Bistability in the Ca^{2+}/Calmodulin-Dependent Protein Kinase-Phosphatase System

Anatol M. Zhabotinsky
Department of Chemistry and Volen Center for Complex Systems, Brandeis University, Waltham, Massachusetts 02454-9110 USA

ABSTRACT A mathematical model is presented of autophosphorylation of Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) and its dephosphorylation by a phosphatase. If the total concentration of CaMKII subunits is significantly higher than the phosphatase Michaelis constant, two stable steady states of the CaMKII autophosphorylation can exist in a Ca^{2+} concentration range from below the resting value of the intracellular [Ca^{2+}] to the threshold concentration for induction of long-term potentiation (LTP). Bistability is a robust phenomenon, it occurs over a wide range of parameters of the model. Ca^{2+} transients that switch CaMKII from the low-phosphorylated state to the high-phosphorylated one are in the same range of amplitudes and frequencies as the Ca^{2+} transients that induce LTP. These results show that the CaMKII-phosphatase bistability may play an important role in long-term synaptic modifications. They also suggest a plausible explanation for the very high concentrations of CaMKII found in postsynaptic densities of cerebral neurons.

INTRODUCTION

Long-term potentiation (LTP) is the most studied experimental model of memory (Bliss and Collingridge, 1993; Huang et al., 1996; Malenka and Nicoll, 1999). Experimental data suggest that LTP relates to intermediate memory, which can be rewritten, as shown by phenomena of depotentiation and long-term depression (Linden, 1994; Bear and Malenka, 1994; Bear, 1996). Because memories of such type often consist of bistable elements, it is natural to ask whether some of the biophysical and biochemical systems, which participate in LTP, can operate as bistable switches. Lisman (1985) suggested that elements of the synaptic memory can use bistability of protein phosphorylation, and analyzed a bistable system that consisted of a protein kinase capable of intermolecular autocatalytic autophosphorylation and a phosphatase that dephosphorylates the kinase.

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) may be a key component of such a bistable molecular switch. It is known that autophosphorylation of CaMKII results in Ca^{2+}/CaM-independent (autonomous) kinase activity (Braun and Schulman, 1995; Hanson and Schulman, 1992; Soderling, 1995). During induction of LTP, short-term high elevations of Ca^{2+} concentration produce autophosphorylation of CaMKII that lasts 30 min or longer (Fukunaga et al., 1993, 1995; Miller and Kennedy, 1986; Ouyang et al., 1997). As long as CaMKII remains autophosphorylated it is able to phosphorylate the LTP related targets, such as AMPA receptors (Barria et al., 1997), even after the return of [Ca^{2+}] to its resting level (Lisman et al., 1997).

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Address reprint requests to Dr. Anatol M. Zhabotinsky, Department of Chemistry, Brandeis University, 415 South St., Waltham, MA 02454-9110. Tel.: 781-736-2531; Fax: 781-736-2516; E-mail:zhabotinsky@brandeis.edu.

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Although a crucial role of CaMKII autophosphorylation in induction of LTP is widely accepted, its participation in maintenance of LTP remains controversial (Giese et al., 1998; Kennedy, 1998; Lisman, 1994; Lisman et al., 1997). To prove the latter, it is necessary to show that a permanent level of autonomous activity of CaMKII lasts in the involved synapses as long as LTP does. Some indirect supporting evidence exists. Zhao et al. (1999) have found that a lasting increase in the in vivo phosphorylation level of CaMKII accompanies long-term memory in chicks. Also, it seems almost evident that long-term autophosphorylation of CaMKII should be connected with some specific structures in neurons. The basal autonomous CaMKII activity constitutes ~5% of the maximum activity in neurons, while it is only ~0.03% in the purified enzyme (Hanson and Schulman, 1992). This relatively high level of autonomous activity cannot be maintained in cytosol of neurons, where protein phosphatase activity is relatively high and essentially Ca^{2+}-independent (Strack et al., 1997). Thus, it takes only several minutes to dephosphorylate CaMKII added to cell extracts from the hippocampal CA1 region (Fukunaga et al., 2000). The postsynaptic densities (PSD) (Harris and Kater, 1994; Ziff, 1997) are the structures that could maintain high levels of CaMKII autophosphorylation because protein phosphatase activity in PSD is Ca^{2+}-dependent in vivo and should be rather low at the resting level of Ca^{2+} (Mulkey et al., 1994; Strack et al., 1997). Indeed, ~10% of CaMKII remained phosphorylated in the total PSD fraction when isolation was done in the presence of inhibitors of protein phosphatases (Strack et al., 1997).

In view of these data, it is of interest to determine whether CaMKII autophosphorylation can be bistable in a wide range of [Ca^{2+}] and whether such bistability can play a role in LTP.

Several mathematical models have been developed to study the dynamics of CaMKII autophosphorylation in response to variations of intracellular calcium concentration (Lisman and Goldring, 1988; Michelson and Schulman, 1994; Matsushita et al., 1995; Dosemeci and Albers, 1996;

Coomber, 1998; Kubota and Bower, 1999). They showed a strong dependence of the level of autophosphorylation on the amplitude and duration of Ca^{2+} pulses. Recently, Okamoto and Ichikawa (2000) have demonstrated that CaMKII autophosphorylation is bistable in their model when [Ca^{2+}] varies from 0.28 to 0.33 μM. This range is quite narrow and does not include the resting Ca^{2+} concentration.

Here, I develop a simple model of autophosphorylation of CaMKII in the presence of a phosphatase. I show that if the total concentration of CaMKII subunits is significantly higher than the phosphatase Michaelis constant, two stable steady states of CaMKII autophosphorylation can be found over a wide range of concentrations of the intracellular calcium. This range includes the resting Ca^{2+} concentration, and is wide enough to ensure that the background firing activity cannot induce transition from the low- to the high-phosphorylated states of CaMKII. I demonstrate that Ca^{2+} transients that induce such transitions are in the same range of amplitudes and frequencies as the Ca^{2+} transients that induce LTP. Moreover, concentrations of CaMKII, which are necessary to produce such a bistability, are shown to be of the same order of magnitude as the values found in the postsynaptic density. These results show that the CaMKII-phosphatase bistability can play an important role in the long-term synaptic modifications. They also suggest a plausible explanation for the very high concentrations of CaMKII found in postsynaptic densities of cerebral neurons.

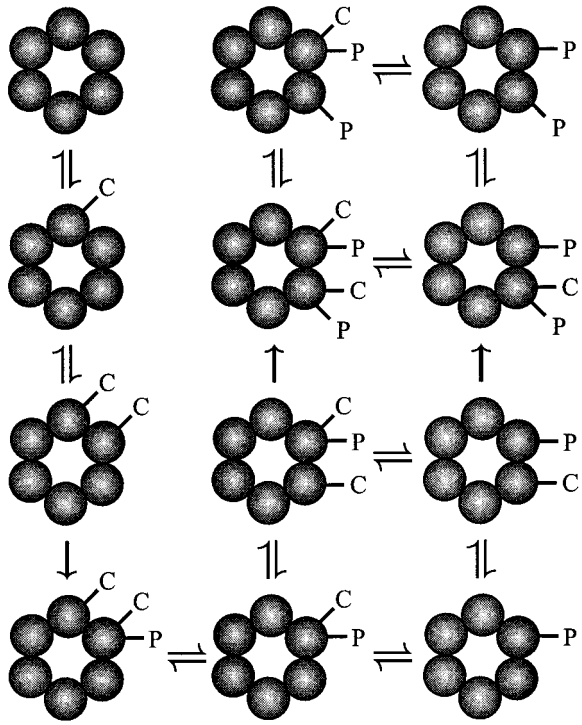

FIGURE 1 Schematic of the first two steps of autophosphorylation of CaMKII holoenzyme. C designates the Ca^{2+}/calmodulin complex, P is orthophosphate.

