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Short communication

Neurosecretory cell-based biosensor: Monitoring secretion of adrenal chromaffin cells by local extracellular acidification using light-addressable potentiometric sensor

Qingjun Liu[a,b], Ning Hu[a], Fenni Zhang[a], Hua Wang[a], Weiwei Ye[a], Ping Wang[a,b,*]

[a] Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, PR China

[b] State Key Laboratory of Transducer Technology, Chinese Academy of Sciences, Shanghai 200050, PR China

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ABSTRACT

Vesicular exocytosis plays an important role in many physiological processes. The dense-core vesicles release of chromaffin cells is a suitable model for the presynaptic process in neurosecretory cells. In this study, light-addressable potentiometric sensor (LAPS) was introduced as a label-free recording method for vesicle release by the local extracellular acidification. The chromaffin cells are directly cultured on the sensor surface. After cells and LAPS hybrid system is established, the events of vesicular exocytosis are recorded. Protons stored in the vesicles and co-released with transmitters, induced a brief acidic shifts in the cell-sensor cleft. Under the stimulation of the KCl and acetylcholine (Ach), the signals presented the different amplitude and exocytosis rate, and reflected the specific features of the exocytosis. The result indicates that neurosecretory cell-based biosensor will provide a useful platform for neurosecretion mechanism research by monitoring the exocytotic activities with extracellular acidification sensing.

1. Introduction

Vesicular exocytosis is a ubiquitous biological mechanism involved in many significant physiological processes. It plays an especially important role in communication pathways of neurosecretory cells. Cells release transmitters and hormones by exocytosis of vesicles. Among the cell models that have provided insight into the molecular machinery underlying the successive steps of secretory pathway, adrenal chromaffin cells, have become a well established model for neurosecretion studies (e.g. Bader et al., 2002; Cárdenas, 2004). And, chromaffin cells remain a powerful model to address new and still open questions in the field of secretion, by offering opportunities to combine the recent biophysical techniques studying vesicle resolution and specific biochemical modifications involved in exocytosis (e.g. García et al., 2006; Livett, 2009).

With the development of biological studies, proton has been identified to be released as co-transmitters during synaptic signaling of neurosecretory cells (DeVries, 2001; Palmer et al., 2003; Shuba et al., 2008; Zhang et al., 2009). The postsynaptic potentials were found accompanied by brief acidic shifts. Protons stored in the vesicles and co-released with transmitters to modulate presynaptic Ca^{2+} channels in excitation-secretion coupling. Therefore, the synaptic transmission is accompanied by significant pH-shifts, which may conceivably occur when the highly acidic contents of synaptic vesicles (pH 5.5) are emptied into the synaptic cleft.

For cell-based biosensors, cells are not only considered as sensing elements, but also as rich information sources accessed using techniques such as cell impedance spectra, microenvironment evaluation, and electrophysiological signals, which are surveyed in microelectronics sensor chips (Bousse, 1996; Rudolph and Reasor, 2001). These are complemented with other novel technologies that could be employed for cell measurements. Light-addressable potentiometric sensor (LAPS) is a utility sensor, which is attractive for H^+ sensibility and simple structure (Hafeman et al., 1988). Due to the high H^+ sensibility, the Molecular Device Corporation invented the Cytosensor (microphysiometer) based on LAPS to measure the protons secretion rate from cells, and have been widely used in several aspects of the biomedical field, such as receptor analysis and drug analysis (Hafner, 2000; Wang and Liu, 2009). However, the microphysiometer monitors the metabolism of groups of cells, with the acidic metabolites, such as carbon dioxide and lactic acid of energy metabolism, acidify cellular environments. The conventional microphysiometer is difficult to confirm the target spot of single cell, saying nothing of bioactive units as synapse.

* Corresponding author at: Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, PR China. Tel.: +86 571 87952832; fax: +86 571 87952832.
E-mail address: cnpwang@zju.edu.cn (P. Wang).

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Now, we use LAPS to indicate secretion from a single chromaffin cell. When exocytosis vesicles fuse with the cell membrane, their content is released into the cell-sensor cleft. Because of the low pH 5.5 and the high buffer capacitance of a vesicle, a local drop pH can be expected. The change of the electrical surface potential leads to a change of modulation photocurrent in the LAPS. With light-addressable feature of sensor, we can detect the stimulus-induced secretion signals by the microscope positioning single cell. Thus, we aim to establish a utility platform based on sensor to study the cellular exocytosis.

