Characterization of Slow Wave Activity in Ex-vivo Porcine Small Intestine Segments

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Abstract— **The motility of the gut is central to digestion and is coordinated, in part, by bioelectrical events known as slow waves. While the nature of these events is well defined in-vivo, the temporal response of ex-vivo gastrointestinal myoelectrical activity without perfusion is poorly understood. To achieve a fundamental understanding of ex-vivo electrophysiology, slow wave activity was measured from excised porcine intestinal segments and characterized over time. In this study, slow wave frequencies and amplitudes, along with the duration of sustained activity were quantified. Slow wave amplitudes and frequencies decreased from initial values of 46 ± 34 µV and 9.6 ± 5.9 cpm to electrical quiescence over a period of 12.2 ± 2.3 minutes. Mean slow wave amplitude and frequency uniformly declined before electrical quiescence was reached. This study demonstrated the successful acquisition of gastrointestinal myoelectrical activity in excised tissue segments without perfusion. Key slow wave characteristics may contribute to future diagnostics, transplantations and treatments for motility disorders.**

Clinical Relevance— **The ability to characterize excised slow wave activity in organs lacking perfusion will be a critical advancement in developing clinical solutions. Findings will assist in establishing the efficacy of bioelectrical activity in excised tissue samples for organ transplantation. In addition, the ex-vivo setting can be used to represent compromised electrophysiological states to evaluate novel therapies.**

I. INTRODUCTION

The motility of the gastrointestinal (GI) tract is governed by rhythmic electrophysiological events known as slow waves [1]. An improved understanding of slow wave characteristics at different physiological and environmental conditions is vital for therapeutic advancements in treating GI disorders which are associated with impaired electrophysiology [2], [3].

Fundamental GI electrophysiological investigations have been established in in-vivo, and in-vitro conditions [4]–[6]. In-vivo studies involve the experimentation on living organisms. In contrast, in-vitro studies primarily involve the experimentation of cellular and tissue-level segments in an artificial environment, such as a perfusion bath. Further to these conditions, ex-vivo studies involve the experimentation outside of its natural environment, typically without artificial perfusion [7], [8].

Following centuries of in-vivo GI investigations [5], [9], the normal human gastric slow wave frequency has been established at approximately 3 cycles per minutes (cpm) with an amplitude varying between 0.25-2.5 mV [10], [11]. Small intestinal human slow waves are known to occur at a frequency between 8-12 cpm with an amplitude of approximately 0.3 mV [12], [13]. In addition, in-vitro studies have been conducted on a range of different species to investigate the characteristics of slow waves [14], [15].

A recent study has quantified the myoelectrical activity of the ex-vivo human uterus without the aid of any perfusion [7]. In that study, active biopotentials were registered consistently in all uteri for at least 2 hours. Therefore, it is of interest that the viability of this technique is tested in the GI tract to establish the slow wave characteristics of resected GI tissues for transplantations and potential clinical applications.

As outlined by a previous study [7], understanding the ex-vivo electrophysiological function is critical in ascertaining the viability of GI organ transplantation [16], [17]. Furthermore, establishing the state and behavior of excised tissue electrophysiology will provide a platform to test novel therapeutics such as pacing. It has been shown that compromised GI pacemaker cells termed as interstitial cells of Cajal (ICC) are associated with a number of functional motility disorders [18], [19]. Therefore, the impairment of ICC function due to excision of tissue, and lack of perfusion can be used to simulate functional motility disorders and test the efficacy of novel clinical solutions.

In this study, we measured slow wave activity from intestinal segments without perfusion to determine the time course over which viable slow wave activity occur. These measurements will form the basis for future experimental studies to interrogate the electrophysiology of the GI tract when perfusion or neural innovation is compromised.

II. METHOD

A. Experimentation

Ethical approval for animal studies was granted by the University of Auckland Animal Ethics Committee. Following, unrelated investigations, tissue samples were obtained immediately after euthanasia. Porcine jejunal segments of approximately 15 cm were excised.

The ex-vivo experimental setup of the jejunal segment is illustrated in Fig. 1. Flexible printed circuit array(s) previously utilized in in-vivo GI investigations were used to record myoelectrical activity [20]. The arrays consisted of 32-64 recording sites with 4-5 mm inter-electrode spacing. Unipolar electrograms were recorded with the reference electrodes placed on the far end of the intestine. A heat lamp was used to maintain the temperature, and evaporation was

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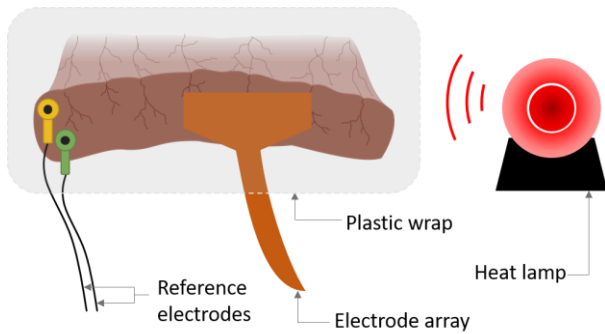
Figure 1. Experimental setup used to measure the slow wave activity in excised jejunal segments. An electrode array was placed on the serosa of the intestine segment. Temperature levels were maintained with a heat lamp and the evaporation of tissue biofluid was minimized with plastic film.

