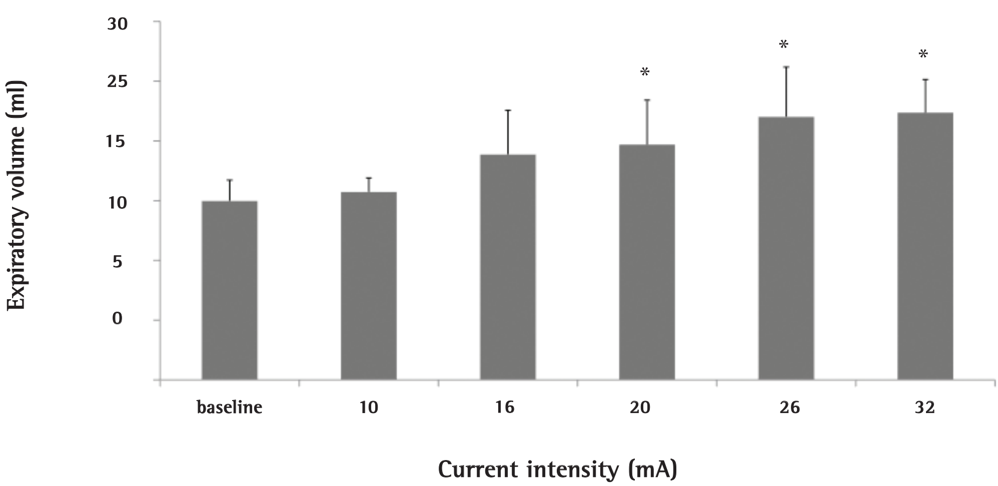

Figure 2 - Mean expiratory volumes at current intensities of 10, 16, 20, 26, and 32 mA.

*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.

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 reduces power consumption. Using that model, we were able to achieve expiratory volumes as high as 149% from baseline values. The findings are consistent with those of experimental and clinical studies.[5,6,9-11] 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.[10]

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.

References

1. Oliveira AS, Pereira RD. Amyotrophic lateral sclerosis (ALS): three letters that change the people's life. For ever. Arq Neuropsiquiatr. 2009;67(3A):750-82. http://dx.doi.org/10.1590/S0004-282X2009000400040 PMid:19722069
2. World Health Organization [homepage on the Internet]. Geneva: World Health Organization. [cited 2010 Apr 01]. World Health Statistics 2009. [Adobe Acrobat document, 149p.]. Available from: http://www.who.int/whosis/whostat/EN_WHS09_Full.pdf
3. Glenn WW, Holcomb WG, Gee JB, Rath R. Central hypoventilation; long-term ventilatory assistance by radiofrequency electrophrenic respiration. Ann Surg. 1970;172(4):755-73. http://dx.doi.org/10.1097/00000658-197010000-00020 PMid:5458627 PMCid:1397282
4. Geddes LA, Voorhees WD, Babbs CF, Deford JA. Electroventilation. Am J Emerg Med. 1985;3(4):337-9. http://dx.doi.org/10.1016/0735-6757(85)90061-0
5. Geddes LA, Voorhees WD, Lagler R, Riscili C, Foster K, Bourland JD. Electrically produced artificial ventilation. Med Instrum. 1988;22(5):263-71. PMid:3054441
6. Reid WD, Blogg T, Wiggs BJ, Paré PD, Pardy RL. Diaphragmatic plate electrode stimulation of the hamster diaphragm. J Appl Physiol. 1989;67(4):1341-8. PMid:2676954
7. DiMarco AF. Phrenic nerve stimulation in patients with spinal cord injury. Respir Physiol Neurobiol. 2009;169(2):200-9. http://dx.doi.org/10.1016/j.resp.2009.09.008 PMid:19786125
8. Glenn WW, Phelps ML, Elefteriades JA, Dentz B, Hogan JF. Twenty years of experience in phrenic nerve stimulation to pace the diaphragm. Pacing Clin Electrophysiol. 1986;9(6 Pt 1):780-4. http://dx.doi.org/10.1111/j.1540-8159.1986.tb06627.x PMid:2432480
9. Onders RP, Elmo M, Khansarinia S, Bowman B, Yee J, Road J, et al. Complete worldwide operative experience in laparoscopic diaphragm pacing: results and differences in spinal cord injured patients and amyotrophic lateral sclerosis patients. Surg Endosc. 2009;23(7):1433-40. http://dx.doi.org/10.1007/s00464-008-0223-3 PMid:19067067
10. Tedde ML, Onders RP, Teixeira MJ, Lage SG, Ballester G, Brotto MW, et al. Electric ventilation: indications for and technical aspects of diaphragm pacing stimulation surgical implantation. J Bras Pneumol. 2012;38(5):566-72 http://dx.doi.org/10.1590/S1806-37132012000500005 PMid:23147048
11. Peterson DK, Nochomovitz ML, Stellato TA, Mortimer JT. Long-term intramuscular electrical activation of the phrenic nerve: efficacy as a ventilatory prosthesis. IEEE Trans Biomed Eng. 1994;41(12):1127-35. http://dx.doi.org/10.1109/10.335861 PMid:7851914
12. Dunn RB, Walter JS, Walsh J. Diaphragm and accessory respiratory muscle stimulation using intramuscular electrodes. Arch Phys Med Rehabil. 1995;76(3):266-71. http://dx.doi.org/10.1016/S0003-9993(95)80614-8
13. DiMarco AF, Onders RP, Kowalski KE, Miller ME, Ferek S, Mortimer JT. Phrenic nerve pacing in a tetraplegic patient via intramuscular diaphragm electrodes. Am J Respir Crit Care Med. 2002;166(12 Pt 1):1604-6. http://dx.doi.org/10.1164/rccm.200203-175CR PMid:12471076
14. Peterson DK, Nochomovitz M, DiMarco AF, Mortimer JT. Intramuscular electrical activation of the phrenic nerve. IEEE Trans Biomed Eng. 1986;33(3):342-51. http://dx.doi.org/10.1109/TBME.1986.325720 PMid:3957387
15. Schmit BD, Stellato TA, Miller ME, Mortimer JT. Laparoscopic placement of electrodes for diaphragm pacing using stimulation to locate the phrenic nerve motor points. IEEE Trans Rehabil Eng. 1998;6(4):382-90. http://dx.doi.org/10.1109/86.736152 PMid:9865885
16. Onders RP, Dimarco AF, Ignagni AR, Aiyar H, Mortimer JT. Mapping the phrenic nerve motor point: the key to a successful laparoscopic diaphragm pacing system in the first human series. Surgery. 2004;136(4):819-26. http://dx.doi.org/10.1016/j.surg.2004.06.030 PMid:15467667

17. Ghedini RG, Margarites A, Felix EA, Xavier RG, Andrade CF. Development of a new experimental model of intramuscular electrical stimulation of the diaphragm in rabbits. Acta Cir Bras. 2010;25(6):475-8. http://dx.doi.org/10.1590/S0102-86502010000600003 PMid:21120276

18. Tedde ML, Vasconcelos Filho P, Hajjar LA, de Almeida JP, Flora GF, Okumura EM, et al. Diaphragmatic pacing stimulation in spinal cord injury: anesthetic and perioperative management. Clinics (Sao Paulo). 2012;67(11):1265-9. http://dx.doi.org/10.6061/clinics/2012(11)07

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 *Hospital de Clínicas* – 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 *Hospital de Clínicas* – 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 *Hospital de Clínicas* – 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 *Hospital de Clínicas* – 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 *Hospital de Clínicas* – 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 *Hospital de Clínicas* – Porto Alegre, Brazil.