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Control of Na^+ Spike Backpropagation by Intracellular Signaling in the Pyramidal Neuron Dendrites

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Abstract

The integrative function of neurons depends on the somato-dendritic distribution and properties of voltage-gated ion channels. Sodium, potassium, calcium, and hyperpolarization-activated cyclic nucleotide-gated K^+ (HCN) channels expressed in the dendrites can be modulated by a number of neurotransmitters and second-messenger systems. For example, activation of protein kinases leads to an increase in dendritic excitability by removing a slow inactivation of Na^+ channels and decreasing the activity of transient K^+ channels in the apical dendrites of hippocampal pyramidal neurons. Consequently, action potentials propagating along the dendrites can be modified significantly by a variety of neuromodulatory synaptic inputs.

Index Entries: Dendrites; excitability; pyramidal neurons; backpropagation; intracellular signaling; G protein; protein kinase; calcium.

Introduction

In the central nervous system (CNS), neuronal excitability is precisely controlled by a variety of ion channels, pumps, and transporters. During this past decade, many intensive studies have examined the intracellular signaling pathways controlling excitability. These studies revealed that membrane-associated proteins are highly regulated by a number of neurotransmitters and second-messenger systems. Activation of metabotropic receptors such as metabotropic glutamate receptors, muscarinic acetylcholine receptors, and adrenergic receptors can affect the voltage-dependence, the speed of gating, and the probability of opening of various ion channels at the final steps of their signal cascades. Of particular interest are mechanisms underlying the control of excitability in the dendrites, because dendrites are the primary locus of synaptic integration and plasticity. Historically, modulatory signals and their effects on ion channels were studied by using several cell lines and/or sympathetic ganglion cells. Recent advances in experimental techniques such as dendritic

patch-clamp and high-speed fluorescence imaging allow direct access to dendrites in central neurons. Moreover, combining these techniques with molecular biological methods, including the use of mutant animals, became popular in the past few years. We now know that several kinds of voltage-sensitive channels are found in the dendrites of cortical and hippocampal pyramidal cells *(1)*, cerebellar Purkinje cells *(2,3)*, midbrain dopaminergic cells *(4)*, and thalamo-cortical relay neurons *(5)*, although only indirect evidence is available about specific channels in the fine branches *(6,7)*. Accumulating evidence indicates that the dendrites of neurons in the CNS no longer just passive structures for synaptic inputs, but are active units for information processing. This review examines how intracellular signals affect activities of voltage-gated ion channels and how these modulatory systems can alter excitability. Particular emphasis is placed on results concerning the dendrites of hippocampal CA1 pyramidal neurons. To evaluate the excitability of dendrites, this review focuses on characteristics of antidromic invasion of action potentials, such as activity- and distance-dependent attenuation of spike amplitude in the apical dendrites. Findings obtained from pyramidal neurons discussed here may provide a framework for studying many of other less accessible neurons.

Voltage-Gated Ion Channels in the Apical Dendrites and Their Modulation

Many of the electrophysiological experiments on neurons have been done under the assumption that all kinds of ion channels present in the soma are also expressed on the dendrites. This assumption is probably not true in cortical and hippocampal pyramidal neurons. Specific channel types and their distributions have recently been intensively examined using whole-cell and cell-attached recordings from the soma and the apical dendritic arbor. Although recordings are limited to dendrites with a diameter of 1 μm or more, differences in the kinetics of dendritic channels and their uniform or non-uniform distributions have been shown along the dendritic tree.

