Role of Ca^{2+} store in AMPA-triggered Ca^{2+} dynamics in retinal horizontal cells

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Fura-2 fluorescent calcium imaging was applied to measure $[Ca^{2+}]_i$ in freshly dissociated horizontal cells of carp retina, and a model containing endoplasmic reticulum (ER) membrane processes and plasma membrane processes was constructed for quantitative analyses of the AMPA-triggered calcium dynamics. A transient increase followed by a sustained steady level of $[Ca^{2+}]_i$ was observed when 100 μM AMPA was applied, while the initial transient increase of $[Ca^{2+}]_i$ was suppressed by exogenously applied ryanodine. The model analyses results suggest that the AMPA-triggered calcium dynamics involves a number of cytoplasmic and endoplasmic processes that interact with each other. It also suggests that calcium store is an important part contributing to the transient calcium signal. *NeuroReport* 15:2311–2315 © 2004 Lippincott Williams & Wilkins.

Key words: AMPA receptor; Calcium-induced calcium release; Calcium store; Dynamic model; Horizontal cell

INTRODUCTION

Calcium regulation is fundamental in many cellular functions, including cell growth, electrical activity, neurotransmitter release, excitation-contraction coupling and muscle contraction [1]. In particular, intracellular Ca^{2+} serves a crucial role in the synaptic plasticity of the vertebrate CNS [2]. The concentration of free intracellular calcium ($[Ca^{2+}]_i$) in excitable cells is determined primarily by two factors, calcium dynamics between the cytoplasm and extracellular environment, and calcium dynamics between internal calcium stores and the cytoplasm. In the enzymatically dissociated retinal horizontal cells, $[Ca^{2+}]_i$ is affected by the activation of Ca^{2+}-permeable AMPA receptors, the opening of voltage-dependent Ca^{2+} channels, the control of Na^+/Ca^{2+} exchanger, and the regulation of Ca^{2+} pump [3–5]. It was also found in teleost retina that horizontal cells contained ryanodine-sensitive non-mitochondrial intracellular calcium stores, which could further generate Ca^{2+}-release induced by Ca^{2+} entering through the plasma membrane [6,7].

In the present study, Ca^{2+} signals induced by exogenously applied AMPA were investigated on freshly dissociated horizontal cells of the carp retina using fura-2 based Ca^{2+}-imaging technique. To explore possible effects that intracellular calcium store might exert on the Ca^{2+} regulation in retinal horizontal cells, a compartmental model was constructed, and the model output was compared to the experimentally observed AMPA-triggered calcium dynamics.

MATERIALS AND METHODS

Cell isolation: Horizontal cells were enzymatically dissociated from retinas of adult carp (*Carassius auratus*, 15–20 cm body length) [8]. The eyes were enucleated and hemisected after the fish had been decapitated. Isolated retina was cut into 8–12 pieces and incubated for 20 min at room temperature in 4 ml Hank's solution (see below) containing 25 U/ml papain (E. Merck) activated by 1 mg/ml L-cysteine (Bo'ao, Shanghai). After rinsing the retinal pieces were stored in the Hank's solution at 4°C until being used. To obtain dissociated horizontal cells, the retina pieces were gently triturated with fire-polished glass pipettes in Ringer's solution.

Intracellular Ca^{2+} concentration measurement: $[Ca^{2+}]_i$ was measured using a fura-2 imaging system. Fura-2/AM (Sigma) was dissolved in dimethyl sulfoxide (DMSO, 5 mM stock solution) and added to the cell suspension at a final concentration of 5 μM. The cells were incubated at room temperature for 10 min to allow for adherence and fura-2 loading. The cells were then continuously superfused with Ringer's solution at a rate of 1 ml/min for 10 min prior to experiment. The horizontal cells, identified as cone dominant type [8], were examined. Excitation light at 340 nm and 380 nm for Ca^{2+} imaging was supplied by a high speed scanning polychromatic light source (Hamamatsu, Japan). The 340 nm and 380 nm wavelength lights were given alternately, and relevant fluorescence image pairs (F_{340} and F_{380}) were acquired every 5 s. $[Ca^{2+}]_i$ is presented as the ratio (F_{340}/F_{380}) in this study. The control of monochromator and shutter, together with data acquisition, were executed with the Aquacosmos 1.2 software.