2. Experiment and method

The LAPS chip and detecting setup were similar to the system we have detailed (Liu et al., 2006). The LAPS consists of an electrolyte-insulator [SiO_2/Si_3N_4]-semiconductor [Si] (EIS) structure that is fit for detecting the biophysiological parameters of extracellular microenvironment. The LAPS chip is coated with a thin silicon oxynitride as the insulating layer, which can effectively separate the silicon substrate from the electrolyte. The insulating layer interacts with protons in the solution to form a group of silanol (Si—OH) and silamine (Si—NH_2) (Hafner, 2000). Thus, the protons affect the sensor surface potential.

As is shown in Fig. 1A, if a voltage is applied onto the sensor, an electric field is formed at the silicon–insulator interface. By the modulated infrared light illuminating at the front side of LAPS, a photocurrent is generated. At the atomic level, this photocurrent corresponds to a hole-electron pair creation by radiation absorption from the laser. Since a field potential is formed in the chip, holes and electrons move in opposite directions, resulting in the local current. The photocurrent can outflow from backside aluminum layer. The cells were directly cultured on the LAPS. Exocytosis of chromaffin cells occurs after appropriate stimulation by chemical substances. The cell responded to them and resulted in the vesicle fuses within the plasma membrane. (Fig. 1B) The protons contained in intracellular vesicles, may thus be released into the extracellular medium (Schroeder et al., 1996; Amatore et al., 2008). The change of the surface potential induced by H^+ is coupled to the bias voltage applied to LAPS, and photocurrent magnitude reflected surface potential.

Chips with chromaffin cells were installed into the LAPS setup under microscope. And then we used KCl and acetylcholine (Ach) to stimulate cells to determine whether the secretion of catecholamine was affected by different stimulants. The sensor was filled with Tris-buffered Tyrode's solution (in mM): 140 NaCl, 2.8 KCl, 5.0 $CaCl_2$, 1 $MgCl_2$, 3.0 Tris (adjusted to pH 7.4 with HCl). Cells were stimulated by application of either an isotonic K^+-based Tyrode's solution (in mM): 62.8 KCl, 5.0 $CaCl_2$, 1.0 $MgCl_2$, 3 Tris (pH 7.4), or by bath Tyrode's solution supplemented with agonists of 100 μM Ach. A simple cycle protocol was employed for exocytosis measurement. The spontaneous activities of cells were recorded as the control group for 5 min. Then the KCl or Ach solution was added to stimulate the cells with the vesicle release for 10 min. Recovery was measured after washing cells gently five times at 1 min intervals by the external solution.

3. Results

3.1. Detecting performance of LAPS for acidification

The pH value in the solution is determined by measuring the current-voltage (I–V) curve in LAPS. The I–V curve results presented a good linearity and repeatability in our study. Linearity ranged from −300 mV to 700 mV, with the slope of the I–V curve was about 4.1 ± 0.3 nA/mV (Fig. 2A). Then the most sensitive point was calculated as working voltage for the long-time constant voltage detecting. Based on the I–V result, the calibration experiments were carried out to determine the relationship between pH and photocurrent. Fig. 2B displayed solution with pH 5, 6, 7 and 8 in the long-time detecting under the working voltage. In the cell experiment, 4 kHz modulation frequency of the laser light was used in the LAPS system, which was based on the calibration and test results. The detecting system set the amplification mode of the detecting system. The signals were offset by removing the baseline photocurrent and expanded the photocurrent with 100 times for detecting faint secretion signals. The signal-to-noise ratio (SNR) of the system was about 20 dB in the linear working region, which can satisfy the requirement of cell experiments.

3.2. Secretion recording of chromaffin cells by LAPS

After cells exposure to KCl, an immediate and large level of secretion was induced. LAPS recorded signals of the cell secretion for about 10 min. And, there were some burst of exocytotic events followed in those substantial responses. After stimulated the cell with Ach, a series of events was also monitored. While, the amplitudes of response signals were larger than those caused by KCl. And, the signals burst in a short time. Those spikes indicate release of secretory vesicles, representing the very responses to stimulus (Fig. 3A).

