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Quantitative Evaluation of Electrodes for External Urethral Sphincter Electromyography during Bladder-to-Urethral Guarding Reflex

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Abstract

Purpose—Accuracy in the recording of external urethral sphincter (EUS) electromyography (EMG) is an important goal in the quantitative evaluation of urethral function. This study aim was to quantitatively compare electrode recordings taken during tonic activity and leak point pressure (LPP) testing.

Methods—Several electrodes, including the surface electrode (SE), concentric electrode (CE), and wire electrode (WE), were placed on the EUS singly and simultaneously in six female Sprague-Dawley rats under urethane anesthesia. The bladder was filled via a retropubic catheter while LPP testing and EUS EMG recording were done. Quantitative baseline correction of the EUS EMG signal was performed to reduce baseline variation. Amplitude and frequency of one-second samples of the EUS EMG signal were measured before LPP (tonic activity) and during peak LPP activity.

Results—The SE, CE, and WE signals demonstrated tonic activity before LPP and an increase in activity during LPP, suggesting that the electrodes accurately recorded EUS activity during tonic activity and during the bladder-to-EUS guarding reflex, regardless of the size or location of detection areas. SE recordings required significantly less baseline correction than both CE and WE recordings. The activity in CE-recorded EMG was significantly higher than that of the SE and WE both in single and simultaneous recordings.

Conclusions—These electrodes may be suitable for testing EUS EMG activity. The SE signal had significantly less baseline variation and the CE detected local activity more sensitively than the other electrodes, which may provide insight into choosing an appropriate electrode for EUS EMG recording.

Keywords

external urethral sphincter; urodynamics; electromyography; rat; muscle

Introduction

Electromyography (EMG) has several important clinical applications, including external urethral sphincter (EUS) EMG testing in urodynamics [1;2]. The recording of EUS EMG has several clinical [3-6] and experimental uses [7]. In particular, with the development of animal models of stress urinary incontinence [8], the recording of EUS EMG has been vital to the study of the response of the EUS in maintaining urinary continence [9].

Several electrodes have been used to study EUS EMG, including the concentric electrode (CE) [9], surface electrode (SE) [10;11], and wire electrode (WE) [12;13] for a variety of purposes. However, little is known about the consistency of different electrodes in recording EUS activity and in detecting the guarding response. In this study, we quantitatively assessed and compared EUS EMG recordings during bladder filling and during leak point pressure (LPP) testing to evaluate the ability of the electrodes to detect EUS EMG and the bladder-to-EUS guarding reflex.

Materials and Methods

The animal study was approved by the Cleveland Clinic Institutional Animal Care and Use Committee. Six female virgin Sprague-Dawley rats (200–250 g) were anesthetized with urethane (1.0 g/kg i.p.). Isoflurane gas (2.5%) was used as a supplemental anesthetic during the surgical procedures. A vertical midline abdominal incision was made and extended to the pubic symphysis, which was opened to expose the EUS.

Electrode placement procedure

Three types of bipolar electrodes were used singly or in pairs to record EUS EMG. A SE (straight end, 1 mm distance between poles, FHC, Bowdoin, ME) was placed on the surface of the EUS at mid-urethra. A CE (pencil-point tip, 0.1 mm distance between poles, FHC) was inserted 1 mm into the sphincter at mid-urethra. A WE was inserted into the EUS bilaterally along the mid-urethra (Fig. 1) using a 30-gauge needle. The WE consisted of 50 μ m diameter, teflon-insulated platinum iridium wires with 2 mm exposed tips (A-M Systems, Carlsborg, WA). The electrodes were connected to an amplifier (Model P511 AC Amplifier, Astro-Med, Inc., Providence, RI; band pass frequencies: 3 – 3 kHz) and a recording system (DASH 8X, Astro-Med, Inc.; 10 kHz sampling rate).

