MODELING NEURONAL ADAPTATION IN THE BRAIN: INTEGRATING RECEPTOR SIGNALING AND ELECTROPHYSIOLOGY

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Abstract

The present study is aimed at understanding the underlying mechanisms of the neuronal adaptation in the brain. An integrated model of signaling dynamics elicited by neuropeptide receptors and the electrophysiology was developed and the interactions were analyzed. The biological system considered is that of brainstem neurons in which the dynamic changes in the neuronal excitability are elicited by Angiotensin II. The model is based on the available kinetics of Gq-protein coupled receptor signaling, known ion channel components and the corresponding models based on Hodgkin-Huxley framework, and relies on experimentally motivated assumptions on the phosphorylation kinetics of ion channels to couple the two domains. This model is in concordance with the experimental data and exhibits an AngII-elicited increase in neuronal excitability. One of the key hypotheses of the integrated model is the compartmentalization of the calcium levels between the membrane ion channels and cytosol. Analysis of the model for different cases of interactions revealed the individual contribution of the key signaling kinases to the overall adaptation dynamics. These experimentally verifiable hypotheses provide insights into the dynamics of interactions between signaling and electrophysiology that underlie neuronal adaptation in the brain.

Keywords

Neuronal adaptation, mathematical modeling, electrophysiology

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INTRODUCTION

One of the fundamental processes underlying brain function is the ability of the neurons to adapt to external inputs in the context of the neuronal state. These adaptive processes span multiple temporal scales ranging from millisecond dynamics of the ion channels, seconds to minutes time scale of the signaling pathways, and tens of minutes to hours time scale of the gene regulation and its feedback onto the signaling pathways and electrophysiology. Quantitative description of these processes is essential to the systems-level understanding of the multi-scale interactions between electrophysiology, signaling pathways and gene regulatory networks.

At an abstract level, a neuron can be considered as a signal processing unit in which the 'output' (e.g., frequency of action potential spikes, termed 'firing frequency') as elicited by the 'input' (e.g., synaptic stimulus) is determined by the physiological 'transfer function' that is conditioned upon the intraneuronal state (levels of various ion channel proteins, their distribution, Ca^{2+} levels, etc.). The intraneuronal state is 'plastic', i.e., it can be altered by various neuromodulatory signaling mechanisms. Such an adaptation at a single neuron level is one of the key molecular modalities by which the neuronal circuit behavior is altered in various brain functions (Kandel et al., 1991).

In this context, one of the key questions is: How do the signal transduction pathways and electrophysiology interact to alter the cellular state thus affecting the neuronal input-output properties? The complexity of this system arises because of the multi-scale bidirectional interactions between signaling and electrophysiology. The present study is aimed at this question by developing and analyzing an integrated model of signaling dynamics elicited by neuropeptide receptors and interactions with the electrophysiology.

The present manuscript is organized as follows: First, the biological system and the experimental evidence on the modulation of ion channels by signaling components are briefly summarized. Second, the model development aspects on separately modeling the signaling and electrophysiology are presented. Third, the details of the integration of the two levels are discussed, highlighting the complexity of the interactions.

BIOLOGICAL SYSTEM AND EXPERIMENTAL DATA

The particular system considered is the Angiotensin II (AngII) receptor signaling and electrical activity in the cardiorespiratory neurons in the Nucleus Tractus Solitarius (NTS) in the brainstem. The NTS is a bilateral, rostro-caudally elongated long, tube-shaped group of neurons in the dorsomedial medulla surrounding the visceral sensory axons. These inputs, within the NTS, subserve a wide range of other integrative activities (e.g., arterial, cardiac, chemoreflex, integration of autonomic and endocrine outflows) that maintain the "internal environment" (Barraco, 1994). The octapeptide AngII is a multifunctional hormone and is involved in stimulation of water and sodium uptake, vasopressin secretion, increased blood pressure and modulation of baroreflexes (de Gasparo et al. 2000; Sumners et al., 2002). AngII acting via Angtiotensin Receptor Type 1 (AT1R) in the NTS influences the baroreceptor reflexes thus modulating cardiac and respiratory dynamics. Thus, factors that regulate the neuronal actions of AngII in the brain will modify the effects of AngII on NTS-dependent cardiorespiratory homeostasis.

