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Calcium signalling in astrocytes and modulation of neural activity

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Abstract

Starting from the experimental data on ATP evoked calcium responses in astrocytes, a biophysical model describing these phenomena was built. The simulations showed, in agreement with the experimental findings, that the intracellular calcium fluxes mediated by the P2X and P2Y purinoreceptors are responsible for the biphasic ATP evoked calcium response in astrocytes. Then, the modulation effects on the neural dynamics arising from the release of glutamate from astrocyte are also investigated. By using a minimal network model describing a neuron coupled to the astrocyte, we demonstrated that the calcium extrusion rate through the astrocyte membrane is critically involved in the generation of different firing patterns of the neuron.

Keywords: Neuron; Astrocyte; Purinoreceptor; Intracellular calcium; Synapse; Neural activity

1. Introduction

Traditionally, astrocytes have been considered as passive elements of the brain, which provide structural and metabolic support to the neurons. During the last decade, the accumulation of a large amount of experimental data characterizing the communication processes between astrocytes and astrocyte-neurons has rapidly changed this old point of view. Astrocytes are nonexcitable cells where transient elevations in cytoplasm-free calcium level can be thought as a sort of calcium excitability.

A wide variety of neurotransmitter receptors are expressed in the astrocytic plasma membrane and experimental findings revealed that astrocytes located near synapses respond to neurotransmitters (including glutamate, GABA, ATP, etc.) with an elevation of their intracellular calcium levels ($[Ca^{2+}]_i$) (Finkbeiner, 1993; Parpura et al., 1994; Porter and McCarthy, 1996, 1997; Kang et al., 1998; Parpura and Haydon, 2000; Wang et al., 2000; Nobile et al., 2003; Fellin and Carmignoto, 2004; Perea and Araque, 2005; Zhang and Haydon, 2005; Koizumi et al., 2005). The $[Ca^{2+}]_i$ increase in these cells mediates the release of glutamate, ATP, and other neuroactive substances that are capable, by a feedback mechanism, of modulating synaptic communication between neurons (Fellin and Carmignoto, 2004; Perea and Araque, 2005; Zhang and Haydon, 2005; Koizumi et al., 2005). In addition, a calcium-independent pathway of glutamate release from astrocytes also occurs, through the activation of the $P2X_7$ receptor (Duan et al., 2003). In the nervous system, ATP is an important extracellular messenger and in turn its action on astrocytes is mediated by ionotropic (P2X) and metabotropic (P2Y) purinoreceptors (Koizumi et al., 2005). Experimental evidences indicate that in the synaptic cleft, the presence of extracellular ATP contributes to synaptic transmission

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by activating postsynaptic P2X receptors (Pankratov et al., 2002; Zhang et al., 2003; Khakh, 2001).

In this paper, some of the above-described phenomena are investigated experimentally and by using a biophysical modeling approach. In particular, the experiments were performed on cultured cortical astrocytes aiming to characterize their calcium-signalling response in the presence of extracellular ATP. ATP induced a biphasic $[Ca^{2+}]_i$ response, consisting in a large initial peak followed by a sustained plateau phase. Starting from these experimental findings, a biophysical model describing the above-described calcium dynamics in astrocyte was built. In agreement with the experimental findings, the model shows that ionotropic and metabotropic ATP receptors play a key role in calcium behaviors. Finally, using a biophysical modelling approach, the last part of this paper is devoted to the study of a minimal neural network model consisting of a pyramidal neuron receiving inputs from an astrocyte. In particular, the neuronal firing properties due to astrocytic release of glutamate are simulated.

The main topic of this study is to compare the results obtained with our model to those obtained recently by Nadkarni and Jung (2003, 2004). By using a specific model of a "dressed" neuron (a single neuron coupled to an astrocyte), these authors showed that the parameter of the model, describing the production rate of IP_3 triggered by the firing of the neuron, is critical for the generation of firing activity even in absence of external stimulation**.** Our more complete model, including plasma membrane Ca^{2+} fluxes, suggests results quite different from those obtained by these authors.

2. Materials and methods

2.1. Calcium measurements in astrocytes

The microfluorimetric calcium responses shown in this paper, concerning the ATP induced calcium elevation in astrocytes, were recently obtained in our group (Nobile et al., 2003). Briefly, the experiments were performed on type-1 cortical rat astrocytes in primary culture. Culture flasks were maintained in a humidified incubator with 5% of CO_2 for 2–5 weeks. During the experiments the cells were continuously superfused using standard solution as described in Nobile et al. (2003) and were maintained at room temperature (20–22 °C). No evidence was found for variability in receptor properties over the period of cell culturing.

Intracellular calcium was monitored using the fluorescent Ca^{2+} indicator Fura-2 AM. Measurements of $[Ca^{2+}]_i$ in single cells were performed by using an inverted fluorescence microscope Nikon TE200 (Nikon, Tokyo, Japan) equipped with a dual excitation fluorimetric imaging system (Hamamatsu, Sunajama-Cho, Japan).

2.2. Biophysical model of calcium signalling in astrocytes

The model describing the calcium dynamics in astrocytes is an enriched version of that proposed by Höfer et al. (2002), and the corresponding scheme is reported in Fig. 1. The calcium fluxes through the membrane are the following: (i) a leakage Ca^{2+} influx described by the rate ν_{LM}; (ii) a capacitive calcium entry (CCE) of rate ν_{CCE}; (iii) an extrusion across plasma membrane described by the rate ν_{OUT}; (iv) an influx of Ca^{2+} mediated by the P2X ionotropic ATP receptor with rate $\nu_{ATP(P2X)}$. According to Höfer et al. (2002), two different biochemical processes mediating the production of IP_3 were included: PLCβ and PLCδ. The first one is responsible of the production of IP_3 in the presence of extracellular agonists and is activated through a G-protein-coupled receptor mechanism with rate $\nu_{PLC\beta}$; the second one describes the production of IP_3 arising by the $[Ca^{2+}]_i$ increase and the corresponding rate is $\nu_{PLC\delta}$. The degradation of the IP_3 is described by the rate $\nu_{IP3(Deg)}$. The binding of the IP_3 molecules to the corresponding receptors on the endoplasmatic reticulum (ER) compartment leads to the release of calcium from the ER stores with rate $\nu_{ER(Rel)}$. The free cytosolic calcium is pumped into the ER with a rate ν_{SERCA}. The active fraction of IP_3 receptors on the ER membrane determine the IP_3-induced release of calcium from the ER stores and the time evolution of the concentration of these receptors is described by the two rates $\nu_{IP3R(Rec)}$ and $\nu_{IP3R(Inact)}$.