EMG recording during leak point pressure testing

A suprapubic polyethylene catheter (PE-50) was inserted and fixed into the bladder dome. The catheter was connected to both a pressure transducer (model P122; Astro-Med, Inc.) and a syringe pump (model 200; KD Scientific, New Hope, PA). The bladder was filled with saline at 5 ml/hr. The bladder was filled and emptied for several normal micturition cycles prior to testing. For LPP testing, a passive bladder pressure increase was made when the bladder contained approximately 0.3 mL by gradually pressing a cotton swab on the bladder until leakage occurred. As soon as one drop of fluid was seen at the urethral meatus, the external pressure from the cotton swab was rapidly removed and bladder pressure rapidly dropped to baseline value, as we have previously described [14]. If a bladder pressure contraction was induced, the results of that test were not analyzed and the bladder was fully emptied via palpation and filling and testing resumed. The LPP test was completed 3–4 times for each electrode combination in each animal. LPP was calculated as baseline pressure subtracted from peak pressure [15]. After testing, the rats were euthanized with pentobarbital (200 mg/kg i.p.).

Signal collection and correction

Tonic activity before the LPP test and activity at the pressure peak during LPP testing were identified and archived in 1s samples (AstroVIEW X, Astro-Med, Inc.). The 1s samples were taken to end at peak pressure during LPP testing, as previously described [11]. The 1s samples were filtered for 60 Hz using a digital notch filter, and traveling baseline signals were corrected as follows (Chart 5 Pro, ADInstruments, Colorado Springs, CO). The signal was first smoothed using a triangular Bartlett window (151 point width) to obtain a signal of baseline changes [16]. This smoothed signal was then subtracted from the original signal to create a corrected signal (Fig. 2a, 2b). The relative amount of baseline correction needed for each signal was expressed as the sum of the amplitude spectrum normalized to the sum of the amplitude spectrum of the original signal.

The signals were rectified and mean amplitude and frequency were calculated after baseline correction, (Myosotic SignalPoint 2007, Myosotic LLC, Woodinville, WA). A threshold for differentiating noise from activity was set at 0.25 μ V. The increase in bladder pressure was calculated to verify that the LPP testing technique was consistent.

Statistics

Mean amplitude and frequency during tonic activity were compared to mean amplitude and frequency during peak LPP activity for the single recordings with each electrode using a paired *t*-test. The amplitudes and frequencies of electrode pairs used in simultaneous recordings were compared using a paired *t*-test. Amplitudes, frequencies, and relative amount of baseline correction of the single recordings, recorded by the different electrodes during both tonic and LPP activity were compared using a one way repeat measures analysis of variance (ANOVA). Contrasts using a *t*-test with a Bonferroni correction were developed to permit selected pairwise comparisons of means. Increases in bladder pressure during single and simultaneous recordings were compared using a one way repeat measures ANOVA as above. To determine if amplitude, frequency, and relative amounts of baseline correction for a particular electrode were affected by the presence of another electrode, comparisons were made between recordings made using the same electrode under single or simultaneous recording conditions using a two way repeat measures ANOVA test. Contrasts using a *t*-test with a Bonferroni correction were developed to permit selected pairwise comparisons of means. In all cases, $p < 0.05$ was considered to indicate a statistically significant difference.

Results

EUS EMG signal baseline correction

A comparison of the single electrode recordings showed that the CE and WE recordings required a significantly greater amount of baseline correction than the corresponding SE recording during the tonic state (Fig. 2c). There was no significant difference in the amount of baseline correction required between the SE, CE, and WE for the single electrode recordings during LPP testing (Fig. 2c). A comparison of the simultaneous recordings indicated that the CE required a significantly greater amount of baseline correction than the SE during the tonic state (Fig. 2d). The CE with WE simultaneous recordings and the SE with WE simultaneous recordings did not significantly differ during the tonic state. There was also no significant difference in baseline correction between any of the simultaneous recordings during LPP testing. The single recording of each electrode was compared to its corresponding simultaneous recordings, and no significant differences in baseline correction were found.