METHODS

Model

It is known that autophosphorylation of Thr^{286} in α-subunits of CaMKII results in Ca^{2+}/CaM-independent (autonomous) kinase activity (Hanson and Schulman, 1992). The autophosphorylation is an intraholoenzyme, intersubunit process (Hanson et al., 1994; Mukherji and Soderling, 1994). CaMKII consists of 8–12 subunits that form a petal structure (Hanson and Schulman, 1992). During autophosphorylation, one subunit acts as a catalyst and another as a substrate. Either binding of Ca^{2+}/calmodulin (Ca^{2+}/CaM) or phosphorylation of Thr^{286} is necessary for activation of the catalytic subunit. The substrate subunit must also bind Ca^{2+}/CaM to be phosphorylated (Hanson et al., 1994).

Here I use a model with many simplifications. I ignore any difference between α- and β-subunits. Binding of ATP to a subunit does not appear explicitly in the model. Binding of Ca^{2+}/CaM and phosphatase to a subunit is independent of the state of other subunits. Activation of subunits by Ca^{2+} obeys the Hill equation. Binding of phosphatase to a phosphorylated subunit and the rate of dephosphorylation is independent of binding of Ca^{2+}/CaM. I consider catalytic activities of the unphosphorylated subunit bound to Ca^{2+}/CaM and the phosphorylated subunit as being equal.

I use a model with asymmetric interaction of the neighbor subunits as proposed by Hanson and Schulman (1992). Essentially, their model is a ring along which autophosphorylation propagates in one direction. I use a model with 10 subunits in simulations and, in what follows, all the coefficients related to the number of subunits in a holoenzyme correspond to the 10-subunit model. Fig. 1 shows a schematic of the first two steps of autophosphorylation of a holoenzyme with six subunits. The left column presents initiation of autophosphorylation. After two neighbor subunits bind $(Ca^{2+})_4$CaM, designated C, the first subunit phosphorylates the second one in the clockwise direction. The following scheme corresponds to the initiation step:

$$4Ca^{2+} + CaM \rightleftarrows C \quad (1)$$

$$P_0 + C \rightleftarrows P_0C \quad (2)$$

$$P_0C + C \rightleftarrows P_0C_2 \quad (3)$$

$$P_0C_2 \rightarrow P_1C_2 \quad (4)$$

Here P_0 is the unphosphorylated holoenzyme and P_1 is the 1-fold phosphorylated holoenzyme. I describe the $(Ca^{2+})_4$CaM activation of subunits by the empirical Hill equation:

$$F = \frac{([Ca^{2+}]/K_{H1})^4}{1 + ([Ca^{2+}]/K_{H1})^4} \quad (5)$$

Here F is the fraction of subunits bound to $(Ca^{2+})_4$CaM and $K_{H1} = [Ca^{2+}]_{50}$ is the calcium Hill constant of CaMKII. The probability of $(Ca^{2+})_4$CaM binding to two neighbor subunits is low when [Ca^{2+}] is significantly less than K_{H1}. In this case, the rate of the initiation step is:

$$v_1 = \frac{10k_1([Ca^{2+}]/K_{H1})^8P_0}{(1 + ([Ca^{2+}]/K_{H1})^4)^2} \quad (6)$$

Here k_1 is the rate constant of Reaction 4, 10 is the statistical factor.

The middle and right columns in Fig. 1 present two routes of propagation of autophosphorylation. Autonomous activity of the CaMKII is usually 60–80% of the (Ca^{2+})/CaM-dependent activity (Hanson and Schulman, 1992). I assume here that catalytic activity of the autophosphorylated subunit does not depend on binding of $(Ca^{2+})_4$CaM. This means that I neglect

differences between the middle and right columns, and in this case the rate of propagation of autophosphorylation depends only on the following reactions:

$$P_1 + C \rightleftarrows P_1C \tag{7}$$

$$P_1C \rightarrow P_2 \tag{8}$$

Correspondingly, the rate of the second autophosphorylation step is:

$$V_2 = \frac{k_1([Ca^{2+}]/K_{H1})^4 P_1}{1 + ([Ca^{2+}]/K_{H1})^4} \tag{9}$$

Here k_1 is the rate constant of Reaction 8, which is the same as of Reaction 4.

In the absence of dephosphorylation, the subsequent steps of propagation of autophosphorylation are identical to the first one because there is always only one autophosphorylating pair in which the unphosphorylated subunit is adjacent to the phosphorylated neighbor in the clockwise direction. However, protein phosphatases dephosphorylate subunits at random. As a result, there is a random distribution of phosphorylated and unphosphorylated subunits in the holoenzyme. I assume that all distinguishable configurations of subunits in the holoenzyme with a given number of phosphorylated subunits exist with equal probabilities. In this case, the effective number of autophosphorylating pairs is:

$$w_i = w_{10-i} = \frac{\sum_1^i j m_j}{\sum_1^i m_j}, \tag{10}$$

where m_j is number of distinguishable configurations with j autophosphorylating pairs.

Values of w_i are: $w_1 = w_9 = 1.0$, $w_2 = w_8 = 1.8$, $w_3 = w_7 = 2.3$, $w_4 = w_6 = 2.7$, $w_5 = 2.8$.

Then, the rate of autophosphorylation of holoenzymes with i phosphorylated subunits is:

$$V_i = v_2 w_i P_i, \tag{11}$$

where

$$v_2 = \frac{k_1([Ca^{2+}]/K_{H1})^4}{1 + ([Ca^{2+}]/K_{H1})^4} \tag{12}$$

is the per-site rate of propagation of autophosphorylation.

Dephosphorylation of subunits proceeds according to the Michaelis-Menten scheme:

$$SP + E \rightleftarrows SPE \rightarrow E + S \tag{13}$$

Here S is an unphosphorylated subunit and SP is a phosphorylated one. Accordingly, the rate of dephosphorylation of holoenzymes with i phosphorylated subunits is:

$$V_{-i} = v_3 i P_i, \tag{14}$$

where

$$v_3 = \frac{k_2 e_p}{K_M + \sum_1^{10} i P_i} \tag{15}$$

is the per-subunit rate of dephosphorylation, e_p is the concentration of the active protein phosphatase, and k_2 and K_M are the catalytic and the Michaelis constants, respectively.

Four protein phosphatases dephosphorylate CaMKII-P: PP1, PP2A, PP2C (Strack et al., 1997), and a specific CaMKII phosphatase (Ishida et al., 1998; Kitani et al., 1999). According to Strack et al. (1997), activity of PP1 is 20% and PP2A is 60% of the total phosphatase activity in cytosol and, correspondingly, 50% and 8% in PSD. PP1 is the only protein phosphatase that dephosphorylates CaMKII in PSD according to Strack et al. (1997) and Yoshimura et al. (1999).