minimized by wrapping plastic film around the intestinal segments. Within 2 minutes of euthanasia, raw data was acquired at 512 Hz using a passive ActiveTwo bioamplifier system (BioSemi, Amsterdam, the Netherlands). Activity was recorded for at least 5 minutes past the point in time where the amplitudes of slow wave events were visually observed to fall below approximately 5 µV, which was then concluded to be electrically quiescent.

B. Signal Processing

The raw signals of all recorded channels were processed using custom software (GEMS v3.0) [21]. Signals were first down sampled to 30 Hz. Then, baseline drift was removed from raw recordings using a Savitzky-Golay filter (5 s window; polynomial order 6), and the common noise, which was defined as the median signal across all channels, was subtracted from all channels [22], [23].

Channels that visually demonstrated periodic slow wave activation were selected for further analysis. The recorded data was analyzed for 1 minute, every 2 minutes from the start of the recording until slow wave events were no longer visible.

C. Signal Analysis

The activation times of individual slow wave events were determined based on the steepest gradient. The slow wave frequency was calculated as the inverse of the period between consecutive slow wave activation times. In addition, the amplitudes of slow waves were computed by calculating the magnitude as the difference between the maximum and minimum value within a 0.5 s window centered around the activation time. Results are presented as mean ± standard deviation (SD).

III. RESULTS

Slow wave activity was successfully acquired from the intestinal segments of all pigs (n=5, 39.5 ± 0.9 kg). Slow wave activity was observed for 12.2 ± 2.3 minutes duration after the intestine was excised (range 9 – 15 minutes).

Representative slow wave recordings from one intestinal segment are shown in Fig. 2 to illustrate the variation in
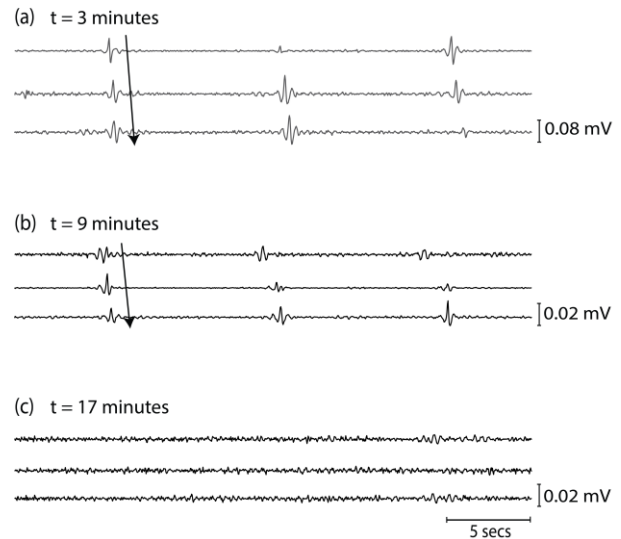

Figure 2. Signal traces representing 30 s of porcine ex-vivo activity at: (a) 3 minutes, (b) 9 minutes, and (c) 17 minutes following excision. The arrows indicate slow wave propagation across 3 adjacent channels during each time period.

amplitude and frequency over time. The highest amplitude was observed towards the start of the recording (Fig. 2(a)), while a decline in amplitude was noted in Fig. 2(b) at 9 minutes, prior to reaching a state of electrical quiescence at 17 minutes, as illustrated in Fig. 2(c). In addition, frequency
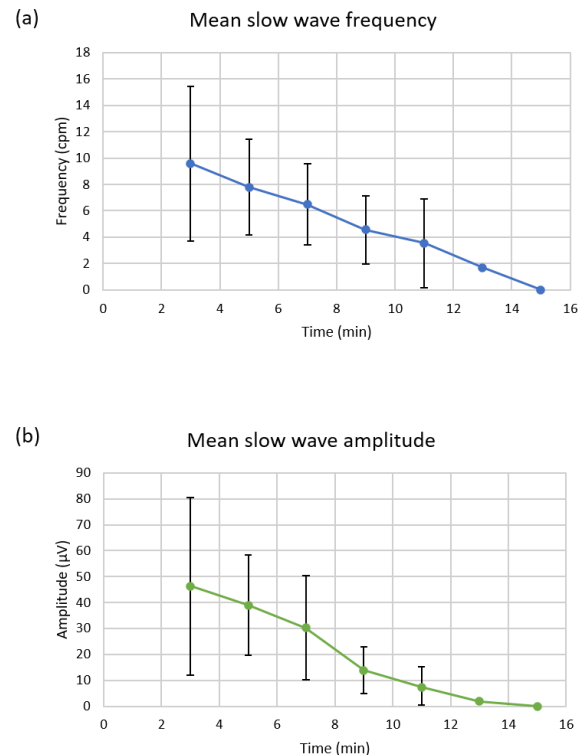