The photocurrent spikes could provide kinetic information to exocytotic events. Each spike may be characterized by amplitude, rise time and decay time. The amplitude depends on the maximum flux of H^+ (with neurotransmitters) released in sensitive detection regions during an event. The rise time is related to the process of secretion, which approximately represents those of the membrane full fusion, while decay time reflects process of the H^+ diffusion release in the buffer. The amplitude, duration and rate of secretory spikes were analyzed under the different experimental conditions in Fig. 3B.

The amplitude and exocytotic rate caused by high K^+ and Ach significantly increased, comparing to the spontaneous control group. The results indicated that spike parameters were different. Ach causes a robust level of secretion by chromaffin cells, which suggests an increased diffusivity of the catecholamine released into the buffer. While, increased extracellular K^+ could cause membrane depolarization by opening of voltage-gated Ca^{2+} channels. Therefore, both of stimulation resulted in more protons generation. Due to the relative variability of these results, there was no significant difference in rise time and decay time of spike between the two groups. It presented that the stimulus have less effect on the duration of exocytotic event. All of these were according with the exocytotic effect of high K^+ and Ach to chromaffin cells.

4. Discussion

Acidic synaptic vesicles produce local extracellular acidification of synaptic clefts, and this effect suggests unique ability for rapid signaling of exocytosis. Vesicle recycling in neurons terminals has been studied by synaptopHluorin (synaptophysin fused with pH-sensitive green fluorescent protein) (Shuba et al., 2008). A recent study found that the pH dependence of quantum dots emission would allow to report of exocytotic events, which was similar to pHluorin-based indicators. The photoluminescence increased by ∼15% when pH was raised from 5.48 (intravesicular) to 7.34 (extracellular) (Zhang et al., 2009).

With measuring cellular signals in label-free analysis, cell-based biosensors provide non-invasive and long-term detection of the functional information of living cells. For exciting cells, silicon-based devices such as field effect transistor (FET) and LAPS have been designed as neuron chips for electrophysiological activities

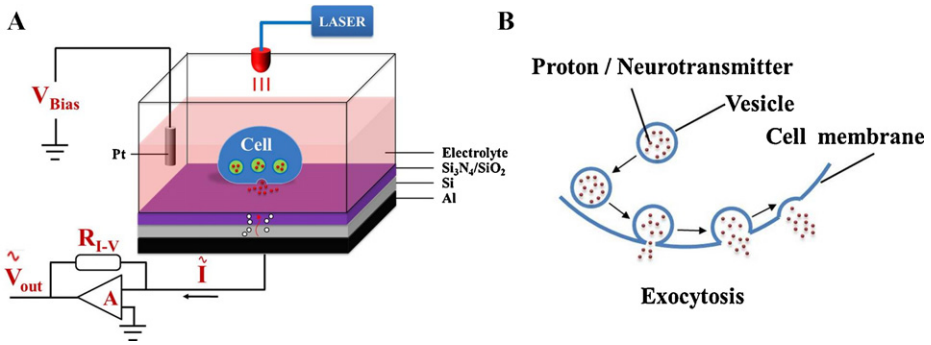

Fig. 1. The detection principle and system of LAPS. (A) LAPS detecting system with chromaffin cells on the sensor. (B) The exocytosis during fusion of vesicles in the cell membrane with H^+ releasing.

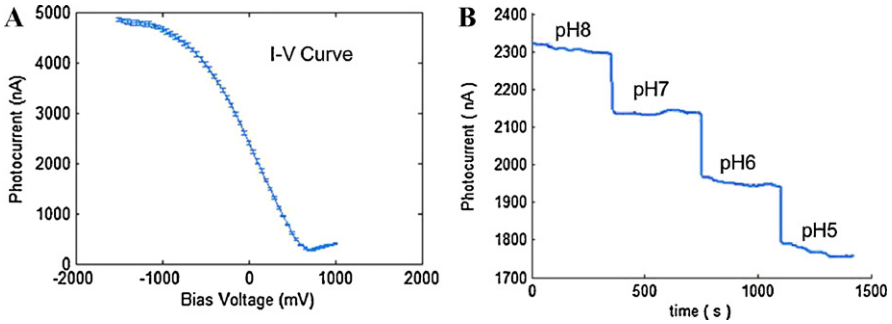

Fig. 2. The test and calibration results of LAPS performance. (A) Linearity and repeatability test result of sensor in the I–V curve scanning. (B) Calibration results between the pH and photocurrent of sensor in the long-time constant voltage detecting.