Na^+ Channels

Voltage-gated Na^+ channel density in the dendrites increases with maturation, especially from 2–8 wk of age and becomes approximately uniform across the soma and at least the first three-fourths of the dendrites *(8)*. This dendritic Na^+ channel has both fast and slow inactivation modes, which are characterized by a fast recovery rate (on the order of 10 ms or less) and by a slow recovery rate (more than 100 ms), respectively. Na^+ channels in the dendrites exhibit a remarkably long recovery time following inactivation *(9,10)*. Recently, Mickus et al. *(11)* found that there is a gradual increase in the magnitude of slow inactivation with distance, reaching a maximum at about 200 μm from the soma. The distribution follows roughly an exponential relationship with a space constant of 126 μm. The author's explanation for this result is not clear. Whether intracellular mechanisms contribute to these kinetic differences might be worth testing. Colbert and Johnston *(12)* reported that application of phorbol esters (10 μ*M*), activators of protein kinases including protein kinase C (PKC), decreases the slow recovery from inactivation of Na^+ channels in the dendrites. This result seems to be in contrast with other findings *(13–15)* that pharmacological activation of PKC decreases peak Na^+ current and slows inactivation of the Na^+ channels in the soma. Colbert and Johnston used a short depolarizing pulse (2 ms) to activate the Na^+ channels instead of the relatively long test pulses (>10 ms), which were used by other groups to analyze inactivating phase of the Na^+ currents. If the channels examined are functionally identical, one possible explanation for this controversy is that PKC may facilitate the transition of a Na^+ channel from open state to a closed state by bypassing inactivation. Another possi-

bility is that different Na^+ channel subunits are differentially distributed across the axonal-dendritic axis. Qu et al. *(16)* reported that coexpression of the β1 subunit accelerated inactivation of type IIA Na^+ currents in Xenopus oocytes, suggesting that subunit composition and its modulation could affect the kinetics of Na^+ currents. It is also possible that the modulatory effect of PKC is isozyme subtype-specific. Little is known about isozyme-specific action of PKC. Recently Yanagida et al. *(17)* reported that both PKCα and PKCε down-regulate Na^+ channels in chromaffin cells.

There are several reports that persistent (non-inactivating) Na^+ currents can be recorded from neocortical and hippocampal pyramidal neurons *(18,19).* In neocortical neurons, persistent Na^+ currents exist in the apical dendrites and contribute to amplification of excitatory synaptic input *(20,21).* Whether this current is due to activation of specific "persistent" Na^+ channels is still unclear. However, a study using cell lines expressed type IIA Na^+ channels and G protein subunits demonstrates that βγ subunits of G proteins directly bind to the C-terminal domain of the Na^+ channel and induce persistent Na^+ currents by stabilizing a gating mode *(22).* For further understanding of the physiology of dendritic Na^+ channels precise histological studies demonstrating the subcellular localization of the Na^+ channel subunits might be needed.

Ca^{2+} Channels

The total density of Ca^{2+} channels also appears to be uniform along dendrites of hippocampal pyramidal neurons, although the relative density of specific types of Ca^{2+} channels differs between soma and dendrites *(8).* Whole-cell and cell-attached patch recordings demonstrate that multiple types of Ca^{2+} channels exist in the soma of hippocampal neurons *(23).* Imaging of Ca^{2+} signals at the dendrites were performed in hippocampal pyramidal neurons to identify the voltage-gated Ca^{2+} channel subtypes in the dendrites *(24,25).* The results indicated that L-type (high-voltage activated, dihydropyridine sensitive) and N-type (high-voltage activated, ω-conotoxin-GVIA sensitive) channels mainly contribute to Ca^{2+} entry into the soma and first 50 μm of the apical dendrite. On the other hand, Ca^{2+} entry into more distal sites is primarily due to T-type (low-voltage activated, Ni^{2+} sensitive) and R-type (high-voltage activated, Ni^{2+} sensitive). The conclusions are generally supported by studies of Ca^{2+}-channel activities obtained from whole-cell *(26)* and dendrite-attached patches *(8).* However, Kavalali et al. *(27)* demonstrated significant contributions of N- and P/Q-type (high-voltage activated, ω-agatoxin-IVA sensitive) Ca^{2+} currents in hippocampal 'dendrosomes' (isolated dendrites). This result agrees well with immunocytochemical studies of Ca^{2+} channel subunits *(28,29).* Because many of the different types of Ca^{2+} channels show similar electrophysiological properties, and because there may be the possibility of age-dependent alterations in the relative abundance of Ca^{2+} types, these classifications may change in the future.