Solutions: Hank's solution contained (in mM) 120.0 NaCl, 3.0 KCl, 0.5 $CaCl_2$, 1.0 $MgSO_4$, 1.0 Na-pyruvate, 1.0 NaH_2PO_4, 0.5 $NaHCO_3$, 20.0 HEPES and 16.0 glucose. Ringer's solution consisted of (in mM): 120.0 NaCl, 5.0 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 10.0 HEPES and 16.0 glucose. Cyclothiazide (CTZ) was prepared in DMSO at 50 mM and diluted to its final concentration in Ringer's solution

(DMSO < 0.5%). AMPA and ryanodine were dissolved in Ringer's solution. The pH value for all solutions was adjusted to 7.4 with NaOH. All the drugs were purchased from Sigma (St. Louis, MO).

Modeling: We constructed a spatially uniform model containing three compartments (Fig. 1) to describe $[Ca^{2+}]_i$ changes: the extracellular environment, the cytosol, and the endoplasmic reticulum (ER). The flow rate of Ca^{2+} influx across the plasma membrane is denoted by ϕ_{in}. It includes Ca^{2+} entry through voltage-gated Ca^{2+} channels (ϕ_{VGCC}) and Ca^{2+}-permeable AMPA receptors (ϕ_{AMPA}). The flow rate of Ca^{2+} efflux across the plasma membrane, denoted by ϕ_{out}, is due to active Ca^{2+} transport against its concentration gradient by the Ca^{2+} pump (ϕ_{pump}) and Na^+/Ca^{2+} exchanger (ϕ_{ex}) located on plasma membrane. The flow rate of Ca^{2+} release from the ER is denoted by ϕ_{rel}, the rate of Ca^{2+}-flow for refilling the ER Ca^{2+} pool is represented by ϕ_{fil}, and a passive leak is denoted by ϕ_{leak} [9]. In these processes, ϕ_{rel} is mediated by ryanodine-sensitive intracellular store, whereas ϕ_{fil} is determined by the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity. These changes can be equated as follows:

$$\frac{d[Ca^{2+}]_{ER}}{dt} = -(\phi_{rel} - \phi_{fil} + \phi_{leak}) \tag{1}$$

$$\frac{d[Ca^{2+}]_i}{dt} = (\phi_{rel} - \phi_{fil} + \phi_{leak}) \cdot c + \phi_{in} - \phi_{out} \tag{2}$$

where c=0.6 is the ratio between the effective volumes of ER and cytosol [10].

The components relevant to $[Ca^{2+}]_i$ regulation by ER (ϕ_{rel}, ϕ_{fil}, and ϕ_{leak}) can be described by the following equations [11,12]:

$$\begin{aligned}
\phi_{rel} &= v_1 \cdot m_1^4 \cdot \left([Ca^{2+}]_{ER} - [Ca^{2+}]_i\right) \\
m_1 &= \frac{[Ca^{2+}]_i}{[Ca^{2+}]_i + k_1} \\
\phi_{leak} &= v_2 \cdot \left([Ca^{2+}]_{ER} - [Ca^{2+}]_i\right) \\
\phi_{fil} &= v_3 \cdot \frac{[Ca^{2+}]_i}{k_{31} + [Ca^{2+}]_i} \cdot \frac{k_{32}^6}{k_{32}^6 + [Ca^{2+}]_{ER}^6}
\end{aligned} \tag{3}$$

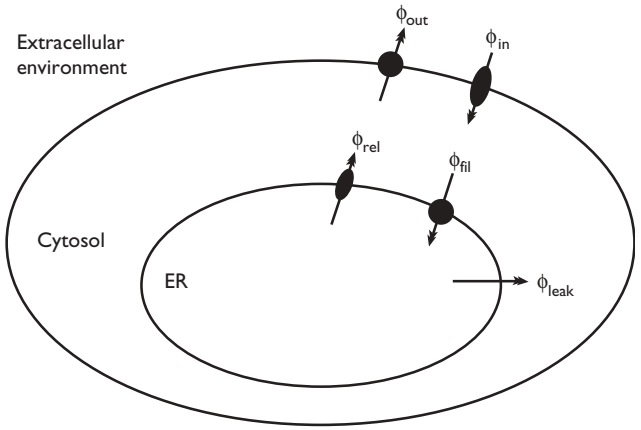

Fig. I. Schematic representation of the major compartments (extracellular environment, cytosol, and ER) involved in Ca^{2+} dynamics and Ca^{2+} exchange fluxes (ϕ) between these compartments.