Activity of PP1 can be controlled by Ca^{2+}/CaM via inhibitor I, calcineurin (CaN), and cAMP-dependent protein kinase (PKA) (Shenolikar and Nairn, 1991; Mulkey et al., 1994). PKA phosphorylates inhibitor 1 (I1) and calcineurin dephosphorylates it (Shenolikar and Nairn, 1991). The Hill number for Ca^{2+} activation of CaN is ~3 (Stemmer and Klee, 1994). Phosphorylated inhibitor-1 (I1P) deactivates PP1 with $K_I = 1$ nM (Endo et al., 1996). The corresponding scheme is:

$$\begin{aligned}
I1 + PKA &\rightleftarrows I1 \cdot PKA \rightarrow I1P \\
3Ca^{2+} + CaM &\rightleftarrows C_3 \\
CaN + C_3 &\rightleftarrows CaN \cdot C_3 \\
I1P + CaN \cdot C_3 &\rightleftarrows I1P \cdot CaN \cdot C_3 \rightarrow I1 \\
SP + PP1 &\rightleftarrows SP \cdot PP1 \rightarrow PP1 + S \\
PP1 + I1P &\rightleftarrows PP1 \cdot I1P \\
SP \cdot PP1 + I1P &\rightleftarrows SP \cdot PP1 \cdot I1P
\end{aligned} \tag{16}$$

I assume that the concentration of free I1 is constant and much less than K_M of PKA, and the concentration of free I1P is much less than K_M of CaN.

The complete model of autophosphorylation of CaMKII in the presence of Ca^{2+}-dependent PP1 is:

$$\begin{aligned}
\frac{dP_0}{dt} &= -v_1 + v_3 P_1 \\
\frac{dP_1}{dt} &= v_1 - v_3 P_1 - v_2 P_1 + 2v_3 P_2 \\
\frac{dP_2}{dt} &= v_2 P_1 - 2v_3 P_2 - 1.8v_2 P_2 + 3v_3 P_3 \\
\frac{dP_3}{dt} &= 1.8v_2 P_2 - 3v_3 P_3 - 2.3v_2 P_3 + 4v_3 P_4 \\
\frac{dP_4}{dt} &= 2.3v_2 P_3 - 4v_3 P_4 - 2.7v_2 P_4 + 5v_3 P_5 \\
\frac{dP_5}{dt} &= 2.7v_2 P_4 - 5v_3 P_5 - 2.8v_2 P_5 + 6v_3 P_6 \\
\frac{dP_6}{dt} &= 2.8v_2 P_5 - 6v_3 P_6 - 2.7v_2 P_6 + 7v_3 P_7 \\
\frac{dP_7}{dt} &= 2.7v_2 P_6 - 7v_3 P_7 - 2.3v_2 P_7 + 8v_3 P_8 \\
\frac{dP_8}{dt} &= 2.3v_2 P_7 - 8v_3 P_8 - 1.8v_2 P_8 + 9v_3 P_9 \\
\frac{dP_9}{dt} &= 1.8v_2 P_8 - 9v_3 P_9 - v_2 P_9 + 10v_3 P_{10} \\
\frac{dP_{10}}{dt} &= v_2 P_9 - v_3 10 P_{10} \\
\frac{de_p}{dt} &= -k_3 I e_p + k_4(e_{p0} - e_p) \\
\frac{dI}{dt} &= -k_3 I e_p + k_4(e_{p0} - e_p) + v_{PKA} I_0 - \frac{v_{CaN}([Ca^{2+}]/K_{H2})^3 I}{1 + ([Ca^{2+}]/K_{H2})^3}
\end{aligned} \tag{17}$$

Here P_i is the concentration of the i-fold phosphorylated CaMKII holoenzyme, e_p is the concentration of PP1 not bound to I1P, e_{p0} is the total concentration of PP1, I is the concentration of free I1P, and I_0 is the concentration of free I1; v_1, v_2, and v_3 are given by the rate Eqs. 6, 12, and 15; k_3 and k_4 are, respectively, the association and dissociation rate constants of the PP1 · ·I1P complex; $V_{CaN} = V_{CaN}/K_{M2}$, V_{CaN} is the activity,

K_{M2} is the Michaelis constant, and K_{H2} is the Hill constant of CaN, $v_{PKA} = V_{PKA}/K_{M3}$; V_{PKA} and K_{M3} are the activity and the Michaelis constant of PKA.

During propagation of autophosphorylation I neglect the initiation terms because the probability of $(Ca^{2+})_4$CaM binding to two neighbor subunits is very low when $[Ca^{2+}]$ is significantly less than $[Ca^{2+}]_{50}$.

I use a model with a Ca^{2+}-independent protein phosphatase in some simulations. Inhibitor 1 is washed out during isolation of PSD. To simulate suggested in vitro experiments with isolated PSD, I set $I_0 = 0$ in Eq. 17. This results in $e_p = e_{p0}$, and is equivalent to elimination of the two last equations in Eq. 17 and treating e_p as a parameter in the remaining equations. I also use this truncated model to simulate kinetics of autophosphorylation and dephosphorylation of CaMKII in cytosol, where 80% of the phosphatase activity is Ca^{2+}-independent, and I can neglect the Ca^{2+} dependence of the remaining 20%. Table 1 gives the values of parameters used in the simulations.

Calcium signaling

In the time-dependent simulations, I use Ca^{2+} dynamics generated by a simple protocol for induction of LTP, which is a single tetanus with frequencies of excitation varying from 5 to 100 Hz, and duration in the range from 1 to 1800 s (Bliss and Collingridge, 1993; Huang et al., 1996). Ca^{2+} response to a single depolarization pulse is modeled by an instant elevation and the following exponential decay. I assume a simple summation of Ca^{2+} pulses during periodic excitation (Helmchen et al., 1996):

$$[Ca^{2+}] = [Ca^{2+}]_{rest} + A \sum_{1}^{n} \exp(-i/(f\tau)) \qquad (18)$$

Here $[Ca^{2+}]_{rest}$ is the resting concentration of Ca^{2+}, A is the amplitude of a single Ca^{2+} pulse, f is the frequency of excitation, τ is the relaxation time of Ca^{2+} decay, and n is the number of pulses in the tetanus. I use the following values of the parameters: $[Ca^{2+}]_{rest}$ is 0.1 μM, τ is 0.2 s (Helmchen et al., 1996; Magee and Johnston, 1997; Majewska et al., 2000). I take A equal to 0.4 μM to simulate background firing. To induce LTP, EPSPs and action potentials must coincide in time. In this case, amplitudes of the single Ca^{2+} pulses become rather large (Yuste et al., 1999). I use A equal to 1.0 μM to simulate induction of LTP.

RESULTS

Fig. 2 shows how the steady-state values of the total concentration of phosphorylated subunits of CaMKII depend on the concentration of Ca^{2+}. In the shaded area of the region of bistability, three steady states correspond to each $[Ca^{2+}]$. The top and bottom steady states are stable and the middle one is unstable. Arrows show directions of evolution of the system in time at constant concentrations of Ca^{2+}. A is the static $[Ca^{2+}]$-threshold for down-switching, and B is the up-switching static threshold. These thresholds are boundaries of the bistability region.

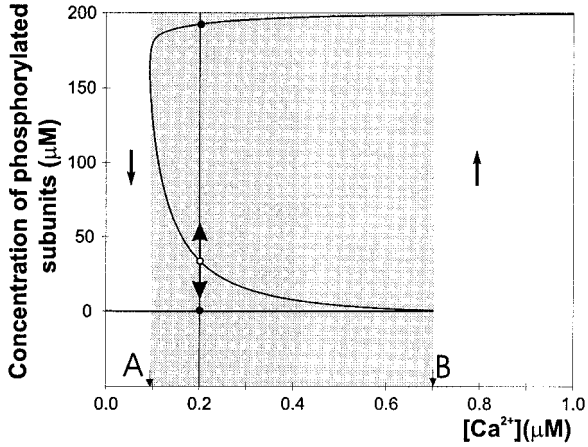

FIGURE 2 Bistability of the steady-state concentrations of phosphorylated subunits of CaMKII. In the shadowed bistability region, the top and bottom branches of the steady-state curve consist of the stable steady states (●), while the middle branch consists of the unstable steady states (○). Arrows show directions of evolution of the system in time at constant concentrations of Ca^{2+}. A is the static $[Ca^{2+}]$-threshold for down-switching, and B is the up-switching static threshold. Parameters (in μM): $K_{H2} = 0.7$, $K_M = 0.4$, $e_k = 20.0$, $e_{p0} = 0.05$, $I_0 = 0.1$.