A set of balance equations describing the time evolution of the relevant variables of the astrocytic calcium model was introduced. Micromolar concentrations of astrocytic $[Ca^{2+}]_i$ is termed Ca_i, Ca_{ER} is the concentration (μM) of the calcium in the ER stores, I is the concentration (μM) of the IP_3, R the fraction of active IP_3 receptors on the ER membrane. Then, the time evolution of these quantities is described by the following equations:

$$\frac{dCa_i}{dt} = \nu_{LM} + \nu_{CCE} + \nu_{ATP(P2X)} - \nu_{OUT} + \nu_{ER(Rel)} - \nu_{SERCA} \quad (1a)$$

$$\frac{dCa_{ER}}{dt} = \beta\left(\nu_{SERCA} - \nu_{ER(Rel)}\right) \quad (1b)$$

$$\frac{dR}{dt} = \nu_{IP3R(Rec)} - \nu_{IP3R(Inact)} \quad (1c)$$

$$\frac{dI}{dt} = \nu_{PLC\beta} + \nu_{PLC\delta} - \nu_{IP3(Deg)} \quad (1d)$$

The values of the corresponding constants are reported in Table 1. One of the most commonly observed mechanisms of driven calcium entry from plasma membrane in non excitable cells, like astrocytes, is the CCE (Putney et al., 2001; Targos et al., 2005). By analogy with a capacitor, the depletion of intracellular stores activates a signalling process leading to the opening of a plasma membrane channel through which calcium enters into the cell. The mechanisms driving the CCE pathway are not fully understood and two relevant open problems are: (a) the identification of the corresponding plasma membrane channels responsible for calcium entry; (b) the understanding of the mechanisms linking the ER depletion to the opening of these CCE channels. Several theories have been proposed to explain

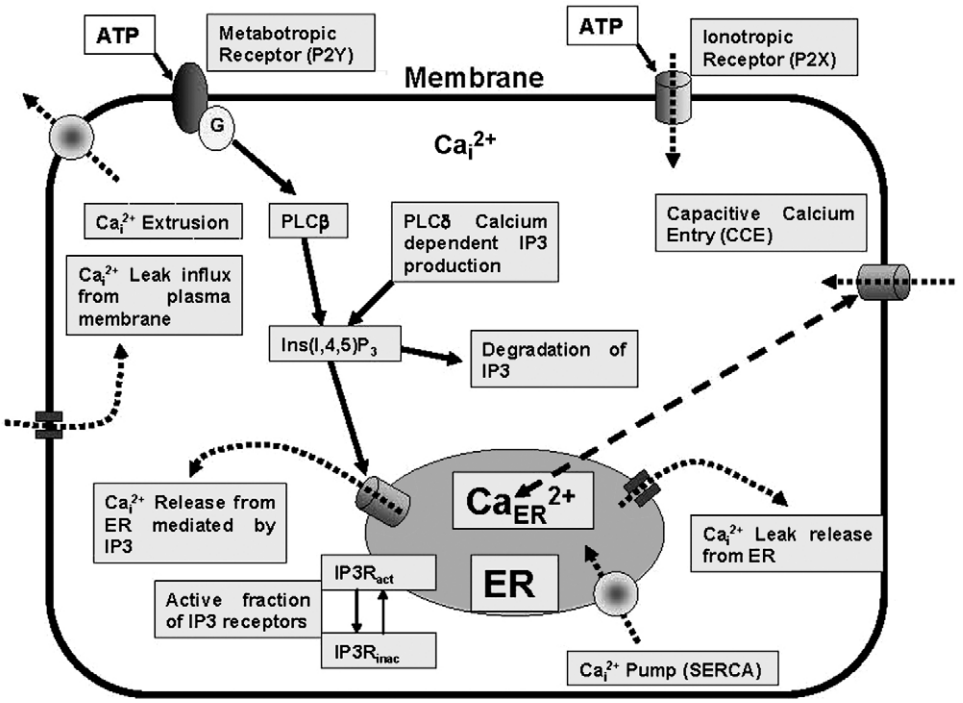

Fig. 1. A schematic diagram showing the main molecular transport and signalling mechanisms described in the astrocyte calcium model.

the communication mechanisms between stores and plasma membrane CCE channels. In general, two main experimental models of channel opening have been proposed: one is based on the hypothesis of a small diffusible messenger released from the depleted ER (Randriamampita and Tsien, 1993), and the second suggests a direct or indirect physical contact between ER proteins and plasma membrane (Berridge, 1995).

