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Influence of Temperature on Pudendal Nerve Block Induced by High-Frequency Biphasic Electrical Current

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Abstract

Purpose—To determine the influence of temperature on the minimal stimulation frequency required to block the pudendal nerve conduction.

Material and Methods—The pudendal nerve block induced by high-frequency, biphasic electrical current was investigated at different temperatures using cats under α -chloralose anesthesia. Urethral pressure was measured to indicate the pudendal nerve activation or block.

Results—As the stimulation frequency was increased above a frequency threshold, the urethral pressure response was diminished and the pudendal nerve was blocked. The minimal stimulation frequency required to block pudendal nerve was reduced from 6 kHz to 4 kHz as the temperature was decreased from 37 °C to 15 °C. At 4 kHz frequency, the maximal temperature below which the pudendal nerve could be blocked was 24.5 °C.

Conclusion—In order to block pudendal nerve conduction at body temperature (37 °C), the stimulation frequency has to be greater than 6 kHz. This study provided a practical guide for blocking the pudendal nerves in order to restore efficient voiding after spinal cord injury.

Keywords

Pudendal Nerve; Block; High-frequency; Stimulation; Temperature

INTRODUCTION

A reversible nerve blocking method using biphasic, charge-balanced, electrical current could have many potential clinical applications, especially in the development of a neural prosthesis for the people with neurological disorders. For example, blocking pudendal nerve conduction during micturition could reduce urethral outlet resistance and improve voiding efficiency in people with detrusor sphincter dyssynergia after spinal cord injury.^{1, 2} Blocking peripheral nerves could also be used to treat chronic pain of peripheral origin,³ or to stop the unwanted motor activity, such as muscle spasms, spasticity, tics and chorea.⁴ In chronic applications a nerve block method employing biphasic, charge-balanced, electrical current will cause less damage to the nerve due to electro-chemical reactions than using uniphasic electrical current.⁵ Therefore, recently attention has been focused on the axonal conduction block induced by high-frequency, biphasic, charge-balanced electrical current.^{1, 4, 6-15}

Previous studies using animals^{1,4,8,11,12,16-19} have shown that high-frequency, biphasic, electrical current applied to peripheral nerves can block nerve conduction. This conduction block is quickly reversible once the stimulation is terminated. However, the minimal stimulation frequency required to induce the nerve block has varied significantly in reports from different investigators.^{1,4,8,11,12,16-19} Our previous studies^{1,11} on the pudendal nerve of cat indicated that a minimal stimulation frequency of 6 kHz is required. But a stimulation frequency as low as 4 kHz has been reported previously to be effective in blocking nerve conduction of large A α fibers (diameter 12-22 μ m) in experiments using cats.¹⁶⁻¹⁸ In a study using frog sciatic nerve⁴ it was shown that nerve conduction could be blocked at a stimulation frequency as low as 1 kHz. More recently, studies^{8, 12} using sciatic nerve of rats have shown that the stimulation frequency has to be higher than 10 kHz. The different nerves used by different investigators might contribute to the frequency differences, since they are composed of axons with very different diameters. However, other experimental conditions may also play a role, including electrode geometry, experimental temperature, etc. Currently the frequency discrepancy has not been explained. Thus, which stimulation frequency should be used in the design of a neural prosthetic device to block pudendal nerve conduction remains undetermined.

Our recent computer simulation studies^{13, 15} using a myelinated axon model indicated that temperature could be one of the factors that influences nerve conduction block. Therefore, in this study we further investigated the influence of temperature on the conduction block of pudendal nerve in cats, which aimed at developing a reversible nerve block method to block pudendal nerve conduction during micturition in order to facilitate voiding after spinal cord injury.^{1,2,11}

MATERIALS AND METHODS

All protocols involving the use of animals in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