Fig. 3 shows how boundaries of the region of bistability depend on various parameters of the system. Fig. 3 *A* displays the bistability domain in the plane concentration of CaMKII (e_k) versus $[Ca^{2+}]$ at two values of K_M. One can see that the bistability region shrinks considerably when e_k decreases. Also, when K_M increases, the right boundary of domain shifts significantly to the left, while the left bound-

TABLE 1 Parameters used in the model (Eq. 17)

Parameter	Symbol	Value	Units	Reference
Total concentration of CaMKII	e_k	0.1–30*	μM	Strack et al., 1997
Total concentration of protein phosphatase	e_{p0}	0.01–1.2*	μM	This paper
Concentration of free inhibitor 1	I_0	0.0, 0.1*	μM	This paper
Activity of calcineurin divided by its Michaelis constant	v_{CaN}	1.0	s^{-1}	This paper
Activity of PKA divided by its Michaelis constant	v_{PKA}	1.0	s^{-1}	This paper
The Michaelis constant of protein phosphatase	K_M	0.4–20*	μM	This paper
The Ca^{2+} activation Hill constant of CaMKII	K_{H1}	4.0	μM	Fährmann et al., 1998; De Koninck and Schulman, 1998
The Ca^{2+} activation Hill constant of calcineurin	K_{H2}	0.3–1.4*	μM	Stemmer and Klee, 1994
The catalytic constant of autophosphorylation	k_1	0.5	s^{-1}	Hanson et al., 1994
The catalytic constant of protein phosphatase	k_2	2.0	s^{-1}	This paper
The association rate constant of the PP1 · I1P complex	k_3	1.0	$\mu M^{-1}s^{-1}$	Endo et al., 1996; This paper
The dissociation rate constant of the PP1 · I1P complex	k_4	1.10^{-3}	s^{-1}	Endo et al., 1996; This paper

*Specific values are given in the figure legends.

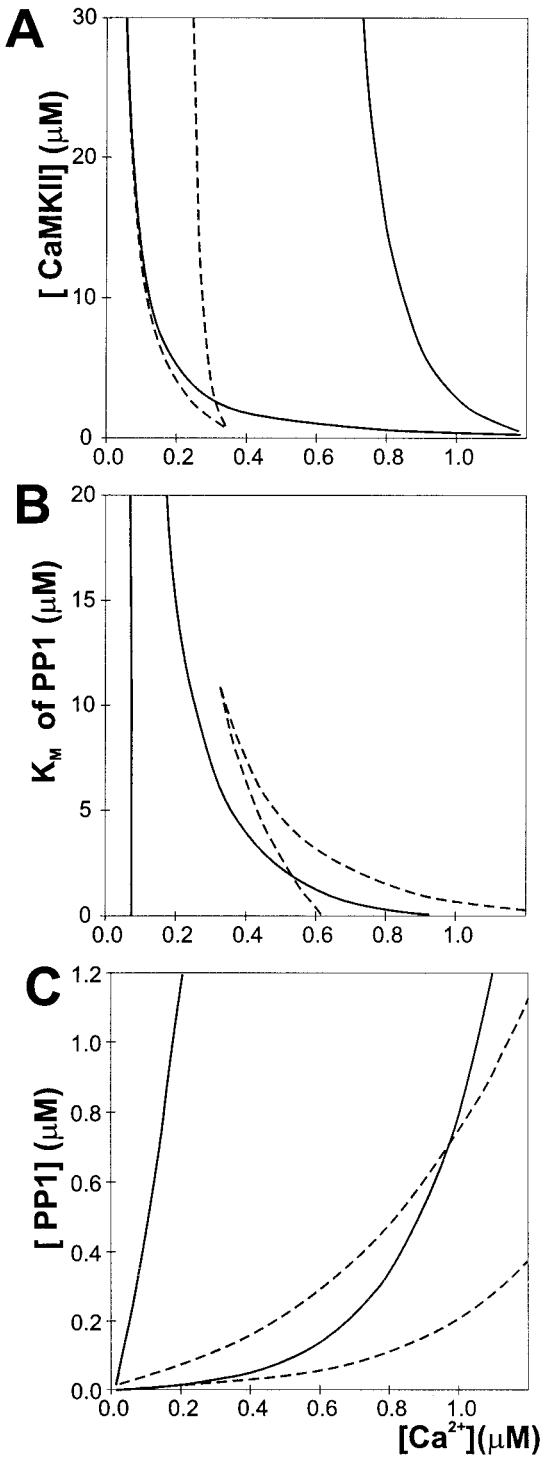

FIGURE 3 Effect of parameters on size and position of the bistability domain. The lines on left correspond to the down-switching thresholds and the lines on right correspond to the up-switching threshold as shown in Fig. 2. Parameters (in μM): $K_{H2} = 1.4$, $I_0 = 0.1$. (*A*) The (e_k, [Ca^{2+}])-plane($e_{p0} = 0.3$ μM): *solid lines*, $K_M = 0.4$ μM; *broken lines*, $K_M = 10.0$ μM. (*B*) The (K_M, [Ca^{2+}])-plane ($e_{p0} = 0.3$ μM): *solid lines*, $e_k = 20.0$ μM; *broken lines*, $e_k = 1.0$ μM. (*C*) The (e_{p0}, [Ca^{2+}])-plane ($K_M = 0.4$ μM): *solid lines*, $e_k = 20.0$ μM; *broken lines*, $e_k = 1.0$ μM.

ary moves only slightly at low values of e_k. Fig. 3 *B* shows in more detail how the region of bistability diminishes when K_M increases. If e_k equals 1 μM, bistability vanishes when K_M is larger than 12 μM. Fig. 3 *C* shows positions of the bistability domain in the plane concentration of PP1 (e_{p0}) versus [Ca^{2+}] at two values of e_k. The domain boundaries shift to the right when e_{p0} increases or e_k decreases. Fig. 3 demonstrates that e_k must exceed 10 μM, and K_M must be significantly lower than 1 μM to obtain a bistability range that includes the resting value of the intracellular [Ca^{2+}] and is wide enough to prevent induction of LTP by random fluctuations of Ca^{2+} concentration.

Fig. 4 *A* shows positions of the bistability domain in the plane (e_{p0}, [Ca^{2+}]) at three values of the calcium Hill constant of calcineurin (K_{H2}). One can see that activity of PP1 has to decrease with decreasing K_{H2} in order to obtain a proper Ca^{2+} range of bistability; if K_{H2} equals 1.4 μM, e_{p0} must be ~0.3 μM, if K_{H2} equals 0.7 μM, e_{p0} must be ~0.05 μM, and if K_{H2} equals 0.3 μM, e_{p0} must be below 0.01 μM. Fig. 4 *B* shows the bistability domain in the plane: (e_{p0}, K_{H2}) at the resting Ca^{2+} concentration equal to 0.1 μM. It demonstrates that the domain is quite large at $e_k = 20$ μM and becomes very small when $e_k = 1.0$ μM.