However, the lack of a clear understanding of the mechanisms underlying this calcium entry pathway leads us to assume, for simplicity, that the rate regulating CCE influx is a nonlinear function of C_{ER} defined as follows:

$$\nu_{CCE} = \frac{k_{CCE}\, H_{CCE^2}}{(H_{CCE^2} + Ca_{ER^2})} \tag{2}$$

Table 1
Model parameters

Description	Symbol	Value
Rate of calcium leak across the plasma membrane	k_0	0.03 μM/s
Rate of calcium leak from the ER	k_1	0.0004 s^{-1}
Rate of calcium release through IP_3 receptor	k_2	0.2 s^{-1}
Rate constant of SERCA pump	k_3	0.5 s^{-1}
Rate of calcium extrusion from plasma membrane	k_5	0.5 s^{-1}
Rate constant of IP_3 receptor inactivation	k_6	4 s^{-1}
Rate constant of IP_3 receptor degradation	k_9	0.08 s^{-1}
Rate constant of PLCδ	ν_7	0.02 μM/s
Half saturation constant for IP_3 activation of the corresponding receptor	K_{ip3}	0.3 μM
Half saturation constant for calcium activation of the IP_3 receptor	K_a	0.2 μM
Half saturation constant for calcium inhibition of the IP_3 receptor	K_i	0.2 μM
Half saturation constant for calcium activation of PLCδ	K_{Ca}	0.3 μM
Ratio of the effective volumes for calcium of cytoplasm and ER	β	35
Half-inactivation constant for CCE influx	H_{CCE}	10 μM
Maximal rate constant for CCE influx	k_{CCE}	0.01 μM/s
Maximal rate of ATP evoked ionotropic calcium flux	$k_{ATP(P2X)}$	0.08 μM/s
Half saturation constant for ATP evoked ionotropic calcium influx amplitude	$H_{ATP(P2X)}$	0.9 μM
Maximal rate of IP_3 production mediated by the metabotropic ATP receptor	$k_{ATP(P2Y)}$	0.5 μM/s
Dissociation constant for the binding of ATP to the P2Y receptor	K_D	10 μM

The adopted values of the constants k_{CCE} and H_{CCE} were chosen to mimic, at best, the experimental features of the calcium response of astrocyte in presence of extracellular ATP.

Astrocytes express a wide variety of neurotransmitter receptors that, when activated by appropriate agonists, evoke a large repertory of responses, including the $[Ca^{2+}]_i$ increase and the gliotransmitters release like glutamate and ATP (Newman, 2003; Volterra and Steinhauser, 2004; Fellin and Carmignoto, 2004; Perea and Araque, 2005; Zhang and Haydon, 2005; Koizumi et al., 2005). ATP participates to calcium signalling by acting on purinergic receptors P2X (ionotropic) and P2Y (metabotropic) (North and Barnard, 1997; Khakh, 2001; Koizumi et al., 2005). To model the effect of extracellular ATP on the astrocytic calcium level, we used the experimental results described by Nobile et al. (2003). These authors showed that in rat cortical astrocytes, the ATP-mediated calcium response consisted in an initial large transient component followed by a sustained plateau phase characterized by a little or no desensitization during subsequent ATP applications. These experimental results indicated that the transient component was due to ATP-evoked calcium release from the ER stores and induced by the activation of the metabotropic ATP receptor (P2Y), while the sustained component of the responses was mediated by the activation of the ionotropic ATP receptor (P2X). Thus, by using these experimental data, the rate of influx of the extracellular calcium in astrocyte mediated by the ionotropic ATP receptor is modeled as follows:

$$\nu_{ATP(P2X)} = k_{ATP(P2X)} G([ATP]_{ex}) \tag{3}$$

where $G([ATP]_{ex}) = [ATP]_{ex}^{1.4}/(H_{ATP(P2X)} + [ATP]_{ex}^{1.4})$, $[ATP]_{ex}$ is the external concentration of ATP (μM), $H_{ATP(P2X)}$ and $k_{ATP(P2X)}$ are defined in Table 1. The $G([ATP]_{ex})$ function was obtained by fitting the experimental data reported in Nobile et al. (2003), which described the amplitude of the sustained calcium response of astrocyte against the extracellular ATP concentration. The transient component of the ATP-evoked calcium response was mediated by the metabotropic ATP receptor activation coupled to G-protein that induced the phospholipase PLCβ activation leading to the IP_3 production. We used a very simple model to describe this process assuming the simple biochemical reaction scheme $ATP + R_{MB} \leftrightarrow ATP - R_{MB}$ in which the ligand binds its receptor (R_{MB}) with forward and reverse rate constants, K_F and K_R, respectively. Let R_0 be the concentration of the metabotropic ATP receptors and $x_F = [ATP - R_{MB}]/R_0$ be the fraction of receptors bound, and then the time evolution of x_F is described by:

$$\frac{dX_F}{dt} = K_F[ATP]_{ex} - x_F\{K_R + K_F[ATP]_{ex}\} \tag{4}$$

being $[ATP]_{ex}$ the external concentration of ATP (μM). The solution of this equation satisfying the initial condition $x_F(0) = 0$ is given by $x_F(t) = [ATP]_{ex}/(K_D + [ATP]_{ex})[1 - \exp(-t/\tau)]$, where $K_D = K_R/K_F$ is the dissociation constant (see Table 1) and $\tau = 1/(K_R + K_F[ATP]_{ex})$. By assuming a fast binding kinetics, the reaction $ATP + R_{MB} \leftrightarrow ATP - R_{MB}$ can be assumed to be in equilibrium and therefore it is:

$$x_F(t) = \frac{[ATP]_{ex}}{(K_D + [ATP]_{ex})} \tag{5}$$

For simplicity, the rate of IP_3 production promoted through this pathway (G-protein and PLCβ activation) is assumed to be determined by

$$\nu_{PLC\beta} = k_{ATP(P2Y)} x_F \tag{6}$$

and the value of the constant $k_{ATP(P2Y)}$ is reported in Table 1.

