Extended Abstracts of the 2012 International Conference on Solid State Devices and Materials, Kyoto, 2012, pp1081-1082

I-7-1(Invited)

Direct Imaging of Acid Release from Biological Specimens on a Solid State 2D Detector

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1. Introduction

Acid is playing an important role in digestion of foods in the stomach and unwanted materials in the tissues. Proteins are cleaved by proteases optimally at a low pH, and calcium salts in the boney materials are readily dissolved in the acidic environments. Hydrochloric acid is known to be secreted from the gastric glands. The pH of gastric juice is reported to be as low as 1–2. This HCl-containing fluid in the stomach sometimes causes an injury to the gastric mucosa itself, resulting in an ulcer. To understand the diseases, it is very basic to consider the local balance between the acid secretion and anti-acid substances like the mucus. A similar situation is true for the osteoclast activity in the bone. In some tissues, the acid release is not known at all but maybe essential to their diseases. To date, however, no devise has been available for measurements of local distribution of the acid secreting activities.

The silicon-based imaging device recently developed by Sawada et al. [1] is able to measure the local pH of solution by using an array of silicon nitrate deposited detectors aligned 2 dimensionally. It produces an electrical output in proportion to the surface potential on each pixel, thus displays the pH of solution with a high spatial resolution (~100 μm). With the chemical microsensor, 2D imaging of not only $[pH]_o$ but also transmitter releases were possible in the living cells without labeling [2, 3]. Here, we tested this device for visualization of pH distribution near the biological tissues and demonstrated their characteristic acid dynamics. We examined cells and tissues of medical interest, i.e., gastric glands (responsible for the gastric ulcer), osteoclasts (responsible for the osteoporosis), and hippocampal neurons (showing the excitotoxic cell death [4]).

2. Materials and Methods

The gastric mucosa [5] was obtained from the guinea pig by scraping the surface of excised stomach. The isolated tissues was cut into small pieces by razor blades, and loaded on the 2D pH detector filled with a physiological saline. The tissue was covered with a fine net to avoid its movement. The mature osteoclasts were prepared by the methods described as previously [6]. Hippocampal tissues were obtained from the neonatal rat, and organotypically cultured on milli-pore membrane filter for 1 week [7]. Then it was placed on the pH image sensor in a slice-side-down configuration. All those preparations were used for the measurements in a recording medium containing 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM *D*-glucose, and 10 mM HEPES (pH = 7.2 with NaOH). The tissues and cells were stimulated by applying a drop of solution containing a chemical stimulant.

A 2D (32 × 32 pixels) pH detector made by a SiN deposition technique (Japan Chemicon, Tokyo) was used [1]. The reference electrode consisted of Ag-AgCl and a bridge made of 3 M KCl solution (Horiba, Kyoto). 2D images obtained with the detector were sequentially recorded and stored in a PC.

3. Results

The assembly of gastric glands loaded on the detector in a side-on manner showed a large release of protons in the mucosal side and a some lowering of pH in the basal side also (Fig. 1). These responses were rather clear when the preparation was kept in a slightly alkaline solution. Sometimes responses were temporary biphasic

The osteoclasts cultured on a filter membrane also showed a clear release of protons when the cell preparations were placed rather closely to the

detector. The lowering of pH near the cells were clear even without stimulation. Histamine (100 μM) stimulation enhanced the response.

The hippocampal slice is known to show the response of excitotoxic neuronal cell death [4,7]. We measured [pH]o during this response by stimulating the tissue with glutamate. The [pH]o of solution -

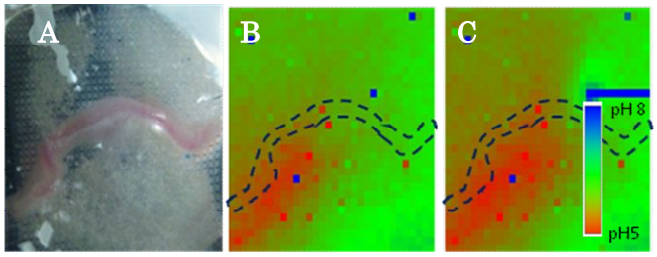

Fig. 1. 2-dimensional $[pH]_o$ imaging of gastric glands. (A) photograph of excised gastric tissue placed on the ion image sensor. $[pH]_o$ image before (B) and after (C) stimulation with 1 mM histamine. Locations of cellular samples were marked by dashed lines in B and C.

measured before placing the slice preparation was about 7.2. After placing the hippocampal slice, the pH was slightly decreased in the area where the somata of pyramidal cells were aligned. When the slice was stimulated by adding a glutamate (1 mM)-containing medium over the membrane filter, the pH did not change in the initial 5 min, but then, a clear decrease in pH was observed (Fig. 2). The low pH signal in the region of CA1 sometimes appeared in a shape of a short arc resembling the line of pyramidal cells. Mostly, the pH response in the CA1 region was larger than that in the CA3 region. The selective decrease of [pH]o in the CA1 region may reflect the glutamate-induced necrotic cell death. These results indicate that rapid diagnosis of the neuronal cell death is possible by the 2D-detection of [pH]o

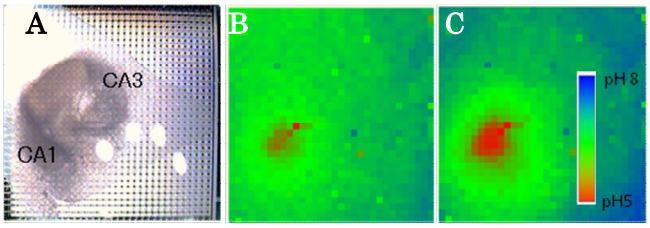

Fig. 2. Glutamate-stimulated neurons of the hippocampal slice. (A) photograph of hippocampal slice placed on the ion image sensor. $[pH]_o$ image before (B) and after (C) stimulation with 1 mM glutamate. Regions of both CA1 and CA3 are indicated in A.

4. Conclusions

Using a 2-dimensional pH imaging device, pH distributions in biological microenvironments were observed at a high resolution. Since the detector is sensitive to the light as well under a proper control, the microscopic image of pH distribution may be compared with light microscopic images of the same preparations. The technique is very promising for future research to reveal pathogenic mechanism in the tissues.

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