EUS EMG amplitude and frequency

EUS EMG recordings made with the CE had significantly higher amplitude than those made with the SE or WE during both tonic activity and LPP testing during single electrode recordings (Fig. 3a, 3b). Simultaneous recordings showed that recordings made with the CE had significantly greater amplitude than those made with the SE during tonic activity and significantly greater amplitude than those made with the WE during both tonic activity and LPP testing (Fig. 4a, 4b). Amplitude of EUS EMG recordings made with the SE or WE were not significantly different during single or simultaneous recordings. When the single recording for each electrode was compared to the corresponding simultaneous recording, no significant difference in amplitude was found. EUS EMG amplitude during LPP testing was significantly greater than during tonic activity using either the CE or WE (Fig. 3b). The same trend was noticed in simultaneous recordings made with all 3 electrodes but the differences were not significant (Fig. 4b).

During single electrode recordings, the frequency of recordings made with the CE was significantly greater than that of recordings made with the SE or WE during both tonic activity and LPP testing (Fig. 3c). Frequencies of EUS EMG recorded with the SE or WE were not significantly different from one another. During simultaneous recordings, the frequency of EUS EMG recordings made with the CE was significantly greater than that of recordings made with the SE during tonic activity (Fig. 4c). EUS EMG frequency during LPP testing was significantly greater than during tonic activity using all 3 electrodes (Fig. 3c). The same trend was noticed in simultaneous recordings made with all 3 electrodes but the differences were not significant (Fig. 4c).

Bladder pressure

Mean bladder pressure increase to urinary leakage was not significantly different among the single or simultaneous recordings using the SE, CE, and WE (Fig. 3d, 4d), indicating that the testing conditions were similar in all cases.

Discussion

The EMG signal contains important objective information regarding muscle function and innervation. Currently, most pharmacological or animal injury studies involving EUS EMG are qualitative, since changes in the EUS EMG signal are often obvious [17;18]. During LPP testing, with increased movement, as well as increased tonic activity, various electrodes may measure EMG differently. In this study we quantitatively analyzed the EUS EMG signal recorded with 3 different electrodes, based on the normalized sum of the amplitude spectrum (amount of baseline correction), mean amplitude, and mean frequency. Quality of signal is important for quantitative analysis and an unstable baseline can invalidate analysis involving thresholding of the signal. We therefore developed a method of creating a corrected signal with a stable baseline and compared the amount of baseline correction required for recordings.

Recordings made with SE required less baseline correction than those made with CE or WE during tonic activity. Therefore, recordings made with SE have a greater chance of providing a cleaner and more readily quantifiable signal during tonic activity in cases when baseline correction is not done. The amount of baseline correction was not significantly different between the electrodes during LPP testing, which may have been due to increased local contraction and movement during LPP testing.

The significantly greater EUS EMG amplitudes and frequencies measured with the CE indicate that the CE is more sensitive than the SE or WE in detecting the firing of motor unit action potentials (MUAP) of the EUS during both tonic activity and during LPP testing. The sources

of MUAPs can be considered to be small dipoles, and the signals of these dipoles diminish rapidly with distance when spread over a greater volume [19;20]. Therefore, the smaller distance between the poles of CE (0.1mm) leads to a greater sensitivity than that of WE and SE whose poles are 1.0 and 1–2mm apart, respectively. The WE and SE record the activity of more motor units than CE but many of these motor units emit a weaker received signal due to signal dampening and may not be counted using the threshold method for determining frequency. The signal dampening may stem from the greater tissue distance the signal must be transmitted through or from different contacts with the EUS made by the different electrodes. Since the CE detects EUS activity more sensitively within a smaller region, it might measure EUS recovery after injury more sensitively. However, several different regions of the EUS would need to be recorded to make a complete assessment of EUS recovery since the CE records only local EMG signals and EUS recovery or pudendal nerve reinnervation may vary with location.