The best-studied system for modulation of voltage-gated Ca^{2+} channels is the G-protein coupled cascade *(30–34).* Although most of the early studies have been done using sympathetic ganglion cells, down-regulation of N- (and probably also P/Q-) type channels by α subunit of $G_{q/11}$ protein and βγ subunits of G_i (and/or G_o) protein, and upregulation of L-type channels by the α subunit of G_s protein appear to be common in various neurons *(35).* Moreover, facilitation of L-type channel activity by Ca^{2+}/CaM-dependent protein kinase II (CaMKII) has been reported *(36).* However, little is known about modulatory mechanisms for dendritic Ca^{2+} channels, except for a report showing that G proteins directly inhibit dendritic Ca^{2+} channels as they do in the soma *(37).* Recently, Delmas et al. *(38)* performed whole-cell and cell-attached patch recordings from both the soma and dendrites of sympathetic neurons and found that the dendrites express N-type channels that have enhanced interactions with Gβγ compared with the somatic counterparts. Inhibition of dendritic

N-type channels by activation of dendritic G protein-coupled receptors occurs more effectively than inhibition of N-type channels in the soma by activation of somatic G protein-coupled receptors. Although immunohistological staining has successfully shown the distribution of Ca^{2+} channels in the dendrites the structure of Ca^{2+} channel subunits and the composition of dendritic Ca^{2+} channels might be different from those of somatic channels. Therefore, it is possible that Ca^{2+} channels expressed in the dendrites may have unique regulatory mechanisms.

K^+ Channels

Transient K^+ channels have somewhat non-uniform distributions along pyramidal neuron dendrites. Hoffman et al. *(39)* reported that the density of transient A-type channels increases fivefold along the first three-fourths of the apical dendrites of hippocampal CA1 pyramidal neurons, although the density of delayed rectifier K^+ channels appears to be fairly uniform. In addition, the transient K^+ current in dendrites was activated near the resting membrane potential of dendrites, and it showed a fast activation and a moderate, voltage-dependent inactivation. Recently, the properties and somatodendritic distribution of voltage-gated K^+ channels in neocortical pyramidal neurons were systematically analyzed *(40,41).* The density of A-type channels in layer 5 neurons increases threefold across the proximal half of these apical arbors. Although the gradient of the channel density as a function of distance from the soma does not look steeper than that in the hippocampal pyramidal neurons because of the difference in total length of the apical arbors, it is similar to that seen in CA1 dendrites. The gradient of the delayed rectifier was shown to be constant for the same range as was shown in CA1 dendrites. Because it is known that both layer 5 pyramidal neurons and hippocampal CA1 neurons show attenuation of backpropagating action potentials, the kinetics and non-uniform distribution of A-type channels might be good explanations for spike attenuation occurring in pyramidal dendrites.

It is well-known that there are many types of K^+ channels that are regulated by G protein-coupled receptors for transmitters and hormones *(42).* On the other hand, intracellular regulation of voltage-gated K^+ channels has not been recognized widely throughout the CNS, although inhibition of A-currents has occasionally been reported in cultured preparations *(43,44).* However, at least in the channels expressed in the dendrites of hippocampal pyramidal neurons, cyclic AMP (cAMP)-dependent protein kinase A (PKA), PKC and mitogen-activated protein kinase (MAPK) systems seem to control activities of A-type K^+ channels *(45,46).* Moreover, modulation of Kv4 channels by PKC in ventricular preparations *(47),* and inhibition of delayed rectifier K^+ currents by activation of CaMKII in cultured cells *(48)* were recently reported. Exploration of the modulation of dendritic A-type K^+ channels by second messengers has been done intensively by Johnston's group *(49).* The activation threshold for the transient channels in the dendrites shifts some 10–15 mV more negative than those in the soma and proximal dendrites. This shift is abolished by bath application of 8-bromo-cAMP, phorbol dibutirate (PDBu), or phorbol 12,13-diacetate (PDA) and makes the activation curves of these dendritic channels very similar to those in the soma. These effects clearly involve activation of kinases because effects of these kinase activators are reversed by the subsequent application of the kinase inhibitor H7, and application of inactive phorbol 4-alpha appears to have no effect. By contrast, the activation curve of the sustained K^+ current in the dendrites is not significantly different from that in the soma, and appears unaffected by 8-bromo-cAMP or phorbol esters *(39).* The MAPK inhibitors PD 098059 and U0126-20 both produced a significant negative shift of the activation curve *(45),* suggesting that MAPK is constitutively active in dendrites. Existence of constitutively active MAPK in the dendrites has also been reported from biochemical studies *(50).* Although activation

of both PKA and PKC can lead to activation of MAPK in neurons, further analysis will be required to know how these kinases work in combination. A recent report by Colbert and Pan *(51)* demonstrates that arachidonic acid (AA) blocks transient K^+ channels including A-type channels and augments sustained outward currents in the apical dendrites of hippocampal CA1 pyramidal neurons.

