Neuromodulation of Dendritic Action Potentials

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Hoffman, Dax A. and Daniel Johnston. Neuromodulation of dendritic action potentials. J. Neurophysiol. 81: 408–411, 1999. The extent to which regenerative action potentials invade hippocampal CA1 pyramidal dendrites is dependent on both recent activity and distance from the soma. Previously, we have shown that the amplitude of back-propagating dendritic action potentials can be increased by activating either protein kinase A (PKA) or protein kinase C (PKC) and a subsequent depolarizing shift in the activation curve for dendritic K⁺ channels. Physiologically, an increase in intracellular PKA and PKC would be expected upon activation of β-adrenergic and muscarinic acetylcholine receptors, respectively. Accordingly, we report here that activation of either of these neurotransmitter systems results in an increase in dendritic action-potential amplitude. Activation of the dopaminergic neurotransmitter system, which is also expected to raise intracellular adenosine 3’,5’-cyclic monophosphate (cAMP) and PKA levels, increased action-potential amplitude in only a subpopulation of neurons tested.

INTRODUCTION

Action potentials back-propagating into the dendrites of CA1 neurons of adult animals become progressively smaller in amplitude the farther they travel from the soma and may even fail to propagate beyond distal branch points. This decrement in action-potential amplitude is found in vivo and in vitro in both the hippocampus and neocortex (Andreasen and Lambert 1995; Buzsáki et al. 1996; Callaway and Ross 1995; Kamondi et al. 1998; Magee and Johnston 1997; Spruston et al. 1995; Svoboda et al. 1997; Tsubokawa and Ross 1997; Turner et al. 1991). Although the physiological function of a decrementing action potential is unclear, we have previously reported that a high density of transient K⁺ channels is largely responsible for this phenomenon in the hippocampus, because blocking the channels with 4-aminopyridine greatly increases the dendritic action-potential amplitude (Hoffman et al. 1997). More recently, we have shown that in the distal dendrites, action-potential amplitude can be boosted with either protein kinase A (PKA) or protein kinase C (PKC) activation through downregulation of the K⁺ channels via a depolarizing shift in their activation curve. An increase in intracellular PKA and PKC can be accomplished through the activation of several neurotransmitter systems. We report here the amplification of antidromically stimulated back-propagating action potentials in the distal dendrites of CA1 hippocampal neurons by activation of three neurotransmitter systems, the adrenergic, cholinergic, and dopaminergic systems.

METHODS

Hippocampal slices (400 μm thick) from Sprague-Dawley rats, 5–8 wk old, were prepared as described previously (Hoffman and Johnston 1998). Recordings were made at 31–35°C using an automatic temperature controller (Warner Instrument, Hamden, CT). The following were included in the external solution (as described in Hoffman and Johnston 1998) to block synaptic transmission (in μM): 50 dl-2-amino-5-phosphonovaleric acid (dl-APV; RBI), 20 MK-801 (RBI), 10 6-cyano-7-nitroquinocinaline-2,3-dione (CNQX; RBI), 10 bicuculline (Sigma), and 10 picROTOXIN (Sigma). Isoproterenol (1–2 μM, Sigma), carbachol (1 μM, Sigma), 6-Cl-PB (10 μM, Sigma), and H7 (300 μM, Sigma) were dissolved directly into the bath solution with the addition of 1 μM ascorbate. Whole cell recording pipettes (8–14 MΩ) were filled with (in mM) 120 KMeSO₄, 20 KCl, 10 N-2-hydroxethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 0.2 ethylene glycol-bis(β-aminoethyl)ether-N,N,N’,N’-tetraacetic acid (EGTA), 2 MgCl₂, 4 Na-ATP, 0.3 tris(hydroxymethyl) aminomethane guanosine triphosphate (Tris-GTP), and 14 phosphocreatine (pH 7.25 KOH) and coated with silicone elastomer (Sylgard). All neurons exhibited a resting membrane potential between −55 and −75 mV. Series resistance was 25–60 MΩ. Antidromic action potentials were stimulated every 7–20 s by 0.1- to 0.2-ms constant current pulses (Neurolog, Digitimer) through tungsten electrodes (AM Systems) placed in the alveus. Traces are averages of 5–15 sweeps. All recordings were made between 180 and 320 μm from the soma. Significance (P < 0.05) was determined by a two-sample t-test. Error bars represent SE.