Carp retinal horizontal cells exclusively express AMPA subtype of glutamate receptors, which could be Ca^{2+}-permeable [4]. The rate of Ca^{2+}-influx via AMPA receptor can thus be described as follows:

$$\begin{aligned}
\phi_{AMPA} &= v_4 \cdot m_4 \cdot \left([Ca^{2+}]_o - [Ca^{2+}]_i\right) \\
\frac{dm_4}{dt} &= \frac{M_4 - m_4}{\tau_4} \\
M_4 &= \theta \cdot C_{AMPA}(t)
\end{aligned} \tag{4}$$

M_4 and m_4 are the maximal activation and the activation variables, respectively, where M_4 is proportional to the concentration of exogenously applied AMPA $C_{AMPA}(t)$ with a constant coefficient θ.

The rate of Ca^{2+} flux through the voltage-gated Ca^{2+} channel ϕ_{VGCC} can be equated as:

$$\begin{aligned}
\phi_{VGCC} &= v_5 \cdot m_5 \cdot h_5 \cdot \left([Ca^{2+}]_o - [Ca^{2+}]_i\right) \\
\frac{dm_5}{dt} &= \alpha_{m_5} \cdot (1 - m_5) - \beta_{m_5} \cdot m_5 \\
\alpha_{m_5} &= 33 \cdot \frac{92.7 - V_m}{\exp((92.7 - V_m)/9.6) - 1} \\
\beta_{m_5} &= 3.3 \cdot \exp((-65.2 - V_m)/11.25) \\
h_5 &= \frac{k_5}{k_5 + [Ca^{2+}]_i}
\end{aligned} \tag{5}$$

where m_5 and h_5 are the activation and the inactivation variables, respectively; α_{m_5} and β_{m_5} are forward and backward rate coefficients, respectively [13]. Since the voltage-gated Ca^{2+} channel is known to be inactivated by $[Ca^{2+}]_i$ [5], thus h_5 is dependent on $[Ca^{2+}]_i$, with k_5 being the half inactivation constant.

To calculate the membrane potential of the isolated cell, the relationship between the bath-applied AMPA concentration and the membrane potential of horizontal cells can be described as follows:

$$V_m + \frac{dV_m}{dt} \cdot \tau_6 = -56 + 52 \cdot C_{AMPA}(t)^n / (C_{AMPA}(t)^n + k_6^n) \tag{6}$$

which was based on the dose-response properties of the isolated horizontal cell [8]. The Hill coefficient n is set as 2.2.

The rate of Ca^{2+} efflux across plasma membrane is due to the activity of Ca^{2+} pump (ϕ_{pump}) and Na^+/Ca^{2+} exchanger (ϕ_{ex}) [3], which can be described as:

$$\phi_{pump} = v_7 \cdot [Ca^{2+}]_i / \left(k_7 + [Ca^{2+}]_i\right) \tag{7}$$

$$\begin{aligned}
\phi_{ex} = {} & v_8 \cdot [Na^+]_i^3 [Ca^{2+}]_o \exp(rV_mF/RT) \\
& - v_8 \cdot [Na^+]_o^3 [Ca^{2+}]_i \exp(-(1-r)V_mF/RT)
\end{aligned} \tag{8}$$

where F, R and T are the Faraday constant, the gas constant and the absolute temperature, respectively.

$[Ca^{2+}]_i$ is regulated by a Ca^{2+} buffer:

$$\begin{aligned}
[Ca^{2+}]_i \cdot [buffer]_f \cdot k_9 &= [Ca^{2+}\ buffer] \\
[buffer]_f + [Ca^{2+}\ buffer] &= [buffer]_{total}
\end{aligned} \tag{9}$$

where $[buffer]_f$ and $[Ca^{2+}\ buffer]$ are concentrations of the free buffer and the Ca^{2+}-bound buffer, respectively [14,15].

RESULTS

AMPA-triggered $[Ca^{2+}]_i$ dynamics: During the application of 100 μM AMPA, $[Ca^{2+}]_i$ was transiently increased to a peak level, which was followed by a gradual decrease back to a steady level. It is shown that the ratio value was quickly increased from 0.28 to 1.03 after the application of AMPA, and then returned to 0.70 over a period of 2 min (Fig. 2a).

