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Dialysis membrane-enforced microelectrode array measurement of diverse gut electrical activity

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ABSTRACT

A variety of electrical activities occur depending on the functional state in each section of the gut, but the application of microelectrode array (MEA) is rather limited. We thus developed a dialysis membranes-enforced technique to investigate diverse and complex spatio-temporal electrical activity in the gut. Muscle sheets isolated from the gastrointestinal (GI) tract of mice along with a piece of dialysis membrane were woven over and under the strings to fix them to the anchor rig, and mounted on an 8×8 MEA (inter-electrode distance=150 μm). Small molecules (molecular weight < 12,000) were exchanged through the membrane, maintaining a physiological environment. Low impedance MEA was used to measure electrical signals in a wide frequency range. We demonstrated the following examples: 1) pacemaker activity-like potentials accompanied by bursting spike-like potentials in the ileum; 2) electrotonic potentials reflecting local neurotransmission in the ileum; 3) myoelectric complex-like potentials consisting of slow and rapid oscillations accompanied by spike potentials in the colon. Despite their limited spatial resolution, these recordings detected transient electric activities that optical probes followed with difficulty. In Addition, propagation of pacemaker-like potential was visualized in the stomach and ileum. These results indicate that the dialysis membrane-enforced technique largely extends the application of MEA, probably due to stabilisation of the access resistance between each sensing electrode and a reference electrode and improvement of electric separation between sensing electrodes. We anticipate that this technique will be utilized to characterise spatio-temporal electrical activities in the gut in health and disease.

1. Introduction

A variety of electrical activities occur in the gut due to its high spatio-temporal requirements for motility (Tomita, 1981; Szurszewski, 1987). The gut is an extremely long tube in the body divided into several sections, including the stomach, duodenum, ileum, and colon. Motility features differ greatly even between the subsections of each section and also change depending on their functional state. Circardian rhythms, food intake and many other daily life activities alter an organism's functional state through the autonomic nervous system and hormones. In addition, under pathological conditions, including psychological stress, gut activity changes significantly. For instance, a group of patients with irritable bowel syndrome (IBS) suffers from bowel conditions alternating with constipation and diarrhoea (Spiller et al., 2007).

Multiple control systems of excitable cellular organizations overlap throughout the gastrointestinal (GI) tract, cooperating properly to yield smooth and elaborate movements (Huizinga and Lammers, 2009). Enteric neuron circuitry employs rapid spikes of action potentials, and neurotransmitters evoke neuromuscular junction potentials (Furness, 2006). Smooth muscle cells also spontaneously generate action potentials and electrically respond to neurotransmitters and hormones (Tomita, 1981; Szurszewski, 1987). Furthermore, network-forming special interstitial cells exist (Huizinga et al., 1997; Komuro et al., 1999), both generating and propagating pacemaker potentials in the GI tract (Sanders et al., 2004; Nakayama et al., 2006).

Investigations into mechanisms underlying gut motility thus require a technique to measure and analyse electrical activities spatio-temporally that can handle high diversity and complexity throughout a long GI tract due to regional differences in existing excitable cellular organizations, and changes in their overlapping activities under physiological and pathological conditions. Here we used dialysis

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membranes to enforce MEA, demonstrating several examples of measurements of gut electrical activities: propagation of fast spikes to slow oscillations, as well as very transient potentials upon neuromuscular transmission.

2. Material and methods

2.1. Animals and preparations

Animals were treated ethically, in accordance with the guidelines for proper conduct of animal experiments published by the Science Council Japan. All procedures were approved by the Animal Care and Use Committee of Nagoya University Graduate School of Medicine (Permission #28316). C57BL/6J wild type mice (8–20 weeks old) were sacrificed by cervical dislocation after inhalation of carbon dioxide. The GI tract was quickly resected, and several portions of the stomach, ileum and colon were isolated in a glass dish mounted with silicone rubber. The whole-muscle layer containing the myenteric plexus was isolated using fine forceps and a pair of scissors for microsurgery under a binocular stereoscopic microscope.

2.2. Electrical recordings

An array of 8×8 microelectrodes with low impedance (P515A, Alpha MED Scientific, Ibaraki, Japan) was used to measure a variety of electrical activities underlying gut motility. The inter-electrode distance was 150 μm, and the sensing area was ~ 1 mm^2. The muscle samples were firmly mounted on MEA, with the longitudinal muscle layer facing down, using a small piece of dialysis membrane (Cellulose tube, VISKING, USA) and a slice anchor (SDH series, Harvard Apparatus Japan, Tokyo, Japan). The muscle sheet and dialysis membrane were woven over and under the strings to fix them to the anchor rig except in the MEA region (Fig. 1a). The pores of the dialysis membranes had a molecular weight cut-off of 12,000, and oxygen, ions and energy metabolites were thus able to be exchanged (Fig. 1b), maintaining a physiological environment for electrical activity. The MEA recording chamber (~2 ml in volume) was placed on a heater kept at ~34 °C. In all example measurements shown in this study, the oral and anal ends of the muscles were placed towards the upper and lower ends of the MEA, respectively.

