A chromaffin cell model to simulate calcium dynamics and secretory responses in various conditions.

Akira Warashina[a], Tatsuya Ogura[b], Masumi Inoue[c]

a. Division of Cell Physiology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.

b. Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO, USA.

c. Department of Cell and System Physiology, University of Occupational and Environmental Health, Japan School of Medicine, Kitakyushu, Japan.

Correspondence: Dr. Akira Warashina, Division of Cell Physiology, Niigata University Graduate School of Medical & Dental Sciences, Niigata 951-8510, Japan
Phone: 81-025-227-2065; **Fax:** 81-025-227-0755; **Email:** awarashi@med-niigata-u.ac.jp

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We constructed a chromaffin cell model for computer simulation analyses of Ca^{2+} dynamics and secretory responses in various conditions. The model includes mechanisms involved in nicotinic excitatory synapse, voltage-dependent Na^+, K^+ and Ca^{2+} channels, Ca^{2+}-activated K^+ channel (SK type), buffered Ca^{2+} diffusion, Ca^{2+} uptake into and release from intracellular Ca^{2+} stores, extrusion of Ca^{2+} at the plasma membrane, fluorescent Ca^{2+} indicator and Ca^{2+}-triggered exocytosis. Membrane current properties and various modes of action potentials reported in the literature were simulated with the model. The model was also applied to simulate our experimental results obtained from chromaffin cells in the perfused rat adrenal medulla. Observed Ca^{2+} responses and secretory responses induced by electric stimuli to the splanchnic nerve or by 40 mM K^+ in the perfusate were adequately simulated. Moreover, in isolated rat chromaffin cells bathed in a Ca^{2+}-deficient medium, profiles of muscarine-induced Ca^{2+} transient were found to be altered from oscillatory to monophasic modes with increasing the muscarine concentrations, and the half-decay time of monophasic response became shorter in thapsigargin-treated cells. These characteristics were reproduced by simulation of Ca^{2+} dynamics involving Ca^{2+} stores with $InsP_3$ receptor/channel and Ca^{2+} pump. Thus, the presented model may provide a useful tool for analyzing and predicting quantitative relations in various events occurring in stimulation-secretion coupling in chromaffin cells.

MATERIALS AND METHODS

Monitoring of Ca^{2+} responses and secretory responses in the perfused rat adrenal medulla. The adrenal gland isolated from Wistar rats (330-390 g) was recurrently perfused for 60 min through the adrenal vein with 2 ml Krebs solution containing 10 μM fura-2 AM. After about half of the adrenal cortex was removed, the gland was mounted on a chamber. Chromaffin cells in the adrenal medulla were stimulated either by transmural electric stimulation of the presynaptic nerve elements in the tissue or by an elevation of KCl concentration in the perfusate. Changes in $[Ca^{2+}]_i$ of chromaffin cells were observed from an exposed portion of the adrenal medulla using an inverted fluorescence microscope. Catecholamines secreted in the perfusate were measured with a carbon fiber electrode.

Measuring $[Ca^{2+}]_i$ responses in isolated rat chromaffin cells. Changes in $[Ca^{2+}]_i$ in isolated rat chromaffin cells loaded with fluo-3 were measured with a laser confocal scanning unit (Zeiss LSM410).

Modeling of chromaffin cell functions.

***Synaptic mechanism*:** The time course of synaptic current in nicotinic transmission was parameterized with the rising and decaying time constants reported by Kajiwara *et al*.[1].

Voltage-dependent channels: Na^+ and K^+ channels of Hodgkin-Huxley's type were modeled so as to reproduce experimental results reported by Kajiwara *et al*.[1]. Cell averaged Ca^{2+} current properties in chromaffin cells were also modeled.

***Ca^{2+}-dependent K^+ channel*:** SK-type channel was modeled based on experimental results reported by Park [2], in which the channel gate was assumed to open depending on binding of Ca^{2+} with a Hill-type kinetics.

Buffered Ca^{2+} diffusion: Intracellular buffered Ca^{2+} diffusion was calculated by a simplified method, in which the model cell was divided into N (6 in this study) compartments by N-1 concentric spheres. The movement of Ca^{2+} with a rate associated with the Ca^{2+} diffusion constant occurred due to the Ca^{2+} concentration difference in adjacent compartments. The Ca^{2+} buffering action with a fixed Ca^{2+} buffer took place in each compartment.