AMPA receptors on horizontal cells desensitize rather rapidly [8], which may help to prevent excessive Ca^{2+} entering into the cells. CTZ, a chemical which blocks the desensitization of AMPA receptors, was thus applied at a concentration of 50 μM [16]. The application of 100 μM AMPA, in the presence of 50 μM CTZ, induced an initial overshoot of $[Ca^{2+}]_i$ wherein the ratio value increased from 0.32 to 0.89 (Fig. 2b). The amplitude of the peak $[Ca^{2+}]_i$ response to AMPA application was similar, in the presence and absence of CTZ (0.69 ± 0.150 and 0.67 ± 0.138, respectively, $p > 0.05$, $n=5$). However, with the application of CTZ, this sharp $[Ca^{2+}]_i$ peak was followed by a much slower $[Ca^{2+}]_i$ accumulation, with the ratio value raising from a trough of around 0.70 to a peak much higher as compared with the transient one, which was ~ 1.70 in this case (Fig. 2b). Consistent results were found in a total number of 5 cells.

Simulation of AMPA-triggered calcium dynamics: Using the parameter values (Table 1) chosen referring to previous findings [4,5,10,13,17], the AMPA-triggered $[Ca^{2+}]_i$ changes were calculated by applying the model. Application of AMPA (100 μM) resulted in an increase of $[Ca^{2+}]_i$, with the transient peak level reaching 1.87 μM and the following sustained plateau at 0.77 μM (Fig. 3a). At the same time, $[Ca^{2+}]_{ER}$ gradually decrease from the resting level of 242 μM to the steady level of 47 μM (Fig. 3b). As shown in Fig. 3c–d, when AMPA was applied, a small and transient influx of Ca^{2+} was originated through the AMPA receptors and voltage-gated Ca^{2+} channels, which was accompanied by a significant Ca^{2+} release from the intracellular Ca^{2+} store. The Ca^{2+} efflux mechanisms, related to Ca^{2+} pump and Na^+/Ca^{2+} exchanger, were activated at a later phase, which counteracted the increase of $[Ca^{2+}]_i$.

Effect of calcium store: In order to examine the effect of Ca^{2+}-induced Ca^{2+} release (CICR) in AMPA-triggered

Table 1. Parameter values of the model equations.

Components and parameters	
Ryanodine receptor (ϕ_{rel})	
Rate constant v_1	$= 0.5\,[1/s]$
Activation constant k_1	$= 0.2\,[\mu M]$
Passive Ca^{2+} leak (ϕ_{leak})	
Rate constant v_2	$= 0.00758\,[1/s]$
SERCA (ϕ_{fil})	
Rate constant v_3	$= 10.8\,[\mu M/s]$
Activation constant k_{31}	$= 0.1\,[\mu M]$
Inactivation constant k_{32}	$= 250\,[\mu M]$
$V_{ER}/V_{cyt}c$	$= 0.6$
Ca^{2+}-permeable AMPA receptor (ϕ_{AMPA})	
Rate constant v_4	$= 8.75e\text{-}004\,[1/s]$
Time constant τ_4	$= 0.1\,[s]$
$[Ca^{2+}]_o$	$= 2.0\,[mM]$
Constant coefficient θ	$= 10.0$
Voltage-gated Ca^{2+} channel (ϕ_{VGCC})	
Rate constant v_5	$= 0.0212\,[1/s]$
Inactivation constant k_5	$= 0.3\,[\mu M]$
Dose-response curve	
EC_{50} k_6	$= 18\,[\mu M]$
Time constant τ_6	$= 0.1\,[s]$
Ca^{2+} pump (ϕ_{pump})	
Pumping rate v_7	$= 5.22\,[\mu M/s]$
Pumping constant k_7	$= 0.4\,[\mu M]$
Na^+/Ca^{2+} exchanger (ϕ_{ex})	
Scaling coefficient v_8	$= 0.017\,[1/s/cm^2/mM^3]$
$[Na^+]_i$	$= 8\,[mM]$
$[Na^+]_o$	$= 120\,[mM]$
r	$= 0.59$
Ca^{2+} buffer	
Binding rate k_9	$= 20\,[1/\mu M]$
$[Buffer]_{total}$	$= 5\,[\mu M]$