The experimental setup is shown in Fig.1. One female and two male cats (5.1 to 6.0 kg) were used under α -chloralose anesthesia (60 mg/kg i.v., supplemented as needed). The temperature of the animal was maintained at 35-37°C using a heating pad. The ureters were cut and drained externally. A catheter (5 F) was inserted through the bladder dome into the proximal urethra and secured by a ligature near the bladder neck. The catheter was attached to both an infusion pump and a pressure transducer via a T-connector. The urethra was infused continuously with saline solution at the rate of 1-2 ml/min. The back pressure in the urethral perfusion system caused by contractions of urethral sphincter was recorded via the pressure transducer. Pudendal nerves were accessed posteriorly in the sciatic notch and cut bilaterally to eliminate any effect of the pudendal-to-pudendal spinal reflex on the experiment results. A small pool was formed around the pudendal nerve (usually on the left side) by retracting skin flaps and the pool was filled with mineral oil. A tripolar cuff electrode [shown as Stim. A in Fig.1, Micro Probe, Inc., NC223(Pt)] was placed around one pudendal nerve (usually on the left side). The electrode leads were made of platinum wires (diameter 0.25 mm) with a distance of 2 mm between the leads. A second pair of stainless steel hook electrode (shown as Stim. B in Fig.1) was placed on the pudendal nerve at a location central to the tripolar cuff electrode in order to test whether stimulation A could block the urethral response induced by stimulation B. A digital thermometer was used to monitor the temperature of the small mineral oil pool. The probe of the thermometer was placed adjacent to the cuff electrode and the pudendal nerve. The temperature of the mineral oil pool was adjusted between 14 °C and 37 °C by manually infusing cold or warm mineral oil into the small pool. The temperature of the mineral oil pool was maintained in a range of ± 0.5 °C during each electrical stimulation period.

The high-frequency blocking stimulation tested in this study was biphasic, charge-balanced continuous rectangular wave (see Fig.1). At a certain temperature (15, 20, 27, or 37 °C), the high-frequency stimulation (10 seconds duration) was tested at different frequencies ranging from 1 kHz to 10 kHz in 1 kHz increments to search for the minimal blocking frequency. The intensity of the high-frequency stimulation was set at about 1.5 times of the blocking threshold determined at temperature of 37 °C. The determination of blocking threshold was fully described in our previous study,¹¹ which involved testing on both intensity and frequency. After the minimal blocking frequency was determined at different temperatures, stimulation frequency of 4 kHz was chosen to test for a nerve block effect at different temperatures ranging from 14.5 °C to 36.5 °C in 2 °C increments. The same test was repeated 3 times in each animal, and the data were presented as mean $\pm$ standard error across all animals.

The high-frequency, biphasic stimulation waveforms (1-10 kHz) were generated by a computer with a digital-to-analog circuit board (National Instruments, AT-AO-10), which was programmed using LabView programming language (National Instruments). Linear stimulus isolators (World Precision Instruments, A395) were used to deliver the biphasic constant current pulses to the nerve.

RESULTS

As shown in Fig.1, the blocking stimulation A (Stim. A) was used to block the urethral pressure responses induced by the exciting stimulation B (Stim. B). When the intensity of high-frequency blocking stimulation was above the blocking threshold, the urethral pressure induced by the high-frequency blocking stimulation alone was gradually reduced as the stimulation frequency increased (see Fig.2 A). During the 10 second high-frequency stimulation, a frequency high enough to suppress the urethral pressure response at the end of the stimulation (for example, 8 kHz as shown in Fig.2 A) could also block the pudendal nerve conduction (see Fig.2 B). Fig.2 B shows that the same stimulation (8 kHz, 2 mA) in the same animal blocked the urethral responses induced by the centrally located stimulation B (see Fig.1). The urethral pressure responses could not be blocked by stimulation A if the stimulation B was moved to a location on the pudendal nerve distal to the stimulation A (see Fig.2 C). This further indicated that pudendal nerve block rather than urethral muscle fatigue was responsible for the loss of the urethral pressure responses during the high-frequency stimulation (see Fig.2 A). At stimulation intensity of 1.5 times of the blocking threshold as used in this study, Fig.2 and our previous study¹¹ have shown that nerve conduction block occurred when the urethral pressure response was completely suppressed at the end of 10 second stimulation. Therefore, in this study the urethral pressure at the end of 10 second stimulation was measured to indicate the blocking effect (see Fig.4 B).