***Ca^{2+} extrusion*:** The extrusion of Ca^{2+} by Ca^{2+} pump and Na^+/Ca^{2+} exchanger was assumed to occur in combination by following Michaelis-Menten's kinetics.

***Intracellular Ca^{2+} store*:** Functions of an $InsP_3$-sensitive Ca^{2+} store along with muscarine- induced $InsP_3$ generation were modeled [3]. The Ca^{2+} uptake into the store was expressed by a Hill-type equation (n=2), and the Ca^{2+} leak from the store was assumed to be proportional to the difference of Ca^{2+} concentrations between the store and the cytosol. The $InsP_3$ receptor/channel was designed to activate cooperatively with $InsP_3$ and Ca^{2+}, with a subsequent inactivation due to increased cytosolic Ca^{2+} concentrations. The Ca^{2+}-dependent inactivation was assumed to be removed in the presence of high concentrations of $InsP_3$.

***Secretory mechanism*:** A two-step secretory scheme proposed by Heinemann *et. al*.[4] was adopted. In the scheme, secretory granules in a reserve pool migrate into a release-ready pool in a Ca^{2+}-dependent manner and then secreted by exocytosis with a rate proportional to the third power of the submembrane Ca^{2+} concentration. The values of kinetic parameters provided by the original study were used without modification.

Detailed mathematical formulas and parameter values involved in the above-mentioned mechanisms are shown elsewhere[5]. Calculations of the modeled mechanisms were carried out using the NEURON simulation environment (http://www.neuron.yale.edu/neuron/).

RESULTS AND DISCUSSION

Simulations of membrane current properties and various modes of action potentials. Simulations using the present model reproduced basic electrophysiological properties of rat chromaffin cells reported in the literature, which included EPSP-induced action potential[1], aborted action potentials during a prolonged current injection[1], repetitive action potentials at elevated resting membrane potentials and SK channel current tails observed following 1-s depolarizing pulses to different voltages[2]. This indicates that the mechanisms for ionic channels and Ca^{2+} dynamics were adequately formulated in the model.

Simulations of Ca^{2+} and secretory responses in the perfused rat adrenal medulla. Chromaffin cells in the perfused rat adrenal medulla were stimulated transsynaptically and changes in fura-2 fluorescence intensity were observed. The record in Fig. 1A*a* was obtained with transmural application of 10 pulses at 400-ms intervals and that in Fig. 1B*a* with 20 pulses at 100-ms intervals. To simulate these results, the corresponding numbers of EPSPs were generated in the model cell. The EPSP-induced action potentials (Figs. 1A*d* and 1B*d*) and increases in the Ca^{2+} concentration in the submembrane compartment (solid line in Figs. 1A*c* and 1B*c*) and the inner compartment (dotted line). The cell-averaged Ca^{2+} responses calculated for fura-2 fluorescence signals are shown in Figs.1A*b* and 1B*b*, which mimic the observed Ca^{2+} responses in Fig. 1A*a* and 1B*a*.