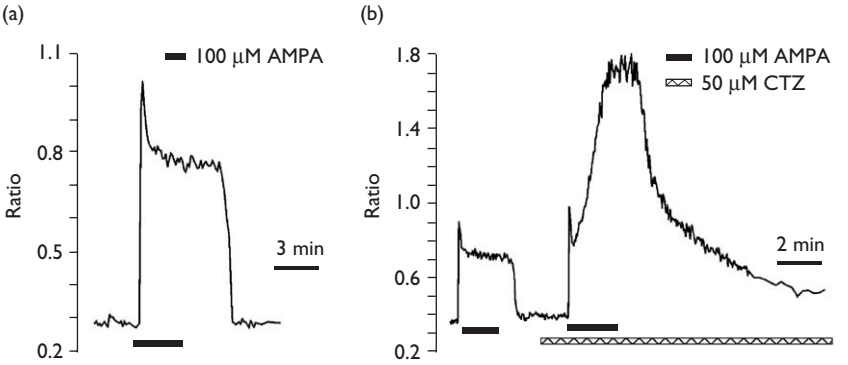

Fig. 2. AMPA-triggered $[Ca^{2+}]_i$ dynamics measured in the isolated horizontal cell and the effect of CTZ. (**a**) When AMPA was applied, a rapid increase of $[Ca^{2+}]_i$ was induced, which was followed by a gradual recovery to a steady level. (**b**) The application of AMPA in the presence of CTZ greatly enhanced the amplitude of the slow component of $[Ca^{2+}]_i$ increase, and left the leading transient component intact.

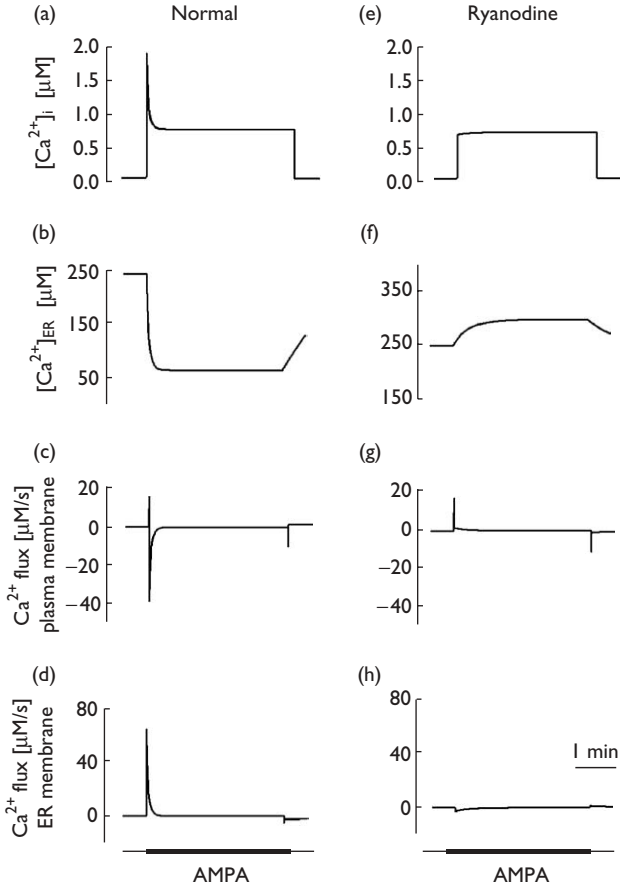

Fig. 3. Simulation of AMPA-triggered Ca^{2+} dynamics using the compartment model of horizontal cell. In rows (**a**) and (**e**) the changes of $[Ca^{2+}]_i$ during the application of AMPA; (**b**) and (**f**) $[Ca^{2+}]_{ER}$ dynamics during the application of AMPA; (**c**) and (**g**) the summed flow rate of Ca^{2+} across the plasma membrane; (**d**) and (**h**) the summed flow rate of Ca^{2+} across the ER membrane. In columns (a–d) changes in response to AMPA application; (e–h) AMPA-induced changes when the ryanodine receptor was inactive. Upward deflections show the Ca^{2+} influx into cytosol.