For local control of dendritic excitability possible contributions of other types of K^+ channels such as Ca^{2+}-activated K^+ channels *(52)* are suggested because of their nonuniform distribution along the dendritic arbor, although these channels do not seem to contribute to repolarization of dendritic-action potentials.

I_h Channels

Expression of nonselective cation "hyperpolarization-activated cyclic nucleotide-gated K^+ (HCN) channels" is widely accepted *(53).* The subcellular distributions of these channels are not clear yet. However, the predominant distribution of HCN channels in the apical dendrites of hippocampal and neocortical pyramidal neurons has recently been suggested by three electrophysiological studies *(54–56).* I_h (the net current passing through HCN channels) has been recorded from the apical dendrites under cell-attached patch clamp. The current density in the most distal regions is nearly seven-fold larger than that in the soma. Fluorescent imaging of intracellular Na^+ also showed that hyperpolarization increases intracellular Na^+ concentration ($[Na^+]_i$) probably by Na^+ influx associated with I_h, and that fractional changes in $[Na^+]_i$ in response to hyperpolarization at the dendrites are larger than that at the soma *(57).*

Kinetics of the HCN channels are regulated by cAMP and by cyclic GMP *(58).* Cyclic AMP shifts the activation curve of HCN channels in a depolarized direction *(59,60),* and this modulation seems to be induced in a PKA-independent manner. Pharmacological activation of cAMP production in hippocampal pyramidal neurons enhanced I_h without activation of PKA *(61).* Because an increase in amount of the cAMP could, in turn, induce activation of PKA in physiological conditions, it might be interesting to explore the interaction between activity of A-type transient K^+ channels and that of HCN channels in dendrites.

Modulation of Backpropagating Action Potentials by Intracellular Signaling

Because voltage-gated channels in the dendrites are responsible for dendritic excitability, transmitters and modulators whose receptors are coupled to the intracellular signaling system could control excitability of the dendrites through their signal cascades. This raises the question as to what kinds of receptor activation could change dendritic excitability, and how can we monitor the changes? In the past few years, it has become apparent that in neocortical and hippocampal pyramidal neurons, Na^+-dependent action potentials initiated near the soma propagate not only to the axon but also to the apical (and probably to the basal) dendrites *(62).* This backward propagation of spikes is thought to play an important role in associative synaptic plasticity by providing depolarizations that induce large Ca^{2+} entry *(63,64).* The generation and propagation of these action potentials reflects the properties and distributions of various types of voltage-gated ion channels in the axon, soma, and dendrites of the neurons. Therefore, characteristics of backpropagating action potentials, such as activity- and distance-dependent attenuation of spike amplitude, might be useful in evaluating the excitability of dendrites.

Cholinergic Modulation

The medial-septal nucleus and ventral-limb nucleus of the diagonal band collectively provide the cholinergic projections to the hippocampus *(65).* The spatial distribution and subcellular localization of muscarinic acetyl-