The minimal stimulation frequency required to completely block the pudendal nerve was reduced as the temperature was decreased. Fig.3 shows the urethral pressure responses to different frequencies of blocking stimulation A at different temperatures. At a temperature of 37 °C (Fig.3 A), the minimal stimulation frequency required to block pudendal nerve conduction during the 10 second stimulation was 6 kHz. This minimal frequency was reduced to 5 kHz when the temperature was decreased to 27 °C (Fig.3 B). At a temperature of 20 °C (Fig.3 C), the block occurred at the end of the 10 second stimulation with frequency of 4 kHz. Further decreasing the temperature to 15 °C (Fig.3 D) resulted in the block occurring at stimulation frequency of 4 kHz in less than 10 seconds. Fig.3 shows that the minimal blocking frequency changed from 6 kHz at 37 °C to 4 kHz at 15-20 °C. The tests as shown in Fig.3 were repeated 3 times in each animal. Fig.4 A summarizes the experimental results from all 3 animals (N=9). The urethral pressure at the end of 10 second high-frequency stimulation was measured to indicate the blocking effect (see Fig.4 B). At a certain temperature, the urethral pressures induced by stimulation at different frequencies were normalized to the pressure induced by

stimulation at 1 kHz. As shown in Fig.4 A, the normalized pressure-frequency curve was shifted toward a lower frequency when the temperature was decreased from 37 °C to 15 °C. The minimal stimulation frequency to induce a complete block was reduced from 6 kHz to 4 kHz as the temperature was decreased from 37 °C to 15 °C.

Based on the results shown in Fig.4 A, stimulation frequency of 4 kHz was chosen to determine more precisely the temperature threshold at which the nerve block occurred. The frequency of 4 kHz was tested because the urethral pressure response at this frequency changed from 0% to almost 100% when the temperature was increased from 15 °C to 37 °C (see Fig.4 A). Therefore, testing 4 kHz could show a full range of responses.

Fig.5 A shows how the urethral response to the 4 kHz stimulation recovered gradually when the temperature was increased from 14.5 °C to 36.5 °C in 2 °C increments. At temperatures below 24.5 °C, stimulation at 4 kHz could completely block the pudendal nerve conduction within the 10 second stimulation. This test was repeated 3 times in each animal. The results are summarized in Fig.5 B (N=9), in which the urethral pressure measurements were normalized to the value induced at a temperature of 36.5 °C. Fig.5 B shows that the 4 kHz stimulation could completely block the nerve conduction only when the temperature was below 24.5 °C.

In summary, for a certain stimulation frequency there is a corresponding maximal temperature below which the stimulation can completely block the nerve conduction (see Fig.5). For a certain temperature there is a corresponding minimal stimulation frequency above which the nerve can be completely blocked (see Fig.3-4). Therefore, the minimal frequency and the maximal temperature are paired in a one-to-one relationship. At a higher temperature, a higher stimulation frequency is required to completely block nerve conduction.

DISCUSSION

The results presented in this study showed that experimental temperature could be one of the factors that influences the minimal blocking frequency, although other factors might also be involved including electrode geometry (bipolar or tripolar), different nerves (sciatic nerve or pudendal nerve), or different species (frog, rat, or cat). Studies using rat sciatic nerves^{8, 12} showed that consistent nerve block could be achieved at stimulation frequency greater than 10 kHz. However, the experimental temperature was not defined,^{8, 12} although one of these studies¹² stated that the body of each rat was warmed during the tests by radiant heat from above and a heating pad beneath. In the frequency range between 5 kHz and 10 kHz, nerve block was not consistent and variable results were obtained in different rats.¹² This variable result might be partially caused by the un-controlled experimental temperature in different animals. A recent study⁶ using cat pudendal nerve found that nerve block could be observed between 1 kHz and 30 kHz, but the frequency range to induce a complete block varied significantly between animals. Although in this study⁶ the animal's body temperature was maintained between 37 °C and 39 °C using a thermal blanket, the method to control the pudendal nerve temperature was not described after the nerve was exposed for electrode placement. Our previous studies using cats^{1, 11} indicated that at temperatures between 35 °C and 37 °C the minimal stimulation frequency to block the pudendal nerve conduction was around 6 kHz. The pudendal nerve temperature in our previous studies^{1, 11} was controlled by covering the exposed nerve with warm Krebs solution or mineral oil. The minimal stimulation frequency of 4-5 kHz was reported in other studies using cat sciatic nerves¹⁶⁻¹⁸ when the temperature varied between 25 °C and 35 °C, 17-18 or was undefined (presumably at room temperature 20-25 °C). 16 More recently, a study using isolated frog sciatic nerve⁴ reported that nerve block could be observed at a stimulation frequency as low as 1 kHz at room temperature. But more effective or consistent block could be achieved between 3 kHz and 5

kHz. It is unfortunate that the specific room temperature was not defined in this study.⁴ It is worthy noting that the temperature influence could only partially explain the discrepancy of minimal blocking frequency (1-10 kHz) presented in previous animal studies,^{1,4,8,11,12,16-19} since it only caused the minimal blocking frequency changing from 6 kHz to 4 kHz in the temperature range of 15-37 °C based on the results from this study.