Stimulation of the hypothalamic and brainstem neurons by AngII has been shown to result in a transient increase in the electrical activity leading to neuronal adaptation and this response is mediated by AT1R binding to AngII (Li and Guyenet, 1996; Zhu et al., 1997; Richards et al., 1999; Sumners et al., 2002; Sun et al., 2002). The time-scale is of the order of tens of seconds and indicates posttranslational modification, such as phosphorylation of ion channels, rather than altered gene expression. Figure 1 depicts the best characterized signaling pathway downstream of AT1R: the Gq-protein-mediated stimulation of phospholipase C beta (PLC-β) and phosphoinositide (PI) hydrolysis, with subsequent Ca^{2+} mobilization and activation of Ca^{2+}-dependent enzymes, such as Protein kinase C (PKC) and Calcium/calmodulin-dependent protein kinase II (CaMKII).

Pharmacological studies have implicated the signaling kinases, PKC and CaMKII, in modulation of different ion channels. In particular, the delayed rectifier potassium (K_{DR}) current and the A-type potassium (K_A) current decrease upon AT1R activation by AngII (Gelband et al., 1999; Pan et al., 2000, 2001; Wang et al., 1997a-b). The

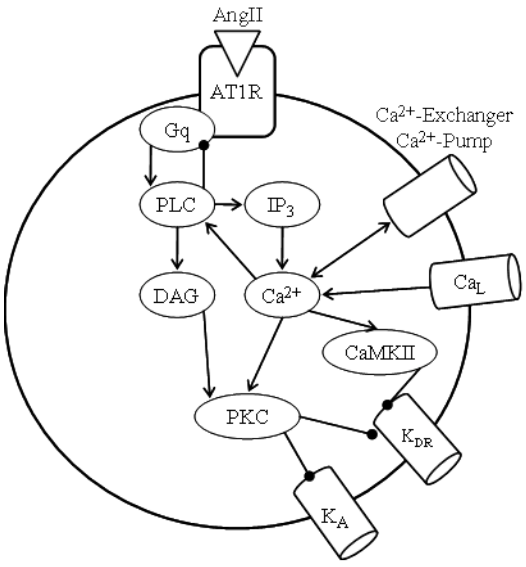

Figure 1: A schematic of the Angiotensin II signaling via AT1 receptor and its effects on various membrane ion channels. Arrows indicate activation/production and filled circles represent inhibition. Different elements of the pathway are described in the text.

inhbition of these channels is presumably via direct phosphorylation of the corresponding potassium channel subunits (Sumners et al., 2002). Phosphorylation of voltage-gated ion channels is a widely observed phenomenon for nearly all known channel subtypes (Levitan, 1985). From a macroscopic (whole neuron) perspective, the effects can include modulation of the maximal conductance and the activation/inactivation variables. In NTS neurons the underlying channel subtype responsible for the modulation of the K_A and K_{DR} are Kv1.4 and Kv2.2 respectively (Pan et al., 2000; Gelband et al., 1999). Inhibition of K_A is due to direct phosphorylation of the Kv1.4-α subunit at sites that could interact with PKC and CaMKII (Hagiwara et al., 2003). While it is not clear whether direct phosphorylation of Kv2.2 occurs, a variety of auxiliary β-subunits have been shown to assemble in complexes with the Kv2.2 (Coetzee et al., 1999). Further, the closely related Kv2.1 is modulated by phosphorylation in several ways (Misonou et al., 2004). Together, this evidence indicates that the K_{DR} channel modulation could be due to direct phosphorylation of the Kv2.2 or related auxiliary subunits.