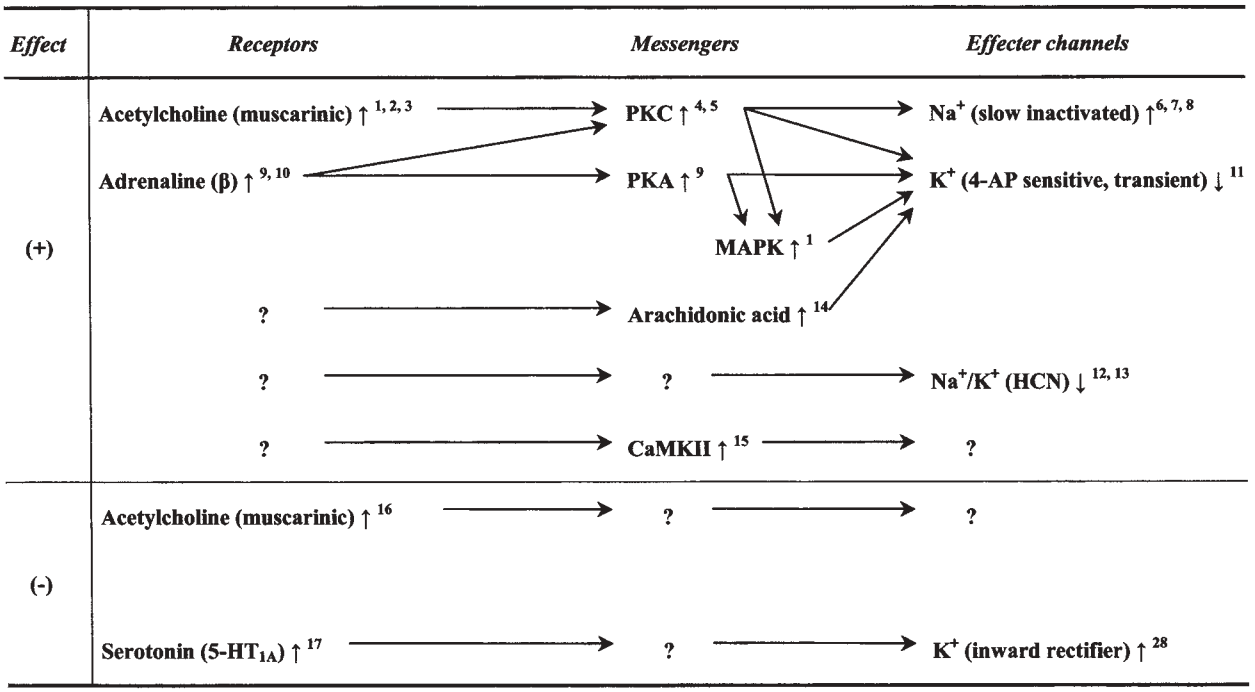

Fig. 1. Possible intracellular signals modulating the backpropagation of action potentials into the apical dendrites of hippocampal CA1 neurons. Upward and downward arrows represent activation (↑) and inhibition (↓) of each receptor *(Receptors),* messenger *(Messengers),* and channel *(Effecter channels).* (+), Facilitation of backpropagation; (–), inhibition of backpropagation; PKC, protein kinase C; PKA, cyclic AMP-dependent protein kinase A; MAPK, mitogen-activated protein kinase; CaMKII, Ca^{2+}/CaM-dependent protein kinase II; 4-AP, 4-aminopyridine; HCN, hyperpolarization-activated cyclic nucleotide-gated K^+ channels. References for each functional molecule are indicated by the numbers in the following list: 1. *(46)* 2. *(79)* 3. *(71)* 4. *(12)* 5. *(57)* 6. *(9)* 7. *(10)* 8. *(11)* 9. *(74)* 10. *(46)* 11. *(39)* 12. *(55)* 13. *(81)* 14. *(51)* 15. *(76)* 16. *(72)* 17. *(88)* 18. *(89).*

choline receptors have been reported in hippocampal neurons *(66–68).* Electrical stimulation delivered to the fimbria and/or the stratum oriens in hippocampal-slice preparations can induce muscarinic receptor-mediated slow depolarizations in CA1 pyramidal neurons *(69,70).* Tsubokawa and Ross *(71)* made the observation that carbachol (~1 μ*M*), an acetylcholine receptor agonist, reduces the frequency-dependent decline in dendritic-action potential amplitude during trains. Because this effect was blocked by application of pirenzepine, a M_1-type muscarinic antagonist, it is suggested that activation of $G_{q/11}$ protein dependent pathways that resulted in activation of PKC and/or Ca^{2+} release from IP_3-dependent internal stores are crucial processes. To obtain this effect, the concentration of carbachol seems to be important because Egorov et al. *(72)* often observed a decrease rather than an increase in dendritic-spike amplitude even when a relatively high concentration of carbachol (10–50 μ*M*) was applied. The decrease in action-potential amplitudes was suggested to be due to suppression of the slow afterhyperpolarization and augmentation of the synaptically evoked slow membrane depolarization, causing inactivation of Na^+ channels. Tsubokawa et al. *(73)* also demonstrated that a phorbol ester (a protein kinase activator) reduced the frequency-dependent decline in action potentials. Furthermore, H-7, a broad kinase inhibitor, reversed the effect of the phorbol ester. Application of an M_2-type mus-