Due to the high frequency electrical artifacts during the stimulation, it is very difficult to investigate the possible mechanisms underlying the nerve conduction block in animal experiments using electrophysiology techniques. However, our previous studies using axonal models and computer simulation^{14,15} have shown that the constant activation of potassium channels under the block electrode is a possible mechanism underlying the nerve conduction block. As the stimulation frequency increases, the potassium channel changes from opening and closing alternatively to opening constantly when the stimulation frequency reaches the threshold level (i.e. the minimal blocking frequency). The dynamics of potassium channel activation is temperature dependent. As the temperature decreases, the potassium channel opens and closes much slower requiring a lower minimal blocking frequency to keep the potassium channel open constantly¹⁵. The results from this animal study agree with the conclusion from our previous computer simulation studies.

Nerve damage will always be a concern when electrical stimulation is applied chronically. However, a biphasic, charge-balanced stimulation waveform was used in this study which is safer than uniphasic, charge-unbalanced stimulation⁵. Also, when the blocking stimulation is applied to treat detrusor sphincter dyssynergia, it will only last 1-2 minutes at the time of voiding for 4-7 times per day. This short stimulation time is also relatively safe for peripheral nerves⁵. Our previous study also showed that the biphasic blocking stimulation (1 minute in duration), which was repeatedly (12 times) applied to the same site on the pudendal nerve during a period of 43 minutes, did not influence the ability of pudendal nerve to induce sphincter contractions in the absence of blocking stimulation¹¹. This suggested that little damage to the pudendal nerve occurred during this period. Furthermore, a stimulation frequency of 4.8 kHz was safely applied in human cochlear implants²⁰. The duration of auditory nerve stimulation is much longer than what is needed to block external urethral sphincter contraction during voiding. Therefore, the nerve blocking method employing a biphasic, charge-balanced stimulation waveform is very promising for use in applications to treat detrusor sphincter dyssynergia and facilitate voiding.

Our study is aimed at designing a neural prosthetic device to reversibly block pudendal nerve conduction and facilitate voiding in spinal cord injured people. Since human body temperature is about 37.5 °C, a stimulation frequency of at least 6 kHz will be needed to block the pudendal nerve based on this study. Therefore, the neural prosthetic device may have to be designed to deliver stimulation frequencies ranging from 6 kHz to 10 kHz in order to have additional flexibility.

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Fig.1.

Experimental setup to test the temperature influence on pudendal nerve block induced by high-frequency biphasic stimulation. EUS – External Urethral Sphincter.

Fig.2.

Pudendal nerve block by biphasic high-frequency stimulation. A. Urethral pressure induced by 10 second blocking stimulation A alone at 2 mA intensity. B. Blocking stimulation A blocked urethral responses induced by stimulation B located centrally to the stimulation A (see Fig.1); C. Blocking stimulation A failed to block urethral responses when stimulation B was moved to a location distal to stimulation A. Black bars in B and C indicate the stimulation durations. Temperature: 37 °C.

Fig.3.

Urethral responses to high-frequency biphasic electrical stimulation of pudendal nerve at different temperatures. Stimulation: 6 mA intensity, 10 sec duration. Urethral infusion rate: 2 ml/min. The black bars under each trace indicate the stimulation duration. The number under each black bar indicates the stimulation frequency in kHz.

Fig.4.

A. Normalized urethral pressure responses change with stimulation frequency and temperature. Stimulation intensity: 1-6 mA. Urethral infusion rate: 1-2 ml/min. N = 9. B. Expanded trace from Fig.3 A showing that the pressures were measured at the end of 10 second stimulation. The black bars under the pressure trace indicate the 10 second stimulation duration.

Fig.5.

A. Temperature influence on urethral response to high-frequency biphasic electrical stimulation of pudendal nerve. Stimulation: 4 kHz frequency, 1 mA intensity, 10 sec duration. Urethral infusion rate: 2 ml/min. The black bars indicate the stimulation duration. The number under each black bar indicates the temperature during the time when the stimulation was applied. B. Summary of the temperature influence on the normalized urethral pressure responses. Stimulation: 4 kHz frequency, 1-6 mA intensity. Urethral infusion rate: 1-2 ml/min. N = 9.