(Fromherz, 2002; Liu et al., 2006). The flow of electrical current in the membrane would give rise to a voltage in the cell-chip junction as was observed with open ion channels. However, when employed to monitor the cell secretion, the sensor chips can be considered as cell-semiconductor synapse (Lichtenberger and Fromherz, 2007). In principle, the sensor signal of beneath an attached cell may indicate a changed voltage drop in the electrical double layer from the oxide surface to the cell-chip junction or a voltage in the cell-chip junction with respect to the bath electrolyte. However, during the vesicle release of a chromaffin cell, there is no flow of electrical current that would give rise to a voltage in the cell-chip junction as is observed with open ion channels. Thus, the signal must be caused by an effect on the electrical double layer caused by local extracellular acidifications.

Here, LAPS was introduced as probes for the exocytosis of vesicles, which has similar structure and detection principle with FET sensor. Compared with limited number of the gate-electrodes in FET, a very interesting feature of LAPS is the complete flatness and simple layout, resulting in the absolute absence of on-plane or out-of-plane contacts and encapsulation (Fanigliulo et al., 1996). This leads to an optimization of the micro-chamber designed for cell-semiconductor hybrid systems, where cells were cultured directly. Since the LAPS surface is laterally unstructured, cells can adhere without any spatial restrictions. And, by illuminating LAPS

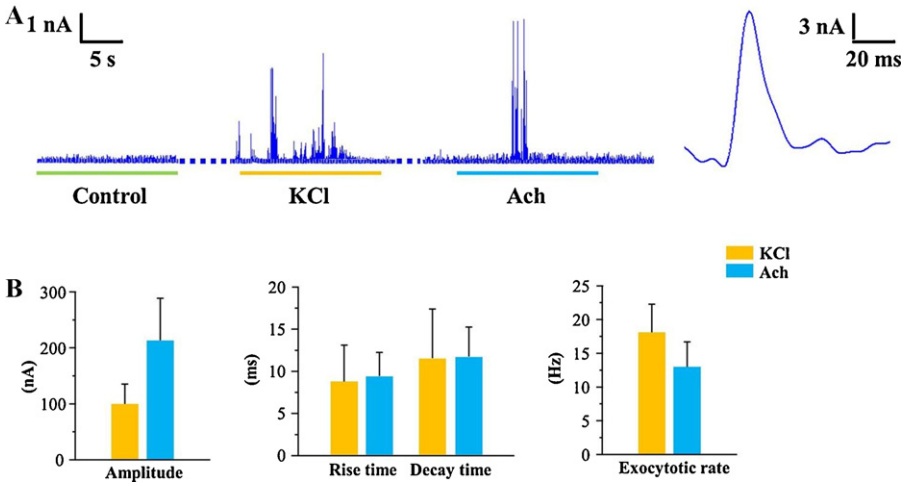

Fig. 3. LAPS detection of the secretion from a single chromaffin cell. (A) Typical signals of cell in the presence of high K^+ solution and Ach solution, with one of the signals was enlarged. (B) Statistic result of the signals' amplitude, duration and exocytotic rate.

surface on one individual cell and recording the local signals, which is proportional to the local surface potential, it should be possible to record the bioactivity of the targeted cell. The whole device and system was non-invasive long-term and label-free.

5. Conclusions

Combining chromaffin cells with LAPS, we engaged in designing a cell-based biosensor for neurosecretory studies with local extracellular acidifications sensing. KCl and Ach were used as stimulus, and we studied the exocytotic events not only by recording secretion of chromaffin cells and LAPS hybrid systems but also by deriving and analyzing significant parameters of response signals. In this investigation, we have presented evidence that LAPS is capable of recording the exocytotic event as a label-free method to study acidic vesicles with transmitters' release. Therefore, with exocytotic events successfully recorded, the neurosecretory cell-based biosensor is a promising sensor technology for studying exocytosis mechanism of neurons.

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