carinic antagonist did not block the carbachol-induced effect. Thus effects of PKA activation seem to be negligible at least in this modulatory pathway. These results support the idea that activation of PKC modulates dendritic voltage-gated channels. Colbert and Johnston *(12)* tested directly the modulation of dendritic Na^+ channels and found that phorbol esters reduce both the slow inactivation of Na^+ channels and the frequency-dependent decline in action potentials. Because activation of PKC also could modulate A-type K^+ channels *(74)* and muscarinic-sensitive K-currents *(75)*, these actions potentially contribute to the decrease in the frequency-dependent spike attenuation. Thus transmitters that lead to activation of PKC may have the potential for enhancing the repetitive firing of back-propagating action potentials in dendrites.

It is important to note, however, that the effect of the cholinergic agonist on the backpropagating trains was not inhibited by application of H-7 *(73)*. This fact indicates that protein kinases downstream of $G_{q/11}$ protein cascades are not necessary to reduce the spike-amplitude attenuation in the dendrites, and that other biochemical pathways can compensate for the functions required. Tsubokawa et al. investigated mechanisms underlying cholinergic modulation of backpropagating spikes by using G_q- and/or G_{11}-protein deficient mice *(76)*. They found evidence that a Ca^{2+}-dependent but $G_{q/11}$-independent cascade is a possible alternative pathway. In mutant mice lacking G_q or G_{11}, reduction of a frequency-dependent decline in dendritic-action potentials is not induced by application of either carbachol or phorbol ester. Instead, large depolarizations applied into the dendrites could reduce the amplitude attenuation of spike trains. This depolarization-induced facilitation of backpropagating spikes also is present in CA1 pyramidal neurons of wild-type mice, suggesting that the pathway is functional normally. Interestingly, this effect occurred gradually within 10 min after depolarization and lasted at least 20–30 min. Because intracellular and/or extracellular applications of Ca^{2+}/CaM kinase II (CaMKII) inhibitors blocked the facilitation, it is likely that increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by depolarization cause persistent facilitation of spike backpropagation by CaMKII-dependent mechanisms. Phorbol ester-induced facilitation is relatively transient and easily reversed by washing with inhibitors, whereas the depolarization-induced effect is rather long-lasting. Whether activation of muscarinic receptors causes Ca^{2+}-entry large enough to activate CaMKII should be examine to test the possibility of cross-talk. Nevertheless, it is a fascinating idea that PKC-dependent systems exert their effects on excitability when the increase in $[Ca^{2+}]_i$ is small, whereas CaMKII-dependent systems then start working to stabilize the effect when the increase in $[Ca^{2+}]_i$ is above some threshold.

Noradrenergic Modulation

Adrenergic neurons in the locus coeruleus give rise to extensive projections that directly innervate large parts of the brain including the hippocampus *(77)* and modulate functions of the target neurons through activation of adrenergic receptors. Consequently, pharmacological activation of adrenergic receptors could change the gain of some types of synaptic plasticity in hippocampal-slice preparations *(78)*. Application of noradrenaline induces no clear changes in the action potentials propagated into the apical dendrites of CA1 pyramidal neurons *(79)*. However, application of isoproterenol, a relatively specific agonist to β-adrenergic receptors, increases the amplitude of dendritic action potentials *(46)*. Isoproterenol-induced increases in spike amplitude are similar to the effect of 8-bromo-cAMP application and are blocked by application of H-7, indicating that β-adrenoceptor activation enhances dendritic excitability through activation of PKA. The application of 8-bromo-cAMP had no effect on the rate of rise of the back-propagating action potentials. This result suggests that the increase in amplitude results mostly from a decrease in the K^+ current and not from an increase in Na^+ current. A positive shift of the activation curve of A-type K^+ channels by