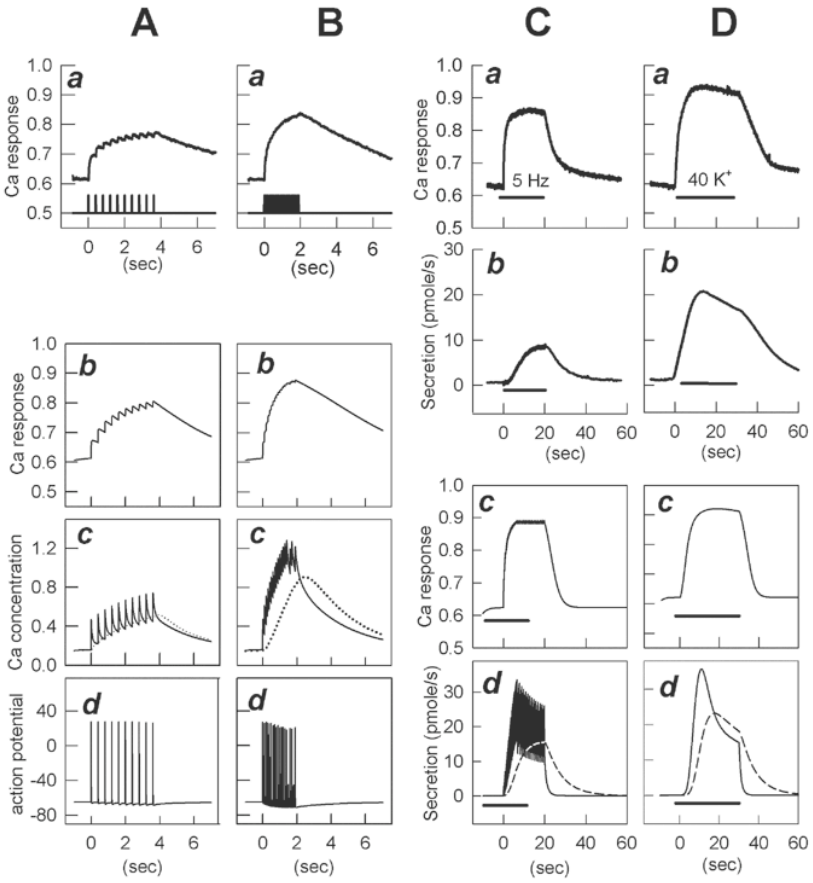

Figure. 1. Simulations of Ca^{2+} and secretory responses in chromaffin cells trassynaptically stimulated in the perfused rat adrenal medulla. (Column A) ***a*)** A normalized change in fura-2 fluorescence (342 nm excitation) intensity associated with 10 transmural stimuli at 400-ms intervals. ***b*)** Calculated cell-averaged Ca^{2+} response. ***c*)** Changes in Ca^{2+} concentrations (in μM) in the submembrane compartment (solid line) and in the 3rd compartment from the cell surface (dotted line). ***d*)** Calculated action potentials (in mV). **(Column B)** ***a*)** Observed Ca^{2+} response to 20 stimuli at 100-ms intervals. ***b, c* & *d*)** Simulation results are displayed in the same fashion as in A. **(Column C)** ***a*)** Observed Ca^{2+} response to transmural stimuli at 5-Hz for 20-s. ***b*)** The simultaneously observed secretory response. ***c*)** Calculated Ca^{2+} response. ***d*)** Calculated secretory response (solid line) was processed with numerical low-pass filter of a time constant of 7.5 s (dashed line). **(Column D)** Observed responses to 30-s stimulation with 40 mM K^+ and simulation results are displayed as in C. The ordinate scale in Figs. 1C***b***, 1C***d*,** 1D***b*** and 1D***d*** is pmole s^{-1} per gland.