Fig. 5 shows kinetics of autophosphorylation during tetanic excitation. During the 10 Hz tetanus (Fig. 5 *A*), the average concentration of Ca^{2+} is well below K_{H1}, and it takes almost 60 s to reach the level of total autophosphorylation of 70 μM, which eventually leads to transition to the top steady state. At this set of parameters, 15 min of excitation at 5 Hz cannot induce transition from the bottom to the top steady state. Fig. 5 *B* demonstrates that the system needs 1 s to reach the level of 92 μM during the 100 Hz tetanus, when the constant component of [Ca^{2+}] is 19.5 μM.

In both cases shown in Fig. 5, the concentration of autophosphorylated subunits continues to rise after the end of excitation, but very slowly. At the resting concentration of Ca^{2+}, the system is almost "frozen" because activity of CaMKII is extremely low. Fig. 6 *A* demonstrates that it takes >500 days to reach the stationary level of 186 μM. However, brain in vivo is not in a steady state, a permanent background firing (BF) is always present, and affects almost every neuron. I model BF by a permanent periodic firing with a low amplitude and frequency of 5 Hz, which corresponds to the major Fourier component of BF found in vivo (Karnup, 1996). Fig. 6 *B* shows that BF greatly accelerates the approach to the top phosphorylated state, which now takes only ~12 h. At the same time, this BF is unable to switch the system from the low to high states of autophosphorylation, but only insignificantly elevates the concentration of phosphorylated subunits in comparison with the low steady-state level. Fig. 6 *B* also demonstrates that BF can maintain the system in the top phosphorylated state even if the Ca^{2+} threshold for down-switching (0.15 μM in this case) is above the resting Ca^{2+} concentration (0.1 μM).

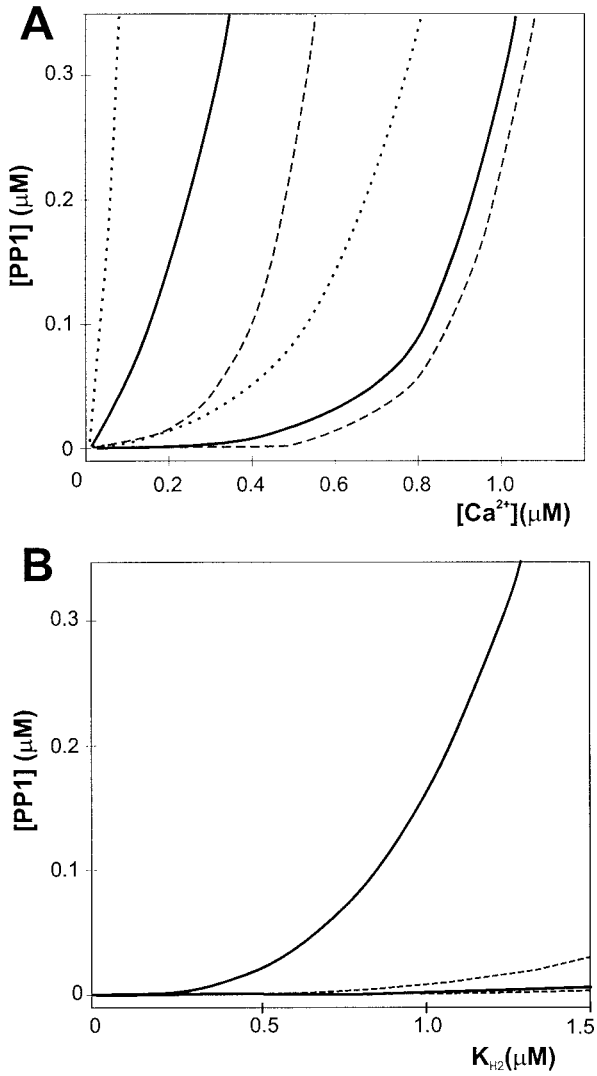

FIGURE 4 Dependence of the bistability domain on the calcium Hill constant of calcineurin and concentration of PP1. Parameters (in μM): $K_M = 0.4$, $I_0 = 0.1$. (*A*) Effect of K_{H2} on position of the bistability domain in the (e_{p0}, [Ca^{2+}])-plane: $e_k = 20.0$; *dotted lines*, $K_{H2} = 1.4$ μM; *solid lines*, $K_{H2} = 0.7$ μM; *broken lines*, $K_{H2} = 0.3$ μM. (*B*) The bistability domains in the (e_{p0}, K_{H2})-plane at the resting Ca^{2+} concentration equal 0.1 μM: *solid lines*, $e_k = 20.0$ μM; *broken lines*, $e_k = 1.0$ μM.

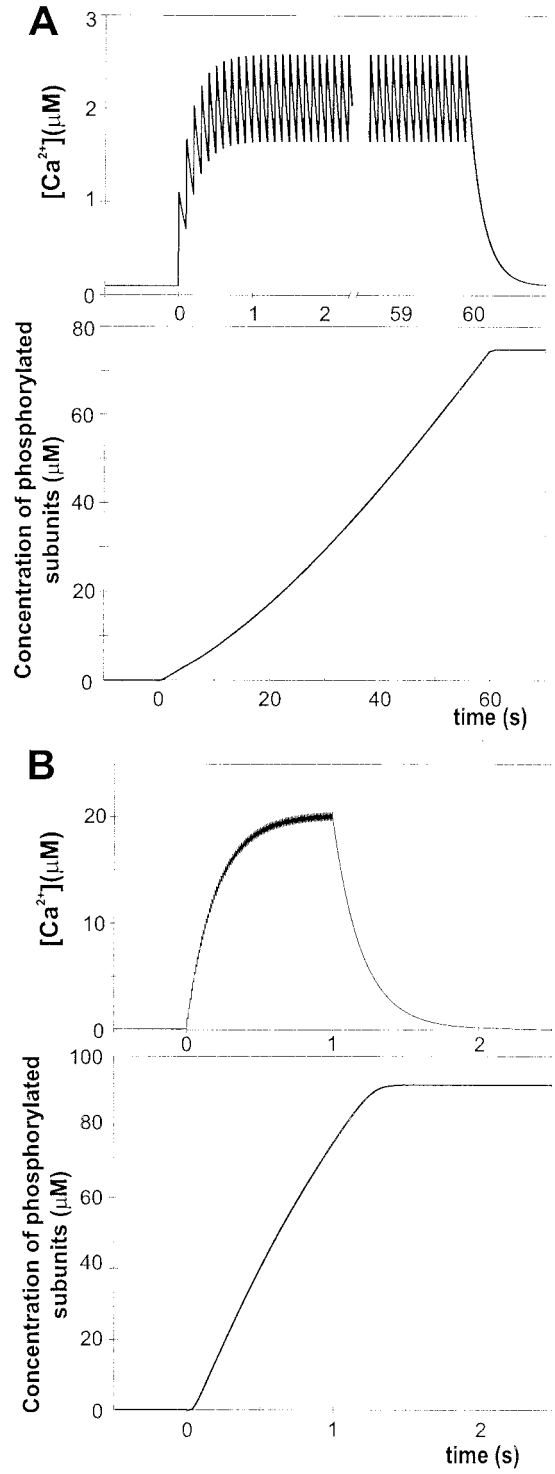

FIGURE 5 Kinetics of autophosphorylation during tetanic excitation. Parameters (in μM): $K_M = 0.4$, $e_k = 20.0$, $I_0 = 0.1$. (*A*) The 10 Hz, 60 s tetanus; the top plot shows the Ca^{2+} transient, the bottom plot displays the total concentration of phosphorylated subunits versus time; parameters (in μM): $K_{H2} = 1.4$, $e_{p0} = 0.3$. (*B*) The 100 Hz, 1 s tetanus; parameters (in μM): $K_{H2} = 0.7$, $e_{p0} = 0.1$. Initial conditions correspond to the low steady state at [Ca^{2+}] = 0.1 μM.