The explicit formulation of the remaining rate terms appearing in Eqs. (1a)–(1d) was taken from Höfer et al. (2002) and are defined as follows:

$$\nu_{LM} = k_0 \tag{7}$$

$$\nu_{OUT} = k_5 Ca_i \tag{8}$$

$$\nu_{ER(Rel)} = \left\{ k_1 + \frac{k_2 R Ca_i^2 I^2}{[(K_a^2 + Ca_i^2)(K_{IP3}^2 + I^2)]} \right\} (Ca_{ER} - Ca_i) \tag{9}$$

$$\nu_{SERCA} = k_3 Ca_i \tag{10}$$

$$\nu_{IP3(Deg)} = k_9 I \tag{11}$$

$$\nu_{PLC\delta} = \frac{\nu_7 Ca_i^2}{(K_{Ca}^2 + Ca_i^2)} \tag{12}$$

$$\nu_{IP3R(Rec)} - \nu_{IP3R(Inact)} = k_6 \left[\frac{K_i^2}{(K_i^2 + Ca_i^2)} - R \right] \tag{13}$$

the meanings and the values of the constants are reported in Table 1. The adopted values of the constants k_0, k_2, and β differ from those used in Höfer et al. (2002).

2.3. Neuron-astrocyte coupling model

The elevation of the intracellular calcium level in astrocytes, promoted by the extracellular glutamate, triggers the release of glutamate from these cells (Parpura et al., 1994; Porter and McCarthy, 1996; Pasti et al., 1997; Parpura and Haydon, 2000). Moreover, recent experimental findings revealed that glutamate released from astrocytes modulates the neural activity by promoting a depolarizing current in neurons (Parpura and Haydon, 2000; Fellin and Carmignoto, 2004; Volterra and Steinhauser, 2004; Perea and Araque, 2005).

Here, we investigate this phenomenon by using a minimal neural network model made up of two coupled units: a pyramidal neuron and an astrocyte. The modulation of the neuron arising from the release of astrocytic glutamate is modeled according to the experimental data of Parpura and Haydon (2000) and by using the mathematical formulation due to Nadkarni and Jung (2003, 2004). Thus, the pyramidal cell model, with a soma compartment assumed of diameter 25 μm, is injected with the following current:

$$I_{(astro)} = A_{astro} H\left[\ln(y)\right] \ln(y), \quad y = \frac{[Ca^{2+}]_i}{nM} - 196.69 \tag{14}$$

where $H(x)$ is the Heaviside function, $A_{astro} = 2.11\ \mu A/cm^2$, $[Ca^{2+}]_i$ is the cytosolic calcium concentration in the astrocyte in nM units. The pyramidal cell was modeled according to Olufsen et al. (2003) and the neuron-astrocyte network model is defined as follows:

$$\begin{aligned}
\frac{dV_P}{dt} &= I_P - I_{Na} - I_k - I_M - I_L + I_{(astro)} \\
\frac{dm_P}{dt} &= \alpha_m(1 - m_p) - \beta_m m_p \\
\frac{dh_P}{dt} &= \alpha_h(1 - h_p) - \beta_h h_p \\
\frac{dn_P}{dt} &= \alpha_n(1 - n_p) - \beta_n n_p \\
\frac{dw_P}{dt} &= \frac{(w_\infty - w_p)}{\tau_w}
\end{aligned} \tag{15}$$

where V_P is the membrane voltage of the pyramidal neuron, I_P is the stimulation current, and $I_{(astro)}$ is defined in Eq. (14). Moreover, $I_{Na} = g_{Na} m_p^3 h_p (V_p - V_{Na})$, $I_K = g_K n_p^4 (V_p - V_K)$, $I_L = g_L(V_p - V_L)$ represent the sodium, the potassium, and the leak currents, respectively; $I_M = g_M w_p (V_p - V_M)$ is the M-current. The corresponding gating variables are defined as follows: $\alpha_m = [0.32(V_p + 54)]/[1 - \exp(-(V_p + 54)/4)]$, $\beta_m = [0.28(V_p + 27)]/[\exp((V_p + 27)/5) - 1]$, $\alpha_h = 0.128 \exp(-(V_p + 50)/18)$, $\beta_h = 4/[1 + \exp(-(V_p + 27)/5)]$, $\alpha_n = [0.032(V_p + 52)]/[1 - \exp(-(V_p + 52)/5)]$, $\beta_n = 0.5 \exp(-(V_p + 57)/40)$, $w_\infty = 1/[1 + \exp(-(V_p + 35)/10)]$, $\tau_w = 400/[3.3\ \exp((V_p + 35)/20) + \exp(-(V_p + 35)/20)]$.

To describe the production of IP_3 in the astrocyte triggered by the discharge of the excitatory cell, we use a very simple mathematical formulation. Let r_{PY} be the rate of production of IP_3 when an action potential is generated by the pyramidal cell and $V_{Th} = -50$ mV be a membrane potential threshold value. Then, we assume that there is a production of IP_3 in the astrocyte only when $V_P > V_{Th}$ and the corresponding rate, to be added to the right hand-side of Eq. (1d) describing the time evolution of the IP_3 concentration, is defined by $\nu_{PY} = r_{PY} H(V_P - V_{Th})$, where $H(x)$ is the Heaviside function. It is worth noting that a similar approach to describe the IP_3 production arising from the neuron discharge has been used by Nadkarni and Jung (2003, 2004).

3. Results

3.1. Calcium dynamics in astrocyte: comparison with the experimental results

First of all, the astrocytic calcium model defined by Eqs. (1a)–(1d) is tested against the experimental data obtained by us (Nobile et al., 2003). The first thing to do is checking whether the model is capable of reproducing the ATP evoked biphasic calcium response as discussed in Section 2.2, and in Fig. 2 the corresponding results are reported. The experimental results reported in the top panel show that the calcium response consists