RESULTS

β-Adrenergic and cholinergic receptor activation increases dendritic action-potential amplitude

In our previous report we found that activation of either PKA or PKC increases dendritic action-potential amplitude through downregulation of dendritic K⁺ channels. In the present study we wished to test the hypothesis that these kinases can be activated by neurotransmitter systems under physiological conditions and augment back-propagating action potentials. To investigate this possibility, antidromically initiated action potentials were recorded in the distal apical dendrites of CA1 pyramidal neurons before and after bath application of various neurotransmitter receptor agonists. We first looked at possible modulation by the β-adrenergic receptor agonist isoproterenol. In all 15 cells tested, we found that application of 1–2 μM isoproterenol resulted in a significant increase in dendritic action-potential amplitude by an average of 56 ± 12% (mean ± SE; Fig. 1). In a dendritic recording 220 μm from the soma shown in Fig. 1A, a 42-mV action potential doubled in amplitude with isoproterenol application. The effect could be reversed upon wash out of isoproterenol and recovered with a second appli-
Isoproterenol application produced a kinase-dependent increase in dendritic action-potential amplitude. A: bath application of 1 μM isoproterenol resulted in a 104% increase in amplitude, from 41 ("Pre") to 84 mV, of an antidromically initiated action potential recorded 220 m from the soma. Wash out of isoproterenol brought the action potential back down to preisoproterenol amplitude (38 mV, "Wash"). With a 2nd application of isoproterenol (dark arrow, "Iso"), the amplitude again increased 2-fold to 80 mV. Three such experiments were performed to show that the effect of isoproterenol was reversible and that a 2nd application would lead to a similar increase in amplitude. B: in a different recording 300 μM from the soma, isoproterenol again doubled action-potential amplitude from 25 to 50 mV. In this experiment, 300 μM H7, a generic kinase inhibitor was included in the control saline during the wash out of isoproterenol. The subsequent 2nd application of isoproterenol failed to lead to a 2nd increase in amplitude (dark arrow, "Iso + H7"). C: summary data. Average percent change in action-potential amplitude and maximal rate of rise are plotted for both the isoproterenol and isoproterenol plus H7 experiments. Isoproterenol alone led to an average increase in action-potential amplitude of 56 ± 12% with a 8 ± 10% change in the rate of rise. In the experiments where the second isoproterenol application was accompanied by H7, no increase in action-potential amplitude was found (−5 ± 4% with a 3 ± 12% change in the rate of rise). In all isoproterenol experiments cells were held near their resting potential (−70 mV). The average preisoproterenol amplitude was 29 mV. The number of cells for each group is in parentheses. Asterisks denote a significant percent increase.

To increase intracellular PKC levels, we chose the acetylcholine receptor agonist carbachol. Qualitatively similar to the isoproterenol experiments, we found that cholinergic activation by bath application of 1 μM carbachol increased dendritic action-potential amplitude in all eight cells tested (Fig. 2, A and C). The average magnitude of the increase, however, was smaller than in the isoproterenol experiments (average, 19 ± 9%; Fig. 2C). In these experiments the cell was hyperpolarized to −80 mV to remove residual Na+ channel inactivation (Colbert et al. 1997; Jung et al. 1997), which has been shown to be decreased by PKC activation (Colbert and Johnston 1998). Under these conditions, the
increase in action-potential amplitude was not accompanied by an increase in rate of rise and thus most likely is due to the downregulation of transient K⁺ channels (Fig. 2C). In experiments without the prior hyperpolarization, however, carbachol increased both the amplitude and the rate of rise, suggesting a mixed action on transient K⁺ channels and Na⁺ channel inactivation (data not shown).

D1/D5 dopamine receptor activation has heterogeneous effects in different neurons

Another neurotransmitter known to act through PKA activation is dopamine (Kebabian and Calne 1979). We thus tested the effect of the D1/D5 dopamine receptor agonist 6-Cl-PB on dendritic action potentials. In 6 of 10 cells we found that 10 μM 6-Cl-PB increased action-potential amplitude by an average of 28 ± 8% (Fig. 2B). In the other four cells, however, no significant change in amplitude occurred up to 20 min after application (average, 0 ± 4%). Averaging all 10 experiments together resulted in a significant 17 ± 7% increase in amplitude with no change in the rate of rise (Fig. 2C). The negative results in 4 of 10 cells could mean that either a subpopulation of dendrites lack D1/D5 receptors or that they are clustered such that, in some cells, their activation does not allow for the critical amount of PKA activation needed to increase action-potential amplitude. The recording location does not seem to be the source of variation because 6-Cl-PB application in recordings from the same location in different cells still produced variable results.