The dynamic up-switching threshold of autophosphorylation depends on amplitude and duration of Ca^{2+} transients and can be characterized by an amplitude-duration function analogous to the classical strength-duration curve for the firing threshold (Noble and Stein, 1966). However, there have been only a few attempts to directly control amplitude and duration of the intracellular Ca^{2+} transients in neurons (Yang et al., 1999). In most of the experiments, the frequency and duration of stimulation are the controlled parameters during induction of LTP (Bliss and Collingridge, 1993). Fig. 5 demonstrates that the shape of Ca^{2+} transients varies greatly with the frequency and duration of stimula-

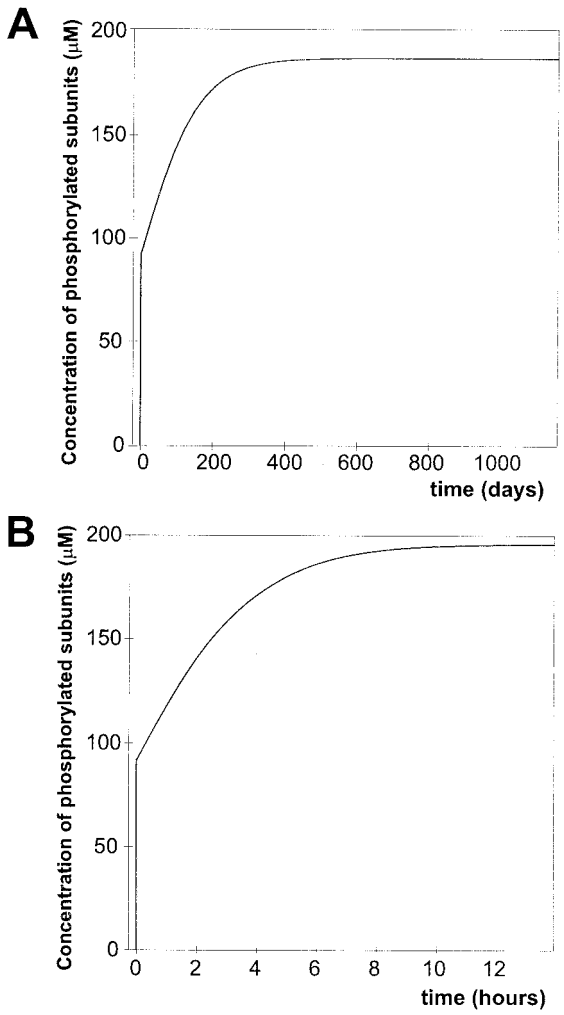

FIGURE 6 Kinetics of transition to the stationary high-phosphorylated state after tetanic excitation. (*A*) Autophosphorylation in the autonomous model ($e_{p0} = 0.04$ μM). (*B*) Acceleration of transition in the model with background firing ($e_{p0} = 0.1$ μm). Parameters (in μM): $K_{H2} = 0.7$, $K_M = 0.4$, $e_k = 20.0$, $I_0 = 0.1$. Initial conditions correspond to the low steady state at $[Ca^{2+}] = 0.1$ μM.

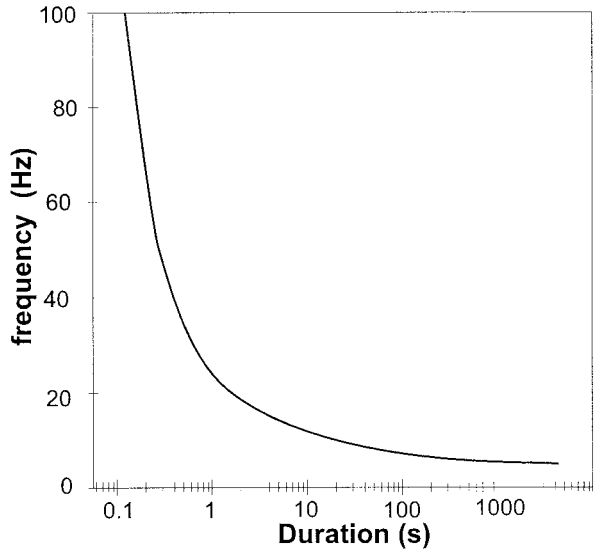

FIGURE 7 The dynamic up-switching threshold plotted in the plane frequency versus duration of tetanic stimulation. Parameters (in μM): $K_{H2} = 1.4$, $K_M = 0.4$, $e_k = 20.0$, $e_{p0} = 0.3$, $I_0 = 0.1$.

tion. Therefore, it is more convenient to plot the threshold curve directly in the frequency-duration plane (Fig. 7). One can see that with decreasing frequency duration of the threshold tetanus increases sharply; the duration threshold is ~0.12 s at 100 Hz, and ~25 min at 5 Hz.

Fig. 8 demonstrates how mutations can affect bistability in our model. Substituting alanine in place of Thr^{35} eliminates inhibitor 1 phosphorylation by PKA and its phosphatase inhibitor activity (Endo et al., 1996). In this case, the term v_{PKAI0} is equal to zero in the model. Fig. 8 *A* shows that the bistability region shifts toward higher concentrations of Ca^{2+}, and the down-switching static threshold now becomes 1.26 μM. Fig. 8 *B* demonstrates that after the end of 100 Hz, 1 s tetanus, the systems drops to the low-phosphorylated state within one hour, contrary to behavior of the "wild type" system shown in Fig. 6 *B*.

Fig. 9 illustrates feasible in vitro experiments that could confirm the existence of such bistability. These can be done with isolated PSD, where I1 is washed out and PP1 becomes Ca^{2+}-independent (Strack et al., 1997; Yoshimura et al., 1999). Fig. 9 *A* shows the steady-state curve of the system with I_0 equal to zero. The system moves along a loop of hysteresis, when $[Ca^{2+}]$ gradually changes. When $[Ca^{2+}]$ increases, the system moves along the ABCDE path; when $[Ca^{2+}]$ decreases, the path is EDFBA. Fig. 9 *B* demonstrates the dynamics of transitions between steady states, which follow sub and superthreshold step-wise changes in $[Ca^{2+}]$. When $[Ca^{2+}]$ jumps from 1.3 μM to 1.8 μM, the concentration of autophosphorylated subunits increases only slightly (*A*), because the system remains on the bottom branch of the curve shown in Fig. 9 *A*; when $[Ca^{2+}]$ jumps from 1.3 μM to 2.2 μM, the system transits to the top steady state (*B*). When $[Ca^{2+}]$ is switched from 2.3 μM to 1.8 μM, the system moves to a new steady state on the top branch of the curve (*C*); when $[Ca^{2+}]$ jumps from 2.3 μM to 1.5 μM, the system transits to the bottom branch of the curve (*D*).

Finally, I show that the model correctly describes kinetics of autophosphorylation of CaMKII in cytosol of neurons. In cytosol, at least 80% of the protein phosphatase activity is Ca^{2+}-independent, the principal phosphatase is PP2A, and the concentration of CaMKII is one order of magnitude lower than in PSD (Strack et al., 1997). Fig. 10 displays behavior of the model with the Ca^{2+}-independent phosphatase and parameters that correspond to the cytosolic system. Fig. 10 *A* demonstrates that bistability is absent in this case. Fig. 10 *B* shows that during the 100 Hz, 1 s tetanus, the concentration of phosphorylated subunits reaches ~4.6 μM, and drops below 0.4 μm in <10 min.