Each sensing microelectrode of the MEA was a ~50 μm square, made by fixing Pt black nanoparticles, thereby increasing the surface area by ~200-folds equivalent to 0.5 mm^2. The capacitance (C_{ME}) and resistance (R_{ME}) of each microelectrode were approximately 0.052 μF and 15 kΩ, respectively. The impedance was thus sufficiently low enough to follow a wide frequency range of electric signals. As communicated previously, the efficacy of signal transmission (*Tr*) was more than 95% for frequencies as low as 0.1 Hz (Fig. 1c: the simulation of *Tr* was modified from Taniguchi et al., 2013). Notably, although the microelectrode remained the same size (50×50 $μm^2$), *Tr* increased considerably at approximately 0.1–1.0 Hz due to the increased surface area provided by the nanoparticles.

A set of 8×8 field potentials was recorded using a multi-channel AC amplifier with high-pass filtering at 0.1 Hz and low-pass filtering at 10 kHz, and the arrayed data were stored in a personal computer through a 14-bit A/D converter with a sampling rate of 20 kHz. The dynamic range of A/D conversion was usually set to ± 1 or 2.5 mV, with a digital resolution of ~0.12 or 0.30 μV.

2.3. Solutions and drugs

The "normal" extracellular solution, a modified Krebs solution, had the following composition (in mM): NaCl 125; KCl 5.9; $MgCl_2$ 1.2; $CaCl_2$ 2.4; glucose 11; and Tris-HEPES 11.8 (pH 7.4). Nifedipine, and atropine were purchased from Sigma-Aldrich (St Louis, MO, USA). Nifedipine at 2 μM and atropine at 1 μM were applied to suppress L-type voltage-gated Ca^{2+} channels and muscarinic receptors of acetylcholine (ACh) in smooth muscle, respectively, in some experiments.

2.4. Data analysis

The arrayed data of field potentials recorded at a sampling interval of 50 μs were appropriately thinned by reducing the digital resolution. For example, to display the time course of slowly oscillating potentials in the colon (Fig. 3c), the digital resolution was reduced to 5 ms, while for analysing electric current-evoked potentials, the stored field potentials were used at the original time resolution. Digital filtering and linear spectrum analysis were performed using commercial add-in software (Kyowa Electronic Instruments, Tokyo, Japan).

In the pseudo-color images, the 8×8 array data of field potentials were thinned by 100-fold in the time domain and processed by a band-pass filter (BPF) at 0.25–10.5 Hz. The 8×8 field potentials were interpolated by calculations using a spline function with 50 points between each potential at certain recording times, using the MATLAB software package (MathWorks, Natick, MA, USA) (Nakayama et al., 2009). The magnitude of field potential is affected by numerous factors, including differences in impedance between sensing electrodes, access resistances between each sensing electrode and reference electrode, and different flows of electric currents due to variable distributions of pacemaker and modulator interstitial cells. To compensate for these factors, the amplitude of the field potential recorded in the n^{th} microelectrode region [ME(n)] was corrected by a normalizing factor [F_N(n)]:

$$LS_{0.25-10.5}(n)/\mathrm{Sum}\,[LS_{0.25-10.5}(1–64)],$$

where $LS_{0.25–10.5}$(n) is the integral of the amplitudes of the linear spectrum in the frequency range between 0.25 and 10.5 Hz for the field potential data at ME(n). This parameter was used to represent the magnitude of pacemaker activity.

Numerical data are expressed as means ± S.D. for the electrically evoked field potentials.

3. Results and discussion

3.1. Spontaneous electrical activity in the ileum

First, we showed an example of dialysis membrane-enforced MEA measurement in isolated ileal muscles of mice in a normal extracellular medium (Fig. 2a,b). As shown in Fig. 1a, an ileal muscle sheet with circular and longitudinal muscle layers was mounted in the MEA chamber with the circular muscle layer facing up attached to the dialysis membrane, while the longitudinal muscle layer (serous membrane) faced down, and was attached to the array of sensing electrodes. In all example measurements including this sample, the oral and anal ends of the muscle sheet were placed towards the upper and lower ends of the MEA, respectively. Therefore, the top-to-bottom and the left-to-right directions represent longitudinal and circular muscle layers, respectively.

An instantaneous 8×8 plot of spontaneous electrical activity corresponding to the sensing area of ~1 mm^2 showed that transient potentials were prominent in the upper half of the MEA, while slow base-line oscillations with a large amplitude overlapped in the middle left region (Fig. 2a). Subsequent off-line signal processing with a band pass filter (BPF) at 5–1000 Hz and a hum noise filter (HNF) of ~60 Hz removed slow oscillations in the baseline and distinguished two types of transient potentials (Fig. 2b). One occurred with an interval of ~2 s, propagating over several microelectrode regions (MEs). The interval of occurrence suggested that the propagating potentials reflect pacemaker activity (Taniguchi et al., 2013). Propagating potentials (left red bar) are shown expanded [ME(19–22), in red square panel]. The other was a series of bursting spike-like fast potentials with only a short delay (right blue bar). The durations of the bursting period were similar in