Figure 3. Variation in *ex-vivo* slow wave (a) frequency and (b) amplitude from 5 intestinal segments at different time intervals after euthanasia at t=0 minutes. A 2-minute delay between euthanasia and the start of recording was attributed to tissue resection, positioning of the intestinal segment and placement of electrodes. The first data point at 3 minutes had the highest mean frequency and amplitude, which declined over time and reached electrical quiescence at 15 minutes.

remained consistent at around 6 cpm in Fig. 2(a-b).

The frequency and amplitude of slow wave activity were quantified and visualized in Fig 3. The graphs indicate the mean and SD of the metrics at each time point for all intestinal segments. Overall, the slow wave frequencies across all intestinal samples exhibited a declining trend (Fig 3 (a)), however, in 2 of the 5 intestinal segments, the frequency remained relatively stable before rapidly reaching electrical quiescence. An average slow wave frequency of 9.6 ± 5.8 cpm was present in the excised segments 3 minutes after resection, and 13 minutes later the frequency dropped to 1.8 cpm. As depicted in Fig. 3(b), the amplitude of all tissue segments depicted a consistent trend of declining magnitude over time. Three minutes after excision, an average amplitude of 46 ± 34 µV was observed across all intestine segments, which gradually dropped to 2 µV at 13 minutes. All samples were electrically quiescent after 15 minutes.

IV. DISCUSSION

Slow wave potentials were successfully acquired from all 5 excised intestinal segments for a mean duration of 12.2 ± 2.3 minutes after excision. Mean slow wave amplitude and frequency uniformly declined before electrical quiescence was reached.

Electrical activity of uteri has been reported to remain active for 2 hours post-resection. However, based on our findings, the ex-vivo slow wave activity of the isolated pig intestine did not persist for such long periods. However, distinct propagation between neighboring electrodes was observed for the entire period the intestine was electrically active. Other studies have shown that perfusing the isolated ex-vivo GI segments can sustain activity for longer periods [6], [24]. For example, some colonic studies have investigated propagating contractions post-resection using high-resolution manometry, while another study focused on observing the effect of neuronal stimulation on these contractions [6], [24], [25].

The slow wave amplitudes of all jejunum segments in this study were lower than previous in-vivo studies, even at the initial point of analysis (mean amplitude 46 µV vs 300 µV) [26]. The ex-vivo tissue segments have a number of differences to in-vivo studies including tissue damage, lack of perfusion, loss of blood, loss of neural innovation and uncoupling from the rest of the intestine. Thereby, the functional and physiological disruption to the natural state of the tissue is a plausible explanation for the lower amplitude and eventual termination of biopotentials in the excised tissue. However, despite efforts to maintain an ideal experimental setting, external factors such as maintaining appropriate temperature and moisture content of the excised tissue which are associated with the experimental setup could accelerate the amplitude decline and activity termination.

Each tissue segment demonstrated variable frequency values. The excision of tissue samples [27] and anesthetics [28] are known to induce irregularities in slow wave frequency. Biochemical changes in post-mortem tissue may further induce irregularities.

While slow waves are primarily governed by ICC that lie within the smooth muscle layers of the GI wall, neuronal pathways and humoral inputs are also regulators of GI myoelectrical activity [29]. However, having established that slow wave activity sustains for approximately 12 minutes after tissue excision, it is evident that activity remained coordinated even after the discontinuity of neuronal pathways along with blood circulation and other signaling pathways. It implies that governing mechanisms that lie within smooth muscle such as ICC on its own can coordinate slow waves, even in the absence of any auxiliary signaling pathways or organ systems. However, it is important to note that these results are based on 5 intestinal segments. Therefore, further research with larger sample size will need to be performed to improve the reliability of these findings to draw well-established electrophysiological conclusions.

Future work involving histological studies will improve the findings by investigating the state of tissue preservation under a transplantation setting. This may assist in testing the efficacy for both organ transplantation as well as skin grafting. In addition, pathological evaluations may help to identify influential functional motility disorders to differentiate irregular behavior from that of the healthy.

Having established the electrophysiological behavior of the unperfused intestine, novel therapeutics can be evaluated with reference to the current findings. The developed experimental framework and recording techniques can be further applied to measure electrophysiological viability in facilitating organ transplantations and organs in ischemic conditions.

V. CONCLUSION

In this study, GI slow waves were measured from excised intestine segments without perfusion. Improving the reliability of the present findings with further animal studies will enable development of an experimental framework under which dysfunctional states can be simulated and upon which novel therapies and clinical solutions can be validated.

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