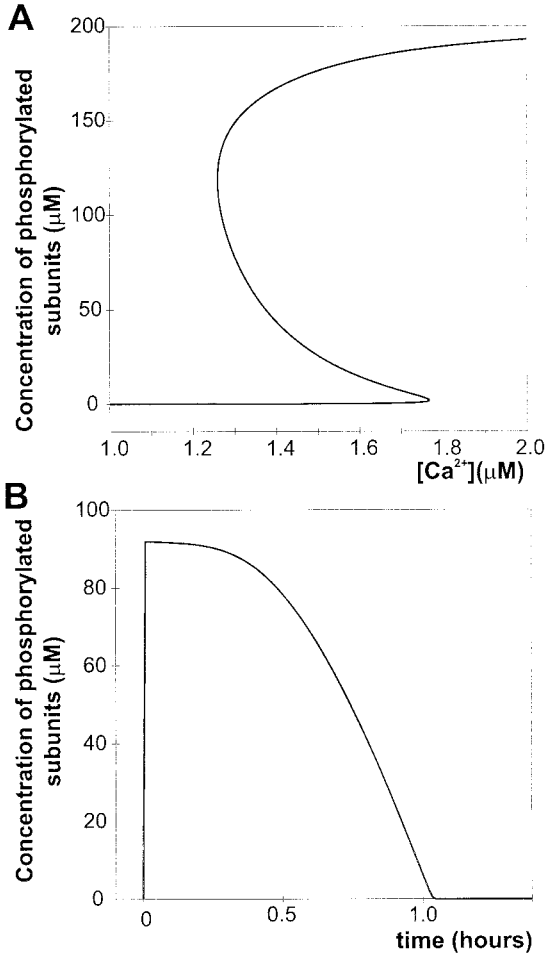

FIGURE 8 Dynamics of autophosphorylation in the system with a mutant inhibitor 1 ($v_{PKA} = 0.0$). (*A*) The steady-state characteristic of the system. (*B*) Kinetics of autophosphorylation induced by 100 Hz, 1 s excitation in the model with background firing. Parameters (in μM): $K_M = 0.4$, $e_k = 20.0$, $e_{p0} = 0.1$. Initial conditions correspond to the low steady state at $[Ca^{2+}] = 0.1$ μM.

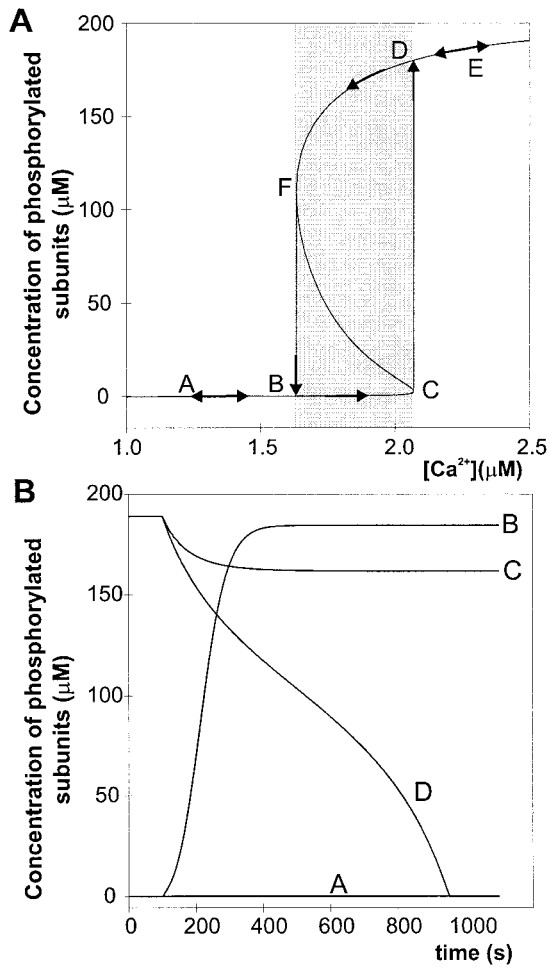

FIGURE 9 Hysteresis and switching in the system with the Ca^{2+}-independent protein phosphatase in vitro. (*A*) The steady-state curve with the hysteretic loop (BCDF). Arrows show direction of movement when $[Ca^{2+}]$ slowly increases or decreases (*B*) Transients after sub- and superthreshold shifts of $[Ca^{2+}]$: *A*, the systems remains on the bottom branch of the bistability curve when $[Ca^{2+}]$ jumps from 1.3 μM to 1.8 μM; *B*, when $[Ca^{2+}]$ jumps from 1.3 μM to 2.2 μM, the system transits to the top steady state; *C*, when $[Ca^{2+}]$ is switched from 2.3 μM to 1.8 μM, the system moves to a new steady state on the top branch of the curve; *D*, $[Ca^{2+}]$ jumps from 2.3 μM to 1.5 μM, and the system transits to the bottom branch of the curve. Parameters (in μM): $K_M = 0.4$, $e_k = 20.0$, $e_{p0} = 0.3$, $I_0 = 0.0$.

DISCUSSION

Recently, all-or-none potentiation was found at CA3-CA1 synapses in hippocampus (Petersen et al., 1998). This bistability of synaptic strength may depend on bistability of phosphorylation of participating receptors and anchoring proteins, which, in turn, may depend on bistability of activity of a major protein kinase at the resting $[Ca^{2+}]$. Bistability of autonomous activity of CaMKII has been analyzed previously. However, Lisman (1985) did not consider the effect of Ca^{2+}, and Lisman and Goldring (1988) assumed propagation of autophosphorylation to be Ca^{2+}-independent. Okamoto and Ichikawa (2000) have found bistability at Ca^{2+} concentrations that are significantly higher than the resting $[Ca^{2+}]$ and did not study bistability in the vicinity of the latter.

My simulations show that two stable steady states of autophosphorylation of CaMKII can arise in the Ca^{2+} concentration range that includes the resting $[Ca^{2+}]$ if the concentration of CaMKII is very high and the phosphatase activity is Ca^{2+}/calmodulin-dependent. These conditions are found in PSD. In cytoplasm, the concentration of CaMKII is relatively low and the protein phosphatase activity is essentially Ca^{2+}-independent. In this case, according to the model, the bistability region is either shifted strongly to the right of the resting concentration of Ca^{2+}, or absent, and the phosphorylated fraction of CaMKII should decay to a very low level after concentration of Ca^{2+} drops to the resting value. The last result corresponds to the experimental observations. Thus, according to the model, high levels of autophosphorylation can be maintained at the resting con-