In summary, AngII-mediated increase in neuronal excitability involves AT1R signaling that activates the kinases PKC and CaMKII, that in turn could modulate specific potassium channels. The role of Ca^{2+} release, which can have substantial effects on the Ca^{2+}-dependent potassium channels, is unclear. Also, the relative dynamic contributions of PKC and CaMKII to the channel modulation are unknown. The present modeling effort is aimed at integrating the experimental data to investigate whether AngII-mediated neuronal adaptation can be sufficiently explained by the known kinetics of the AT1R signaling mechanisms and biophysics of the ion channels expressed in the NTS cardiorespiratory neurons, and also develop quantitative hypotheses towards the aforementioned unclear issues.

MODELING MEMBRANE ELECTRICAL ACTIVITY

The electrophysiology of neurons is commonly modeled using Hodgkin and Huxley (1952)-like framework that regards the neuron as an electrical circuit, with a balance for overall ionic current across the membrane represented as:

$$C\frac{dV}{dt} = \sum_i g_i(V)\big(E_i(V) - V\big) \quad (1)$$

$$g_i(V) = g_{i,\max} m_i^{M_i}(V) h_i^{H_i}(V) \quad (2)$$

where C is the membrane capacitance; V is the voltage across the membrane; and for each ion channel i, g_i is the conductance, E_i is the reversal potential, $g_{i,max}$ is the maximal conductance, m_i is the activation variable, h_i is the inactivation variable, and M_i and H_i are suitable parameters that are dependent on the kinetics of the channel activation/inactivation. The time-varying membrane potential-dependent functions $m_i(V)$, $h_i(V)$ are typically described using nonlinear functions as in Hodgkin and Huxley (1952) or depend on paramterized Boltzmann functions. In the latter representation, the activation is unambiguously characterized by the half-activation voltage (V_{12}) and a activation curve slope factor (k).

Model set-up and tuning

The present electrical model was based on the cardiorespiratory NTS neuron model in Rybak et al. (1997) and consists of a sodium channel (Na), a delayed rectifier (K_{DR}), a fast activating (K_A) and a Ca^{2+}-activated potassium (K_{AHP}) channel, a high threshold L-type Ca^{2+} channel (Ca_L), a non-specific leak channel and an excitatory synaptic input conductance. Initial simulations of this available model did not match the experimental observations that of an increased firing rate upon reduced K_{DR} conductance, increased intracellular Ca^{2+} concentration or increased Ca_L current. The initial model was modified according to available experimental data on the brain stem neurons as follows:

(1) The maximal conductances of the different channel types were adopted from (Rybak et al., 1997). This ensured an NTS neuron model exhibiting the desired firing pattern at nominal conditions.

(2) The activation variables of the K_{DR} and K_A currents were chosen to provide a good fit to whole cell patch-clamp current data (Wang et al., 1997a-b; Gelband et al., 1999). In the modified model, the K_{DR} activation was represented by a fourth order Boltzmann function based on the experimental data on rat brain thalamic relay neurons in Huguenard et al. (1991). The half activation voltage was identified as 2.3mV based on the Kv2.2 current in response to AngII in cultured hypothalamic and brain stem neurons (Gelband et al., 1999).

(3) The change in maximal conductance and half activation voltage of K_{DR} and K_A channels due to AngII treatment were determined from available experimental data (Wang et al., 1997; Pan et al., 2001) indicating that, upon AngII stimulus, the $g_{KDR,max}$ was reduced by ~25% without any effect on $V_{12,KDR}$), whereas $g_{KA,max}$ is reduced by ~20% and $V_{12,KA}$ shifts by approximately +4mV. The channel model fit to the data is shown in Figure 2.

Simulation of channel modulation and neuronal firing rate

In order to determine the contribution of different ion channel to the neuronal firing rate and the influence of the tuning parameter (reversal potential of the leak channel E_{leak}) different AngII treatment related conditions were simulated. The K_{DR}, K_A and Ca_L currents were altered according to experimental observations by either perturbing the maximal conductance (~20%) or the half activation constant (~4mV) of the corresponding channel model. Simulation results indicate that, regardless of the different molecular mechanisms underlying these two channel modification types, there is no significant difference in effect on the firing rate. This indicates that

the frequency modulation is in principle determined by the channel type itself (Figure 3).