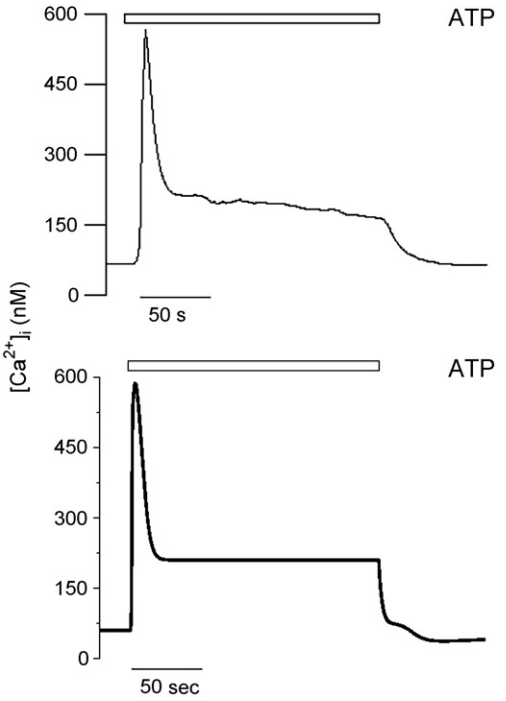

Fig. 2. ATP mediated calcium response in astrocyte. Top panel: experimental data; bottom panel: simulation results. For both panels it is $[ATP]_{ex} = 3\ \mu M$ (horizontal bar).

of two phases: transient and sustained. The corresponding model data are shown in the bottom panel and agree, qualitatively and quantitatively, with the experimental ones. How to explain the molecular mechanisms underlying this biphasic response? In another experiment (Nobile et al., 2003), it was found that: (1) the sustained phase disappears when the extracellular calcium is removed; (2) in the presence of extracellular calcium and for an ATP stimulation lasting about 20 s, the transient peak alone is observed (data not shown). Moreover, the simulations show that during the transient phase, the ER calcium concentration decreases very rapidly (data not shown) suggesting that the P2Y metabotropic ATP receptor mediates this calcium response phase.

To clarify this point, additional experiments were performed by using specific agonists of the P2X and P2Y receptors: the results are reported in the top panels of Fig. 3. When only the specific 2-methylthio-ATP (2-MeSATP) agonist for the P2Y receptor is employed (see top-left panel), the astrocyte calcium response exhibits the transient phase alone. On the contrary, when the 3′-*O*-(4-benzoyl) benzoyl-ATP (Bz-ATP) agonist of the P2X ionotropic receptor (specific for the $P2X_7$) is used (see top-right panel) the sustained response phase is clearly visible, while the transient one is quite absent. For comparison, the results obtained with the model

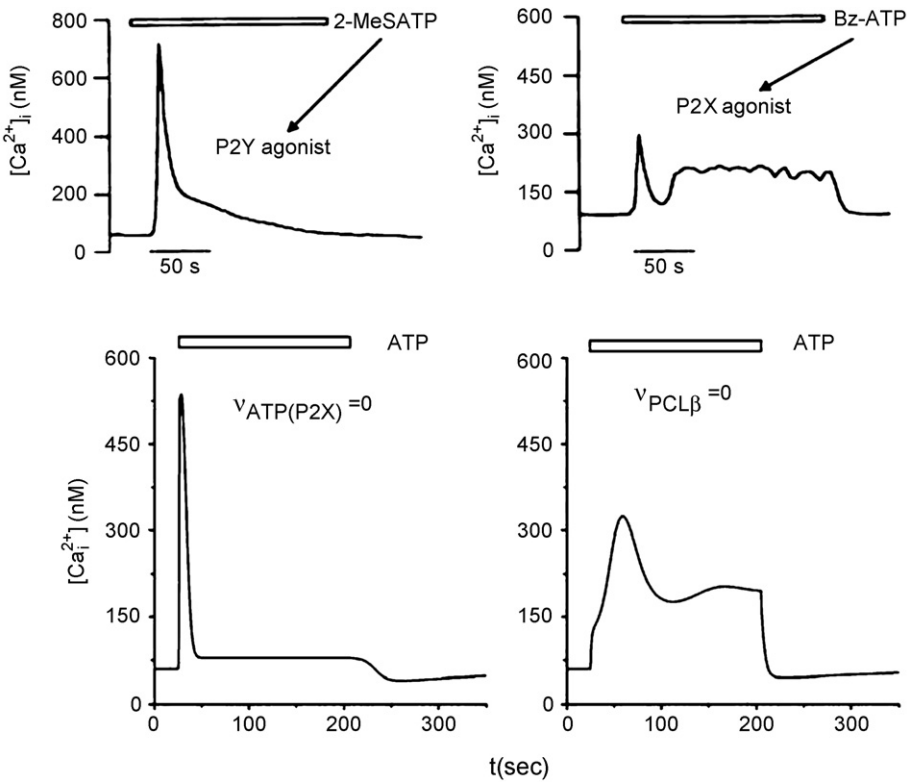

Fig. 3. Experimental results (top panels): different purinoreceptor agonists evoke kinetically variable calcium responses in rat astrocytes. Top-left panel: bath application of 2-MeSATP agonist (10 μM) of P2Y receptor evokes only a transient calcium response in the astrocyte. Top-right panel: application of Bz-ATP agonist (10 μM) of P2X receptor (selective for the P2X7 receptor) evokes a small peak calcium response followed by a sustained calcium plateau (similar to that evoked with ATP). Simulation results (bottom panels): effects of P2X and P2Y receptors mediating the ATP evoked calcium responses in the model. Bottom-left (Bottom-right) panel: calcium response evoked by ATP when the calcium entry through the P2X (P2Y) receptor is set off. For both panels it is $[ATP]_{ex} = 3\ \mu M$ (horizontal bar).

when the membrane calcium flux mediated by the P2X ionotropic receptor is abolished are reported in the bottom-left panel of Fig. 3. Noteworthy, the contribution of CCE calcium entry pathway to the sustained phased is very low. Instead, the results obtained when the P2Y metabotropic receptor is set off are reported in the bottom-right panel and agree qualitatively with the corresponding experimental data. We remark that the extracellular ATP concentration used in these simulations, $[ATP]_{ex} = 3\ \mu M$, is sufficient to mimic the corresponding experimental results: either those evoked by ATP or by specific receptor agonists. Thus, both experimental and simulation findings suggest that the peak corresponds to the release of calcium from the ER store, while the sustained part is mainly determined by the influx of extracellular calcium mediated by the P2X purinoreceptor.