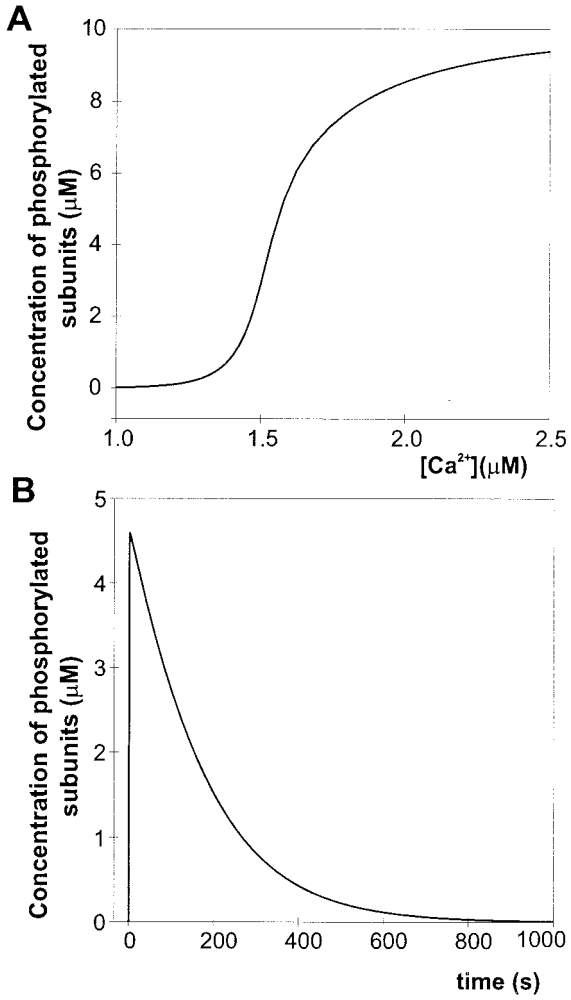

FIGURE 10 Dynamics of autophosphorylation of CaMKII in cytoplasm ($k_3 = 0.0$). (*A*) The steady state characteristic is single valued. (*B*) Kinetics of autophosphorylation induced by 100 Hz, 1 s tetanus. Parameters (in μM): $K_M = 15.0$, $e_k = 1.0$, $e_{p0} = 0.05$. Initial conditions correspond to the low steady state at $[Ca^{2+}] = 0.1$ μM.

centration of Ca^{2+} only in PSD. The model also shows that stimulation protocols, which induce LTP, switch the system from the low-phosphorylated to the high-phosphorylated state, while background firing is unable to switch. However, background firing can maintain the system in the top phosphorylated state even if the Ca^{2+} threshold for down-switching is above the resting Ca^{2+} concentration.

The existence of bistability of the CaMKII autophosphorylation in PSD can be tested in in vitro experiments. If one would maintain isolated PSDs in a medium with ATP and gradually change the Ca^{2+} level in both directions, hysteresis should be observed, approximately as shown in Fig. 9 *A*. The addition of inhibitor 1 should shift the bistability region to lower concentrations of Ca^{2+} and make it wider.

Experiments with transgenic animals can provide evidence that such bistability plays a role in the maintenance of LTP. It is known that substituting alanine in place of Thr^{35} eliminates inhibitor 1 phosphorylation by PKA and its phosphatase inhibitor activity (Endo et al., 1996). According to the model, such a mutation should prevent autophosphorylation of CaMKII at resting $[Ca^{2+}]$ (Fig. 8); that could down-regulate or eliminate LTP.

Bistability is a robust phenomenon in this model. It persists even when any parameter in the model varies by an order of magnitude, or more. However, the parameters significantly affect the position and extent of the Ca^{2+} range of bistability (Figs. 3 and 4 *A*). Where possible, I use parameter values estimated from available experimental data.

Values of $[Ca^{2+}]_{50}$ from 0.7 to 4 μM have been reported for Ca^{2+}/calmodulin activation of CaMKII (Fährmann et al., 1998; Gupta et al., 1992; Kennedy et al., 1983; Kuret and Schulman, 1984). De Koninck and Schulman (1998) have shown that the dissociation constant of Ca^{2+}/calmodulin from the unphosphorylated α-subunit of CaMKII is $\sim$0.08 μM. One can estimate that $[Ca^{2+}]_{50}$ varies from 2 to 8 μM, when the concentration of free calmodulin varies from 40 to 0.2 μM (Huang et al., 1981; Cohen and Klee, 1988). I have chosen $K_{H1} = 4$ μM.

The Hill number for Ca^{2+} activation of calcineurin is 3 ± 0.1, and $[Ca^{2+}]_{50}$ can vary from 0.6 to 1.4 μM, when Ca^{2+}/calmodulin activates purified calcineurin in the presence of 6 μM of Mg^{2+}. When $[Mg^{2+}]$ decreases, $[Ca^{2+}]_{50}$ also decreases (Stemmer and Klee, 1994). I ran simulations with K_{H2} equal to 0.3, 0.7, and 1.4 μM.

The Michaelis constants of PP1 for phosphoproteins vary from 0.21 to 6.8 μM (Johansen and Ingebritsen, 1987), and from 3.8 to 31 μM for catalytic subunit of PP2A (Bialojan and Takai, 1988). I could not find direct data for dephosphorylation of CaMKII. I assume that the Michaelis constants of these phosphatases with CaMKII as a substrate are of the same order of magnitude as for the other phosphoproteins.

The average concentration of the α-subunits of CaMKII is $\sim$10 μM in forebrain according to Erondu and Kennedy (1985), McNeil and Colbran (1995), and Strack et al. (1997). Our estimation of this concentration in PSD is 80 μM on the basis of data published by Suzuki et al. (1994), and 200 μM according to data presented by Strack et al. (1997). The total phosphatase activity toward CaMKII in homogenates of hippocampus is ~ 0.1 $\mu M \cdot s^{-1}$ (Fukunaga et al., 2000).

I chose the value of k_1 according to Hanson et al. (1994). I made reasonable estimates of parameters involved in the Ca^{2+} regulation of the protein phosphatase activity. It is worth mention that the results of simulations are insensitive to absolute values of these parameters as long as they are not too small, and their ratios are kept constant.

Here I use a rather simplified model. The scheme of autophosphorylation is strictly valid only if $[Ca^{2+}]$ is significantly less than K_{H1}. However, $[Ca^{2+}]$ becomes much higher than K_{H1} during high-frequency stimulation. For assurance, I have run several simulations with an extended

model that included additional terms in the autophosphorylation scheme. The difference in results was <10%.

I also neglect any binding of the CaMKII holoenzymes to PSD. However, the accumulation of CaMKII in PSD is a result of such binding. It was shown that CaMKII can bind to the C-terminal tails of subunits of the NMDA receptor in PSD (Gardoni et al., 1999; Leonard et al., 1999; Strack and Colbran, 1998). To be kept in PSD, CaMKII must be either autophosphorylated (Gardoni et al., 1999; Strack and Colbran, 1998; Strack et al., 1997; Yoshimura et al., 1999), or bound to Ca^{2+}/CaM (Shen and Meyer, 1999). One can see that these requirements are the same as for the CaMKII activation. This means that the catalytic and autoinhibitory domains of the CaMKII subunits must dissociate before binding to PSD, and suggests that one of these domains binds the NMDA receptor or another anchor protein. There are two variants, if only one subunit participates in binding. If the catalytic site remains active in the bound state and can interact with its neighbor in the clockwise direction, the bound subunit will become the initiation point of autophosphorylation. As a result, autophosphorylation can proceed faster in comparison with the unbound holoenzyme. However, if the catalytic site becomes incapacitated, the rate of autophosphorylation will decrease approximately twofold. In these two cases, there is a moderate quantitative change in the dynamics of the system due to the binding, and the results remain valid. However, autophosphorylation is severely hindered if the holoenzyme binds two or more anchor sites. In this case, the bistability region shifts to much higher concentrations of Ca^{2+}, and the physiological importance of bistability becomes doubtful.

The very high concentration of CaMKII in PSD can be a result of translocation of CaMKII from cytosol during high elevations of $[Ca^{2+}]$ (Shen and Meyer, 1999; Strack et al., 1997). The preliminary simulations with a two-compartment model show that this translocation affects the characteristics of bistability only if the Ca^{2+}-dependence of CaMKII activity is quite different in cytoplasm and PSD.

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