Activation of K_{DR} reduced the onset of repetitive firing (shifting of the curve to lower E_{leak}), thus stimulating otherwise quiescent neurons (Figure 3). The simulations also revealed a potential bimodal effect of K_{DR} channel modulation: at low firing rates, reduction in K_{DR} current increased the firing rate (the stimulatory effect, consistent with experimental data with AngII stimulus), whereas the effect was reversed into partial inhibition at higher firing rates (no experimental observations were reported in this physiological range).

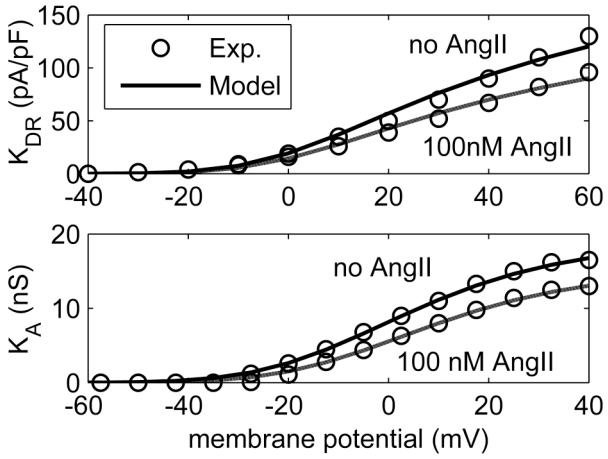

Figure 2: Effect of AngII stimulus on the current-voltage relationship of the potassium channels K_{DR} and K_A. For K_{DR}, the slope of the curve (maximal conductance) is reduced, whereas for K_A, the curve is also shifted to increase the half activation voltage by ~4mV. Experimental data from Pan et al. (2001) and Wang et al. (1997b).

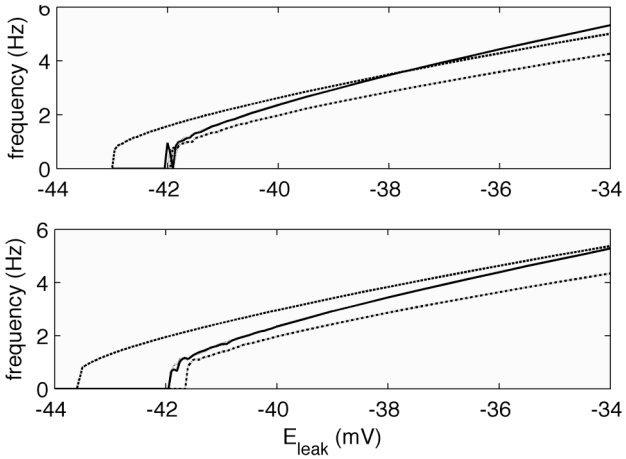

Figure 3: Results comparing baseline neuronal firing rate (solid line) to that of perturbed channel parameters. Top panel: modulation of maximal conductances: 20% reduced K_{DR} (dashed), 20% reduced K_A (dotted) and 20% increased Ca_L (dash-dotted). Bottom panel: increase of half activation voltage by 4mV: K_{DR} (dashed), K_A (dotted), Ca_L (dash-dotted). Results discussed in the text.

Partial inhibition of the K_A channel had no significant effect on the firing rate in the model simulations (Figure 3). An explanation of this lack of response may lie in the inactivity of K_A channel during the inter-spike intervals. The simulations also explored the case of increasing the Ca_L channel current. This had an inhibitory effect on the neuronal firing rate (Figure 3), as it increases intracellular Ca^{2+} and hence subsequently activates K_{AHP} channel to further hyperpolarize the neuron and increase the inter-spike interval (i.e., reduce the firing rate). These results indicate that the increase in total Ca^{2+} current proposed by Wang et al. (1997a-b) may not be due to increase in Ca_L current, but could be of different Ca^{2+} channel subtype. Alternatively, it could be that the K_{AHP} channel present in these neurons has different Ca^{2+}-dependent kinetics from that is considered in the model here. Further experiments on this channel are required to resolve this issue identified in the computational analysis.