$[Ca^{2+}]_i$ dynamics, the initial value of v_1 was set to be zero to simulate the inhibition of ryanodine receptors. As illustrated in Fig. 3e–h, when AMPA was applied to the horizontal cell in which the ryanodine receptor was blocked ($v_1=0$), $[Ca^{2+}]_i$ directly increase to 0.77 μM lacking the initial spike (Fig. 3e). $[Ca^{2+}]_{ER}$ was increased by the continual uptake of SARCA, from about 250 μM to 300 μM (Fig. 3f). These results suggest that calcium store is significantly involved in shaping $[Ca^{2+}]_i$ transient induced by AMPA in horizontal cells. Compared with those under the normal condition, when ryanodine receptor was blocked, Ca^{2+} flux via plasma membrane did not change distinctly (Fig. 3c,g), but the CICR was effectively inhibited (Fig. 3d,h).

Experiments were also performed to confirm the role of Ca^{2+} store in the $[Ca^{2+}]_i$ dynamics. When the cells were preincubated in 20 μM of the CICR blocker ryanodine for 10 min, the application of AMPA resulted in an increase of $[Ca^{2+}]_i$ without the transient peak observed during control ($n=8$; Fig. 4). These results suggest that the transient increase of $[Ca^{2+}]_i$ induced by AMPA is closely related to CICR from ryanodine-sensitive calcium store.

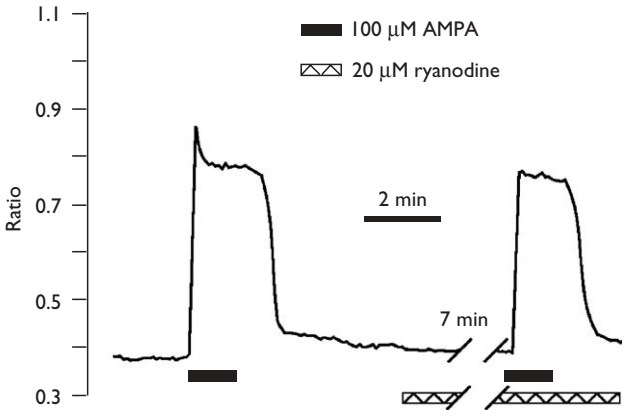

Fig. 4. Effect of ryanodine in the AMPA-triggered $[Ca^{2+}]_i$ dynamics. Pre-application of ryanodine suppressed the transient phase of $[Ca^{2+}]_i$ increase.

DISCUSSION

The ER in neuronal cells appears to be a continuous system that extends throughout the neuron. The release of Ca^{2+} from the ER store upon stimulation regulates a variety of cellular events and processes, such as neuronal excitability and synaptic plasticity. Among the factors, Ca^{2+} itself is capable of triggering Ca^{2+} release from the ER store by activating ryanodine receptors present in the ER membrane [18]. Previous studies demonstrated the existence of ryanodine-sensitive intracellular calcium stores in retinal horizontal cells [6,7]. AMPA induced a transient phase of $[Ca^{2+}]_i$ followed by a gradual decrease back to a steady level in isolated horizontal cell. The transient component maintaining under CTZ perfusion indicated that the desensitization of AMPA receptors is not responsible for the transient increase of $[Ca^{2+}]_i$. The application of ryanodine which inhibited CICR, suppressed the transient increase of $[Ca^{2+}]_i$ induced by AMPA (Fig. 4), suggesting the contribution of the CICR to the transient phase of AMPA-triggered $[Ca^{2+}]_i$ dynamics in the isolated horizontal cell.

The compartmental model of solitary horizontal cells with calcium dynamics has been previously developed [13], in which $[Ca^{2+}]_i$ was regulated only by the plasma membrane Ca^{2+} flow. The model developed in the present study simplified the relationship between Ca^{2+} and other ion channels and clarified the calcium signal interaction between the inner calcium store and cytoplasm in retinal horizontal cells. ER was modeled with excitable membrane components and revealed its significant participation in calcium dynamics of retinal horizontal cells.

CONCLUSION

Ca^{2+} store is significantly involved in shaping neuronal Ca^{2+} dynamics and calcium-induced calcium release from ryanodine-sensitive calcium store should be responsible for the initial transient elevation of $[Ca^{2+}]_i$ during AMPA application on isolated carp retinal horizontal cells.

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