In this study, having all three of the electrodes in the EUS was not feasible because of the small size of the rat EUS. Therefore, paired comparisons were used. Most of the simultaneous recordings showed no significant difference in amplitude between CE and the other 2 electrodes. This may indicate that electrodes within close proximity interfere with each other during simultaneous recordings. However, there were no significant differences when the normalized sum of amplitude spectra, mean amplitude, and mean frequency for the single recording of each electrode was compared to the respective normalized sum of amplitude spectra, mean amplitude, and mean frequency of the same electrode during simultaneous recordings. Therefore, although there may be an effect of a nearby electrode, it is not a large effect.

We also compared amplitude and frequency between tonic activity and LPP testing situations for each individual electrode. A significant increase in activity from tonic activity to LPP testing would indicate detection of increased recruitment of motor units in the EUS. All 3 electrodes detected a significant increase in frequency with LPP. CE and WE detected a significant increase in amplitude. Because amplitude calculated in this study is an average, newly recruited motor unit firings from Onuf's nucleus of lower amplitude during the guarding reflex may offset the increase in amplitude of currently recruited motor units, resulting in a lower than expected change in amplitude between tonic activity and LPP signals. Therefore, mean frequency increase could be more a more sensitive determinant of increased EUS EMG activity compared to mean amplitude.

We conclude that the SE signal had significantly less baseline variation and the CE detected local EUS activity and the response to LPP testing more sensitively than the other electrodes.

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Test sequence	1	2	3	4	5	6
SE	√	√				√
CE		√	√	√		
WE				√	√	√

Figure 1.
Ventral view of electrode placement in the external urethral sphincter and electrode testing sequence.

Figure 2.

Baseline correction of external urethral sphincter (EUS) electromyography (EMG) recording.

a. Example of EMG signal recorded with surface electrodes (SE) at peak pressure during leak point pressure (LPP) testing (top); the same EMG signal after smoothing with a Bartlett triangular window (middle); and resultant signal after subtracting smoothed signal from the original signal (bottom). **b.** Amplitude spectrums of the original (top), smoothed (middle), and baseline corrected (bottom) EMG signals from A. **c.** The relative amount of baseline correction from singular recordings during tonic and peak LPP activity: the sum of the spectrum normalized to the sum of the spectrum of the original signal. **d.** The relative amount of baseline correction from simultaneous recordings during tonic and peak LPP activity as in c. Each bar

represents mean $\pm$ standard error of data from six animals. * indicates a significant difference in baseline correction compared to tonic recordings made with surface electrodes (SE). CE = concentric electrode. WE = wire electrode.

Figure 3.

Comparisons of single electrode external urethral sphincter (EUS) electromyography (EMG) recordings. **a.** Example EMG recordings made with surface electrodes (SE), concentric electrodes (CE), and wire electrodes (WE) during tonic activity and peak leak point pressure (LPP) testing. Note that the CE scale is greater than that of the SE and WE. **b.** Mean amplitude of EUS EMG signals during tonic and peak LPP activity. **c.** Mean frequency of EUS EMG signals during tonic and LPP activity. **d.** Increase in bladder pressure (peak LPP pressure – tonic pressure) during LPP testing. Each bar represents mean $\pm$ standard error of data from six animals. * indicates a significant difference between tonic and LPP activity. † indicates a significant difference compared to respective CE recording.

Figure 4.

Comparisons of simultaneous electrode external urethral sphincter (EUS) electromyography (EMG) recordings. **a.** Examples of simultaneous EUS EMG recordings made with surface electrodes (SE), concentric electrodes (CE), and wire electrodes (WE) during tonic activity and peak LPP testing. Note that the CE scale is greater than that of the SE and WE. **b.** Mean amplitude of EUS EMG signals during tonic and peak LPP activity. **c.** Mean frequency of EUS EMG signals during tonic and peak LPP activity. **d.** Increase in bladder pressure (peak LPP pressure – tonic pressure) during LPP testing. Each bar represents mean $\pm$ standard error of data from six animals. * indicates a significant difference compared to simultaneous CE recording.