MODELING AT1R SIGNAL TRANSDUCTION

The AT1R signaling component was constructed based on the a kinetic reaction model that contains detailed kinetics of the Ins(145)P3 metabolism integrated with Gq-mediated PLC-β activation and Ca^{2+} release by the InsP3 receptor (Mishra and Bhalla, 2002; available as accession #31 in the DOQCS database, http://doqcs.ncbs.res.in). This DOQCS model was tuned based on the data from brain tissue samples. In the present study, this model was further extended by adding descriptions of: (1) a Na^{+}-Ca^{2+} exchanger, and (2) dependency of the transmembrane ion transport on membrane potential based on the common Goldman-Hodgkin-Katz electrodiffusion models (Athanasiades et al., 2000). The parameters were chosen to match the observed resting potential of the NTS neurons, and also to account for the observed AngII-elicited intraneuronal Ca^{2+} response (data from Fernandez et al., 2003).

The key 'outputs' for the purpose of model integration are the activity of the kinases PKC and CaMKII involved in the modulation of the ion channels. These kinases can be activated by phosphorylation/ effector-binding at several domains leading to a variety of active kinase species. In the absence of clear experimental data in neurons, all active kinase forms were considered to be similarly capable of phosphorylating the ion channels, and hence the 'net active PKC (or CaMKII)' was obtained as the sum of all corresponding active forms.

MODELING ION CHANNEL PHOSPHORYLATION

In the present model, the phosphorylation level determined the reduction in the total macroscopic conductance of specific ion channels. Based on the results above from analyzing the electrical model (inhibition of K_{DR} and not K_A had significant effect on neuronal excitability, and Ca_L channel modulation with as yet

unresolved concerns, Figure 3) only the phosphorylation of K_{DR} was considered. The kinases PKC and CaMKII were considered to be acting independently as:

$$\begin{aligned} K_{DR} + n \cdot PKC &\rightarrow K_{DR}^{1} + n \cdot PKC \\ K_{DR} + n \cdot CaMKII &\rightarrow K_{DR}^{2} + n \cdot CaMKII \\ K_{DR}^{2} + n \cdot PKC &\rightarrow K_{DR}^{1,2} + n \cdot PKC \\ K_{DR}^{1} + n \cdot CaMKII &\rightarrow K_{DR}^{1,2} + n \cdot CaMKII \end{aligned} \quad (3)$$

where the superscript of K_{DR} denotes the phosphorylation state. Dephosphorylation was modelled as occurring at a first order rate (k_{PKC} and k_{CaMKII}). The parameter n=4, a simplification under the assumption that each of the four subunits of the channel tetrameric complex must be phosphorylated. The kinetic model was derived based on law of mass action and accounting for the constant total channel concentration (applicable at the tens of seconds time-scales considered here). Based on experimental evidence that indicated substantial reduction in conductance of potassium currents in rat brain (~85-90%; Perez et al., 1996; Hagiwara et al., 2003), a simplifying assumption was made that the phosphorylated K_{DR} channels were completely inhibited in their function, i.e., their conductance is negligible or zero. Thus, the net maximal K_{DR} conductance was obtained by multiplying the basal maximal conductance $g_{KDR,max}$ by the fraction of unphosphorylated channels.

Based on the experiments by Sumners and co-workers, AngII treatment induces about 20-35% reduction in the potassium channel conductance. These experiments also indicate that the basal level of phosphorylation is very low, based on the fact that PKC or CaMKII inhibitors did not have an effect on the potassium currents in the absence of AngII (Zhu et al., 1999; Pan et al., 2001; Sun et al., 2002). The equilibrium constants were chosen such that both PKC and CaMKII can independently cause a reduction in the K_{DR} maximal conductance ($K_{M,PKC}$=0.5, $K_{M,\ CaMKII}$=1.5). The dephosphorylation rate constants were specified as k_{PKC}=10 and k_{CaMKII}=10 through a trial and error procedure to obtain a good fit to the experimental data on the reduction of K_{DR} current over time. A comparison of the simulated K_{DR} dynamics and the experimental data from Pan et al. (2001) is shown in Figure 4. Note that this model is not critically dependent on the direct phosphorylation of alpha subunits. Similar kinetic representations could be employed for the cases in which the phosphorylation could occur at the auxiliary subunits.