In another set of experiments, the calcium response in astrocytes due to successive ATP challenges of about three minutes duration each, was investigated. In this case, the typical calcium response is reported in Fig. 4 and shows that the transient phases have two different amplitudes, the last peak having lower amplitude than the first one. On the contrary, the sustained responses are qualitatively similar. It is worth noting that when the time

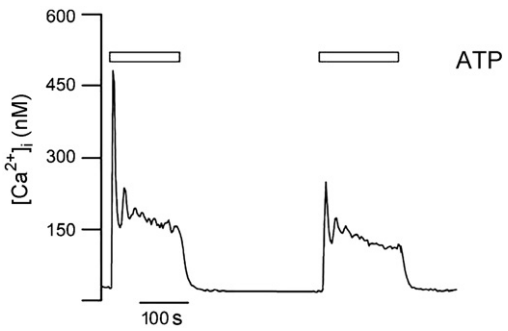

Fig. 4. Experimental results: the transient and sustained phases of the ATP evoked calcium response in rat astrocyte are driven by different molecular mechanisms. The peak of the transient phase is substantially diminished when a second ATP challenge is performed 5 min later, while the sustained phases are not significantly altered. The extracellular ATP concentration was: $[ATP]_{ex} = 3\ \mu M$.

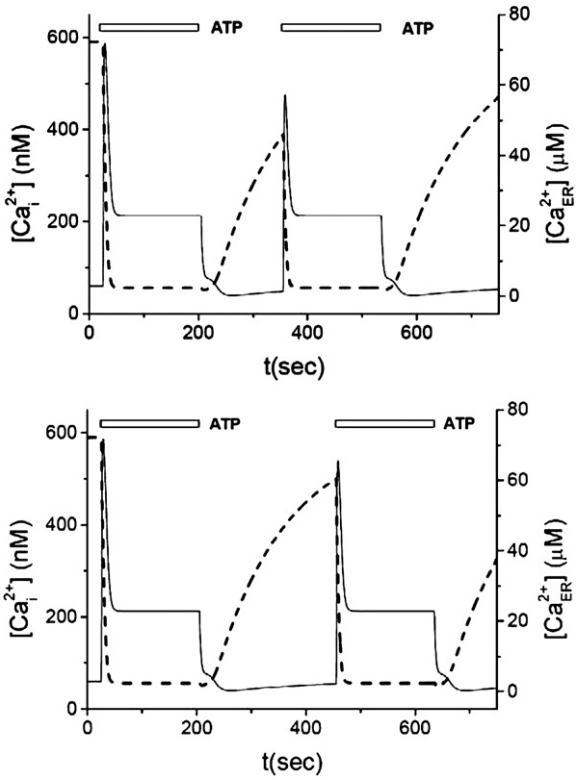

Fig. 5. Simulations results, mimicking those presented in Fig. 4, characterizing the molecular mechanisms underlying the calcium responses evoked by successive ATP challenges. Top (bottom) panel: the time separation between the two ATP applications, each one lasting 3 min, is 330 (430) s. For both panels the thin line represents the cytoplasm calcium level, while the dashed line the ER one. The extracellular ATP concentration was: $[ATP]_{ex} = 3\,\mu M$.

separation between the two ATP challenges increases sufficiently, the amplitudes of both peaks exhibit similar values.

To explain these findings we performed a numerical experiment mimicking the experimental one and the results, reported in Fig. 5, suggest that the different amplitudes of the transient responses are accounted for by the refilling level of the ER stores. In fact, from the top panel of this figure it can be clearly seen that the level of calcium concentration in the ER store associated to the second peak ($[C_{ER}^{2+}] \cong 46\,\mu M$) is lower than that corresponding to the first one ($[C_{ER}^{2+}] \cong 72\,\mu M$). Thus, the amplitude of the transient response, promoted by the second ATP stimulation, will be smaller than that corresponding to the first one because the amount of calcium released from the store is lower. This conclusion is corroborated by the result reported in the bottom panel of Fig. 5. In this case the time separation between the two ATP stimulations is greater and therefore the refilling level of the ER stores is sufficient to guarantee that both peaks have similar amplitudes. Moreover, in agreement with the experimental results shown in Fig. 4, the sustained phase responses elicited by the two stimulations are the same for both panels.

To sum up, the simulation findings are in agreement with the experimental ones and so the model is suitable to investigate these phenomena, besides its predictions can help the understanding of the molecular events underlying calcium dynamics in astrocyte. However, the model could be improved by including other receptor behaviors like P2Y desensitization, etc. and that will be pursued in a future work.

3.2. Astrocytic calcium and modulation of neural activity

As discussed in Section 2.3, the glutamate released from an astrocyte promotes an inward current in a neuron (see Eq. (14)), so an important issue is to investigate the dynamical behavior of the neurons in the presence of these modulator signals. This topic will be discussed in this section by combining the model describing the calcium dynamics in the astrocyte with that describing a pyramidal neuron (see Fig. 6). Recently, a dressed neuron model was proposed and it was shown that the release of glutamate from astrocyte could have profound effects on the firing properties of the neuron. In this model, the calcium dynamics in the astrocyte is described by the Li–Rinzel model (Li and Rinzel, 1994). This model differs substantially from the calcium model used in our paper because we inserted calcium fluxes through the plasma membrane of astrocyte. Specifically, the calcium influx mediated by ionotropic purinoreceptors was considered. On the other hand, a relevant finding obtained with this model is that at large production rate of IP_3 (r_{PY}), the firing activity of the neuron can be switched to a persistent oscillatory state resembling that occurring in the epileptic regime (Nadkarni and Jung, 2003, 2004).