DISCUSSION

The results presented here illustrate that dendritic action-potential amplitude can be increased by activation of three different neurotransmitter systems: the β-adrenergic, cholinergic, and dopaminergic systems. Previously, we have shown an increase in distal dendritic action-potential amplitude by activation of either PKA or PKC, suggesting that the neurotransmitter effects are due to activation of these kinases. The effect on action-potential amplitude most likely occurs through the downregulation of dendritic transient K⁺ channels via a depolarizing shift in their activation curve (Hoffman and Johnston 1998). Several lines of evidence support this conclusion. First, the increase in amplitude is found only in distal dendrites where action-potential amplitude is attenuated by a high density of transient K⁺ channels (Hoffman et al. 1997). Second, the increase in amplitude is not associated with a significant increase in the rate of rise, suggesting that the effect is not due to an increase in Na⁺ conductance. Third, similar increases in spike amplitude were found for both PKA and PKC, which both produce a ~15-mV depolarizing shift in the transient channel activation curve. Finally, a modeling study has found that even a 5-mV shift in the K⁺ channel’s activation curve can increase action-potential amplitude in CA1 dendrites (M. Migliore, D. A. Hoffman, J. C. Magee, and D. Johnston, unpublished observations).

In addition to limiting the back-propagation of action potentials into the dendrites, we have also reported that dendritic transient K⁺ channels act to reduce the amplitude of synaptic potentials and inhibit dendritic action-potential initiation (Hoffman et al. 1997). The question arises as to why neurons go through the energetically costly step of expressing a high density of K⁺ channels in distal dendrites to limit dendritic excitability instead of simply excluding Na⁺ channels. One hypothesis is that there are circumstances where larger amplitude action potentials and synaptic potentials are vital to neuronal function. The present study demonstrates a physiologically relevant case through which the attenuating influence of dendritic K⁺ channels could be relieved. Neuromodulatory inputs into the distal dendrites could act to increase synaptic potential amplitudes, increase the likelihood of dendritic Na⁺ or Ca²⁺ action-potential initiation, direct action potentials to active regions of the dendrite, or simply increase action-potential amplitude at the site of synaptic input. Such changes in dendritic signals may be particularly important in the CA1 region during cholinergically driven theta activity (Huerta and Lisman 1995).

The Ca²⁺ influx associated with back-propagating action potentials sharply increases with depolarization given the steep Ca²⁺ channel activation curve found in distal dendrites (Magee and Johnston 1995). Back-propagating action potentials also unblock N-methyl-D-aspartate receptors leading to supralinear increases in internal Ca²⁺ (Schiller et al. 1998; Yuste and Denk 1995). Thus even the modest increases in action-potential amplitude reported here could lead to large increases in the associated Ca²⁺ influx. The Ca²⁺ influx associated with a back-propagating action potential may be crucial in the induction of synaptic plasticity (Christie et al. 1996; Magee and Johnston 1997; Markram et al. 1997), and the amount of influx may determine the degree and direction of changes in synaptic strength (Schexnayder et al. 1997).

The results reported here are in agreement with field studies that found that isoproterenol increases population spike amplitude (Dunwiddie et al. 1992). In a recent report looking at the effect of carbachol on trains of dendritic action potentials, no change in the amplitude of the first spike in a train on carbachol application was reported (Tsubokawa and Ross 1997). There are a number of methodological differences, however, including the age of animals used, recording temperatures, and initial membrane potentials. The most likely explanation for the different results is the difference in initial action-potential amplitude between the two studies (~60 mV vs. 29 ± 2 mV in our experiments). The larger initial amplitude suggests a smaller initial K⁺ current, which would then have less effect if further reduced by neuromodulation.

Stratum radiatum of the rat hippocampus contains high levels of both muscarinic and β-adrenergic receptors (Adem et al. 1997; Booze et al. 1993; Levey et al. 1991). Neither D1 nor D5 dopamine receptors, however, are found in high density in this region in the rat (Dawson et al. 1986; Meadow-Woodruff et al. 1992). The reliable increase in action-potential amplitude reported for the β-adrenergic and cholinergic but not dopaminergic systems appears to reflect these differences in receptor density.

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