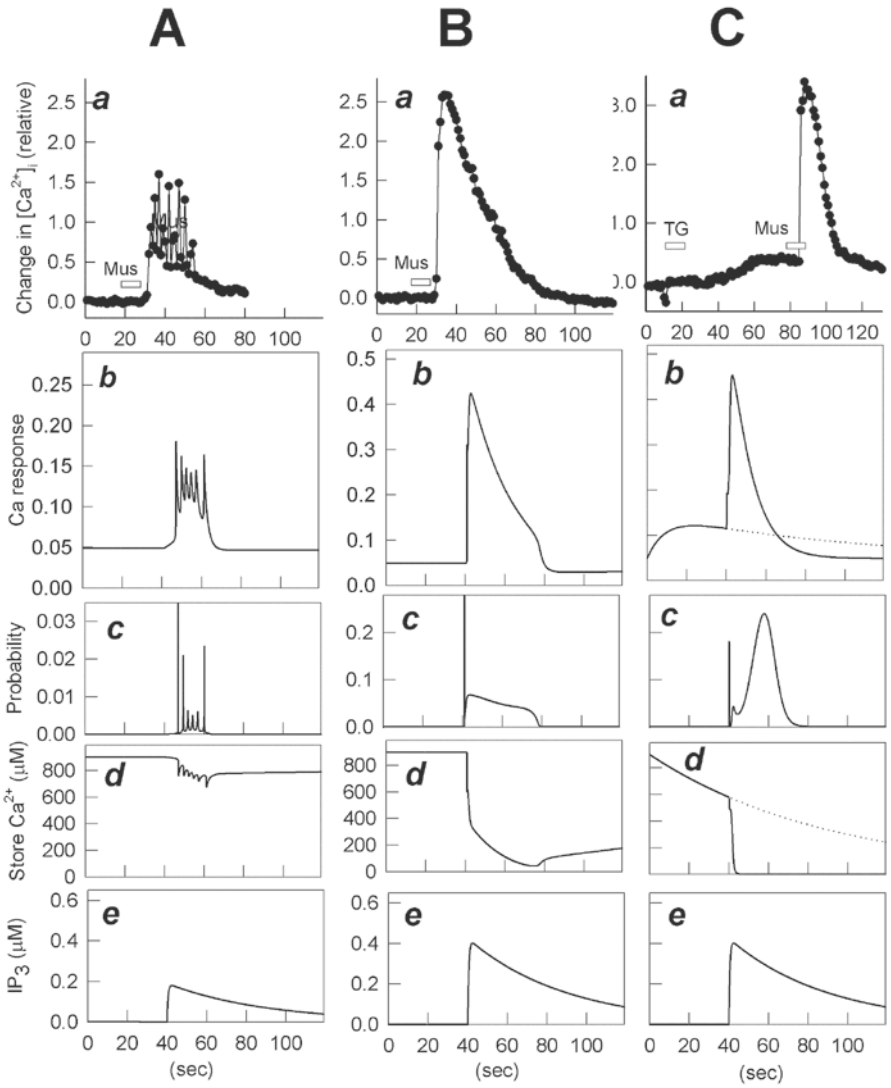

Figure. 2. Simulations of muscarine-induced Ca^{2+} responses in isolated rat chromaffin cells in Ca^{2+}-deficient medium. (Column A) ***a*****)** An oscillatory change in fluo-3 fluorescence intensity induced by 6 μM muscarine. ***b*****)** Calculated oscillatory Ca^{2+} response. ***c*****)** A change in the open channel probability of the Ca^{2+} release channel. ***d*****)** The associated change in the Ca^{2+} concentration of the store. ***e*****)** An assumed change in $InsP_3$ concentration following the muscarine stimulation. **(Column B)** Same as in A except that a cell was stimulated by 30 μM muscarine. **(Column C)** Same as in B except that a cell was pretreated with thapsigargin.

Figures 1C*a* and 1C*b* show the Ca^{2+} response and secretory response elicited by transmural application of 5-Hz pulses for 20 s. The calculated Ca^{2+} response in Fig. 1C*c* roughly approximates the time course of the observed record in Fig. 1C*a*, whereas the calculated secretory response in Fig. 1C*d* does not mimic the observed record in Fig. 1C*b*, exhibiting spiky responses associated with the respective stimuli. Since the experimental detection of catecholamines in the perfusate required a time constant of 7.5 s, the calculated response was processed by numerical low-pass filter with this time constant. The resultant shown by the dashed line in Fig 1C*d* approximates the time course of the observed response in Fig. 1C*b*.

Figures 1D*a* and 1D*b* show the Ca^{2+} response and secretory response observed in chromaffin cells stimulated by 40 mM K^+ in the perfusate. The calculated Ca^{2+} and secretory responses to depolarizing voltage to -30 mV (corresponding to E_K for 40 mM K^+) are displayed in Figs. 1D*c* and 1D*d*.

Simulation of muscarine-induced $[Ca^{2+}]_i$ changes in isolated rat chromaffin cells. Muscarine at 6 μM induced oscillatory changes in fluo-3 fluorescence intensity in isolated rat chromaffin cells in Ca^{2+}-deficient medium (Fig. 2A*a*), whereas 30 μM muscarine elicited monophasic responses (Fig. 2Ba). In thapsigargin-treated cells, the duration of the muscarine-induced monophasic Ca^{2+} responses was significantly reduced (Fig. 2C*a*). Ca^{2+} dynamics following muscarine stimulation were simulated with the model that included Ca^{2+} store function. Due to the assumed gating properties of $InsP_3$ receptor/channel, pulsatory increases in the open channel probability (Fig. 2A*c*) occurred with a low production of $InsP_3$ (Fig. 2A*e*), which caused an oscillatory Ca^{2+} release from the store as indicated by changes in the Ca^{2+} concentration in the store (Fig. 2A*d*) and in the cytosol (Fig. 2A*b*). With a higher production of $InsP_3$ (Fig. 2B*e*), no oscillation of the open channel probability (Fig. 2B*c*) occurred due to $InsP_3$-mediated removal of the Ca^{2+}-induced inactivation of $InsP_3$ receptor/channel. Consequently, a massive Ca^{2+} release form the store took place (Fig. 2B*d*) to produce a monophasic Ca^{2+} response (Fig. 2B*b*). It is noted that a robust reuptake of Ca^{2+} by the store delayed the depletion of the store and prolonged the duration of the Ca^{2+} response.

The reduction of duration of monophasic Ca^{2+} response by thapsigargin was reproduced in simulation with the model in which the Ca^{2+} uptake mechanism was removed ((Fig. 2C*b*).

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