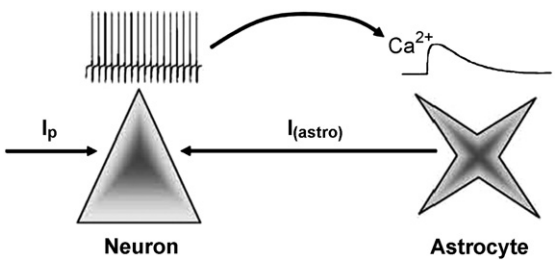

Fig. 6. A schematic diagram showing the main features of the coupling between neuron and astrocyte. The action potentials generated by the neuron injected with a constant current I_P, trigger an increase of the internal calcium of the astrocyte. This event feedbacks an inward current to the neuron ($I_{(astro)}$).

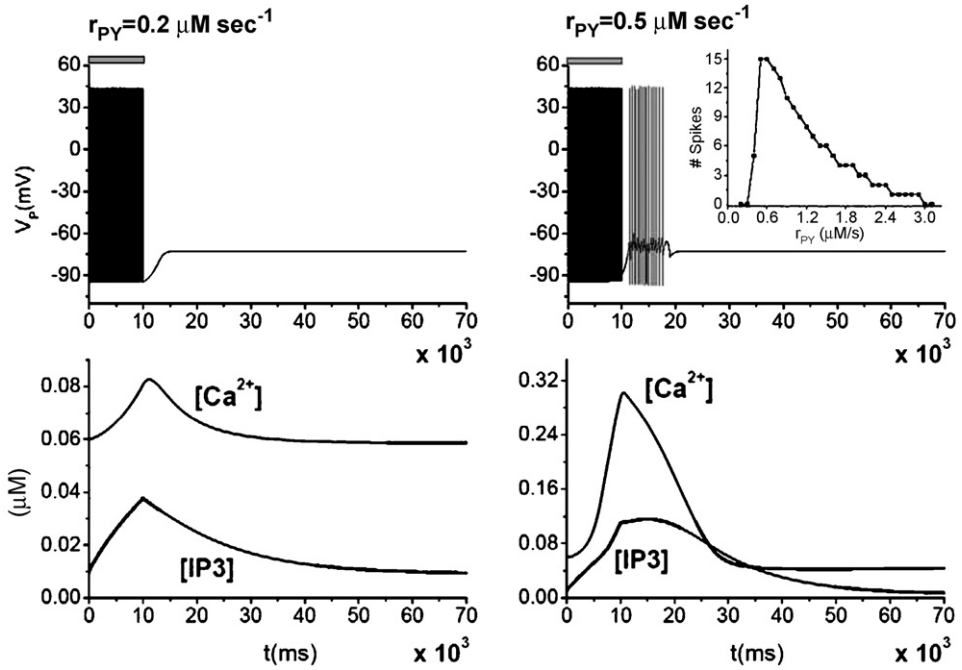

Fig. 7. Time course of the membrane potential of the neuron model and of the $[Ca^{2+}]$ and $[IP_3]$ concentrations in the astrocyte, when the neuron is injected with the current $I_P = 20\ \mu A/cm^2$ for a time interval of 10 seconds (marked with the grey bar). Left (right) panels show the evoked response for an IP_3 production rate $r_{PY} = 0.2\ \mu M$ (0.5 μM). The insert shows the number of action potential fired by the neuron after the stimulation period against the IP_3 production rate r_{PY}. Please note the different ordinate scales of the lower panels. All simulations were performed in absence of extracellular ATP.

Our aim here is to check whether, in absence of extracellular ATP, our astrocyte–neuron network model exhibits such a dynamical state. Therefore, closely following their numerical experiment protocol, we first inject the pyramidal cell with a constant current for a time interval of $T_S = 10$ s then, after the stimulation is terminated, the dynamical behavior of the neuron is followed for the remaining time interval (60 s). The value of the stimulation current is chosen in such a way that the cell-discharging frequency is realistic: the adopted value is $20\ \mu A/cm^2$ and the corresponding firing frequency is about 25 Hz. The results are reported in Fig. 7 and the left panels show that for $r_{PY} = 0.2\ \mu M$, the generation of firing activity in the pyramidal neuron occurs during the stimulation phase alone ($t \leq T_S$). In this case, the elevation of the internal calcium level in the astrocyte is not sufficient to trigger a feedback response on the neuron (see Eq. (14)). The increase of the production rate of IP_3 amplifies the calcium response in the astrocyte and so leads, for $t > T_S$, to the generation of action potentials within a well-defined time window (see right panels of Fig. 7). By performing numerical experiments, with increasing r_{PY} values, dynamical regimes in which neuronal firing is maintained indefinitely for $t > T_S$ were never observed. On the contrary, it was found that for very high values of parameter r_{PY} action potentials were absent in the region $t > T_S$.

To characterize this behavior, the total number of action potentials generated in each time window (located in the region $t > T_S$) were counted and plotted against the parameter r_{PY} (see insert in Fig. 7): there is a sharp increase of the firing activity of the cell in a narrow interval of r_{PY} values that is followed by a slower decrease for high r_{PY} values. We checked that this behavior does not depend on the stimulation level of the cells: numerical experiments performed with different values of I_P lead to qualitatively similar results (data not shown). The overall results obtained with our model contrast with those obtained by Nadkarni and Jung (2003, 2004) for high r_{PY} values. Then, the obvious question to ask is why the predictions obtained with the two models are different for $t > T_S$. Let us remark that to get the dynamical regime in which the neuron fires indefinitely, when the stimulation is set off, needs an intracellular calcium concentration in the astrocyte above ∼0.2 μM. Therefore, considering that our model is equipped with membrane calcium transport mechanisms, the parameters characterizing these fluxes could be involved in the generation