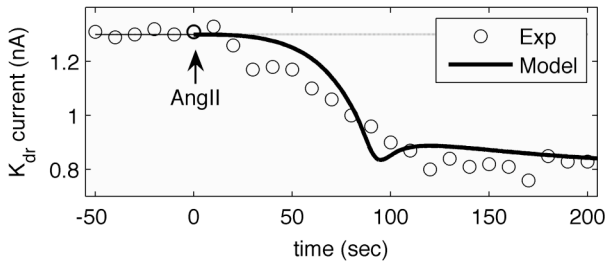

Figure 4: Comparison of the time course data for the KDR current (Pan et al., 2001) and the phosphorylation model results. Arrow indicates the time at which AngII was added to the preparation.

INTEGRATING THE SIGNALING AND ELECTROPHYSIOLOGY MODELS

A naïve 'integration' of the signaling and electrophysiology domains can be achieved by simulating the signaling model to obtain the dynamics of the kinases PKC and CaMKII, utilizing those dynamics to compute the maximal channel conductance dynamics based on the ion channel phosphorylation model, and employing it in the electrophysiology model simulations. While such an approach would lead to a model system that, when simulated, may result in an AngII-elicited increase in neuronal firing frequency, there are several inconsistencies with the actual biological mechanisms. Such a model cannot be analyzed for the key role of Ca^{2+} on the dynamics of the excitability in the system, as the 'signaling Ca^{2+}' and 'electrophysiological Ca^{2+}' would be disjoint in the naïve model.

A rigorous approach would be to consider all the 'bi-directional interactions' between the signaling and electrophysiology domains that arise because of: (1) the kinases PKC and CaMKII phosphorylating the ion channels, (2) the dependency of Na^{+}-Ca^{2+} exchanger on the membrane potential (V), (3) the contribution of voltage dependent Ca^{2+} channels to overall Ca^{2+} balance, and (4) the effects of Ca^{2+} level on the Ca^{2+}-dependent potassium channel conductance. This 'bi-directional' coupling was analyzed to address the questions summarized in the BIOLOGICAL SYSTEM AND EXPERIMENTAL DATA section above.

A key aspect of integration is the 'normalization' of the transport rates and currents in the model components based on the cytosolic volume and membrane surface area. This is because, each ion flux through the outer cell membrane produces an ionic current, contributing to intracellular ion concentrations and the membrane potential, and can be described in chemical or electrical form. Since neurons are highly variable in cell size, the cytosolic volume was calculated based on assumptions about the membrane surface that underlies the electrical model, and the assumption of a spherical soma. A correction of 16% was applied to account for the space claimed by the endoplasmatic reticulum (consistent with Mishra and Bhalla, 2002).

The signaling domain model was modified to consider the membrane potential as a dynamic state variable in the Na^{+}-Ca^{2+} exchanger description. The maximal conductance of K_{DR} channel is dependent on the state variables corresponding to active PKC and CaMKII via the phosphorylation model component detailed above.

The remaining key interaction is that of Ca^{2+} that is based on the release from cytosolic stores in the signaling

model and through ion channel currents in the electrical model. Simulations indicate that the Ca^{2+} level affecting the Ca^{2+}-dependent potassium current (K_{AHP}) has to be considered separately from the free cytosolic Ca^{2+}, as otherwise the large increase in cytosolic Ca^{2+} levels activates the hyperpolarizing potassium current of K_{AHP} beyond saturation and prevents any neuronal action potentials (firing rate = 0, i.e., zero excitability). However, the interplay between K_{AHP} and the voltage-dependent calcium current (Ca_L) underlies the firing frequency adaptation, a key feature of the neuronal behavior. In order to preserve the biologically realistic adaptation behavior and simultaneously avoid the 'zero excitability' problem, the Ca^{2+} in the integrated model was considered in a compartmental approach in which the K_{AHP} and Ca_L channels were assumed to be closely situated, and hence the resulting Ca^{2+} levels from the ion channels were buffered in the neuron and did not *directly* contribute to the cytosolic free Ca^{2+} level that participates in the signaling dynamics. The Ca^{2+} buffer and regulation model corresponding to the K_{AHP}-Ca_L cluster was adopted from Rybak et al. (1997).

The integrated model was implemented in MATLAB® (Mathworks, USA), contains 193 states, and was simulated using ODE15s solver for stiff systems.

Simulation results

A representative simulation in which a dynamic increase in neuronal firing rate was observed in response to stimulation with AngII (step increase from 0 to 100nM at 0s) is depicted in Figure 5. These results are in concordance with the experimental observations summarized earlier.

The relative contribution of PKC- and CaMKII-mediated phosphorylation of the K_{DR} ion channels was considered by simulating an '*in silico* blockade' of either phosphorylation by removing the corresponding reaction from the model (Figure 5). Such a scenario is biologically feasible when the phosphorylation sites of PKC and not CaMKII and vice versa, are unavailable in the modified forms of ion channels (e.g., gene mutations). Blocking PKC-dependent modulation significantly altered the response to result in a faster, but delayed, increase in the firing rate. This is due to the similar dynamic profile of CaMKII activation. However, blocking CaMKII-dependent phosphorylation had an effect on the overall 'gain' but not on the pattern of the neuronal adaptation dynamics. Blocking the modulation by both the kinases resulted in a slow and relatively marginal increase in the neuronal firing rate. However, this response is different from control behavior and indictes the interactions dependent on the non-voltage-gated ion transport mechanisms that are modulated by signaling dynamics.

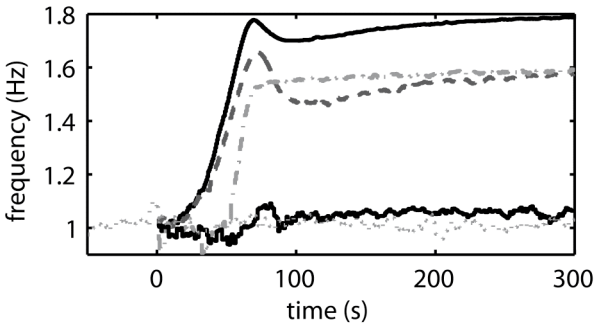

Figure 5. Response of the integrated model to 100 nM AngII stimulus. nominal response (upper solid line), phosphorylation by PKC is blocked (dashed), phosphorylation by CaMKII is blocked (dash-dotted), phosphorylation by PKC and CaMKII are blocked (lower solid line), no AngII stimulus (dotted). The nominal response is in concordance with experimental data and the different blockade results reveal relative contributions of PKC and CaMKII.

CONCLUSIONS

An integrated model of the signaling and electrophysiology of neurons was developed in the present study. This model was based on the available kinetics of Gq-protein-coupled receptor signaling to model response to AT1R activation by AngII, known ion channel components and the corresponding models based on Hodgkin-Huxley framework, and relies on experimentally motivated assumptions on the phosphorylation kinetics of ion channels to couple the two domains. This model exhibits an AngII-elicited increase in neuronal excitability and is in concordance with experimental observations on neuronal adaptation dynamics. One of the key hypotheses of the integrated model is the compartmentalization of the Ca^{2+} levels between the membrane ion channels and cytosol (with the two interacting via a buffer) without which the model cannot exhibit AngII-elicited increase in neuronal excitability. Analysis of the model for different cases in which PKC or CaMKII do not phosphorylate the delayed rectifier potassium channel indicated the key relative contribution of each of these kinases to the overall neuronal adaptation dynamics. In addition to the effect of the voltage-gated channels, the present model also indicates the role of the non-voltage-gated ion transport in the neuronal adaptation. These hypotheses form the basis for experimental validation of the key mechanisms and their contributions to the neuronal adaptation in the brain.

The present integrated model also forms the basis for subsequent application of systems-theoretic methods for analyzing the parametric sensitivity and time scales. Future studies could also extend this model to include the gene regulation network downstream of AT1R signaling to develop an integrated model that spans millisecond to multi-hour dynamics of neuronal modulation and adaptation.

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