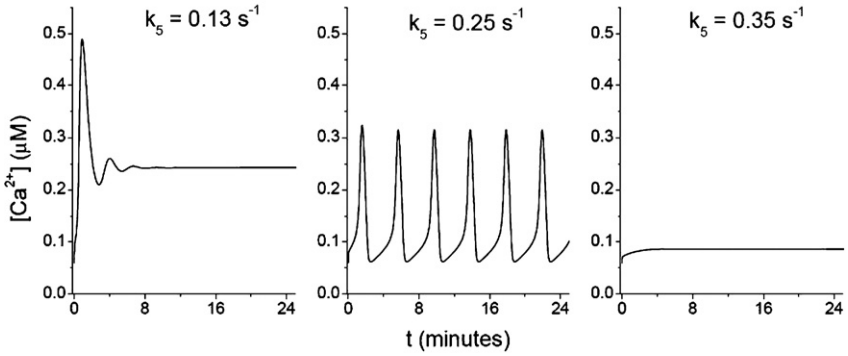

Fig. 8. Time course of the internal calcium level in the astrocyte model (uncoupled from the neuron) for three different values of the calcium extrusion rate, k_5, through the plasma membrane. All simulations were performed in absence of extracellular ATP.

of cytoplasm calcium concentration regimes satisfying the previous constraint. Thus, these qualitative considerations suggest that the discrepancy between our findings and those of Nadkarni and Jung (2003, 2004) could be related to calcium transport mechanisms through the membrane of the astrocyte. Among them a relevant role is played by the extrusion of calcium from the cell, which is described in our model by the parameter k_5 (see Eqs. (1a) and (8)). It is worth noting that recently it has been shown that the membrane calcium transport is a relevant molecular mechanism controlling Ca^{2+} oscillations (Sneyd et al., 2004), and we are going to show that in our case this is the key mechanism to explain the discrepancy between the results obtained with the two models. To this aim we have considered the astrocyte calcium model used here and analyzed, in absence of any coupling with the neuron, the dynamical behavior of the cytoplasm calcium concentration as the parameter k_5 is changed. We find that there are two critical values, $P_1 \cong 0.14\,s^{-1}$ and $P_2 \cong 0.295\,s^{-1}$, of the parameter k_5 separating different dynamical regimes as shown in Fig. 8. For $k_5 > P_2$ ($k_5 < P_1$) the calcium concentration in astrocyte gets a stationary value satisfying the condition $[Ca^{2+}] < 0.1\,\mu M$ ($[Ca^{2+}] > 0.2\,\mu M$), while when $P_1 < k_5 < P_2$ the dynamical regimes is characterized by calcium oscillations occurring through a Hopf bifurcation (see Fig. 8). Then, we have performed a simulation study of the astrocyte–neuron network by using different k_5 values and the corresponding results are shown in Fig. 9. As for the case reported in Fig. 7, the pyramidal neuron model is injected with a constant stimulation current lasting $T_S = 10$ s. As expected the system exhibits dynamical regimes that are the same as those shown in Fig. 8 and thus explain why the parameter k_5 is so critical for determining whether the neuron fires when the stimulation is set off (see Fig. 9). In fact, the left panel shows that for $k_5 < P_1$ the stationary values of the calcium concentration are over 0.2 μM and therefore the neuron fires indefinitely; for $P_1 < k_5 < P_2$ the neuron fires occasionally in correspondence of the calcium peaks (see middle

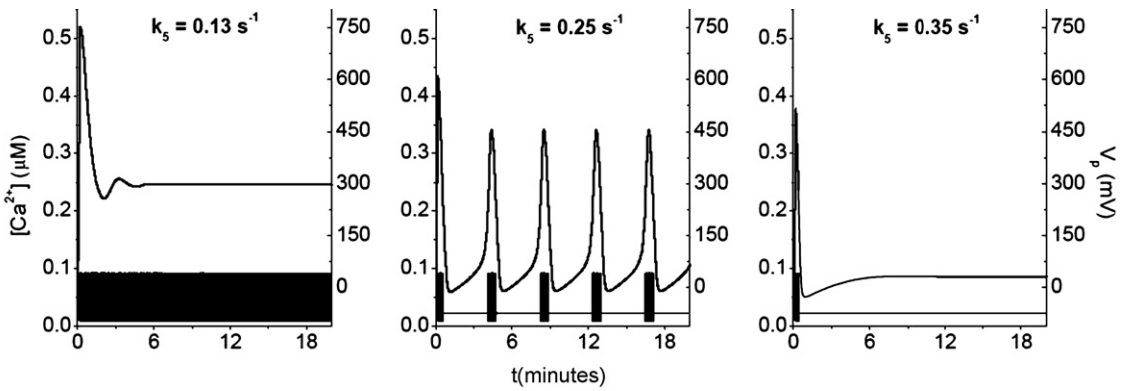

Fig. 9. Membrane potential of the neuron model and $[Ca^{2+}]$ concentration in the astrocyte for three different values of the calcium extrusion rate, k_5, through the plasma membrane. The neuron is injected with the current $I_P = 20\,\mu A/cm^2$ for a time interval of 10 s, while the IP_3 production rate is set to $r_{PY} = 0.5\,\mu M$. All simulations are performed in absence of extracellular ATP.

panel); while for $k_5 > P_2$ the neuron firing is suppressed because the asymptotic calcium concentration is below 0.2 μM.

4. Conclusions

A model of calcium dynamics in astrocyte was build and analyzed. The simulation results show that, in agreement with the experiments, the transient calcium response to extracellular ATP is mediated by the P2Y receptor, while the sustained one by the P2X receptor. Moreover, the model is able to mimic more complex calcium responses of the astrocyte obtained by using different ATP stimulation protocols. A collective model involving an astrocyte coupled to a single neuron was developed and the corresponding simulations results show that the calcium extrusion rate through the astrocyte membrane play a key role in shaping the neural activity.

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