PKA decreases dendritic K^+ currents and thereby increases the amplitude of action potentials *(80).* Adrenoceptors are classified into three groups, which are coupled with $G_{q/11}$ protein (α_1 group), $G_{i/o}$ protein (α_2 group and β_3), and with G_s protein (β group). Because both $G_{i/o}$ protein and G_s protein change activities of adenylate cyclase in opposite directions, the effects of adrenoceptor activation in physiological conditions is highly dependent on the distribution of those receptor subtypes. Bath application of noradrenaline could activate both $G_{i/o}$-coupled and G_s-coupled receptors, which may obscure effects of PKA activation (or inactivation).

One experiment that might be interesting is to see how β-adrenoceptor dependent pathways control activity of HCN channels in the dendrites because dendritic I_h has been suggested to be important for spatial normalization of synaptic potentials in hippocampal *(81)* and neocortical *(82)* pyramidal neurons. Somatic excitatory postsynaptic potentials (EPSPs) amplitudes, which are critical for action potential initiation, are not dependent on the location of synapses *(83),* at least in part because of the non-uniform distribution of I_h. In physiological conditions, several transmitter actions related to cAMP production might control spatial profiles of EPSPs in each branch by regulating HCN channels.

Serotonergic Modulation

Serotonergic inputs to the hippocampus are supplied from the dorsal-raphe nucleus *(84)* and from the median-raphe nucleus *(85)* in the rat brain. Serotonin receptors are also diverse and at least 14 receptor subtypes have been cloned *(86).* In central neurons, $5\text{-}HT_{1A}$ receptors are expressed densely in dendrites *(87).* This receptor subtype is coupled with $G_{i/o}$ proteins, which inhibit adenylate cyclase activity resulting in inhibition of PKA action. Sandler and Ross *(88)* reported an interesting pattern of spike-amplitude modulation in hippocampal dendrites. Application of 5-HT hyperpolarized resting membrane potentials. Therefore, the absolute amplitude of Na^+-dependent action potentials was expected to increase if the peak potentials of the spikes do not change. However, the amplitude of the Na^+ spikes did not change significantly in the apical dendrites because the peak potentials also hyperpolarized. Because hyperpolarization by negative current injection through the recording pipet mimicked the effect of serotonin, a sustained increase in some conductance in the dendrites is suggested to contribute to the shift. One of the candidates is activation of inward rectifier K^+ channels. Takigawa and Alzheheimer *(89)* performed patch-clamp recordings in segments acutely isolated from the soma and the dendrites and found a predominant distribution of G protein-activated inwardly rectifying K^+ (GIRK) currents in the dendrites. This current is activated similarly not only by serotonin but also by adenosine and by GABA via $GABA_B$ receptors.

Future Directions

During this decade, active properties of neuron dendrites have been studied primarily in cortical and hippocampal pyramidal neurons. Characteristics of specific voltage-gated channels and their distributions along the dendrites are being revealed, and these are clearly responsible for backpropagation of action potentials. Now some important questions remain to be clarified. First, what is the physiological role of spike backpropagation? Evidence that the backpropagation is required for some types of synaptic plasticity in CA1 pyramidal neurons has been shown by Magee et al. *(64).* Possible contributions to other neuronal functions are not reported yet. One key function of backpropagating spikes might be to increase dendritic $[Ca^{2+}]_i$. A recent report by Nakamura et al. *(90)* demonstrated that large Ca^{2+} release from the internal stores were induced at the dendrites when metabotropic glutamate receptors (mGluRs) were activated together with backpropagating spikes. The amount of dendritic $[Ca^{2+}]_i$ increase by back-

propagating action potentials without activation of mGluRs was significantly smaller than that by synergistic activation. This finding clearly indicates that backpropagation could facilitate Ca^{2+}-dependent mechanisms caused by mGluR activation. Interactions between mGluR-related signaling and action potentials in the dendrites might be an interesting theme for future study. Second, how active properties of the dendrites contribute to the synaptic integration is still not clear. A recent report by Magee and Cook *(83)* shows that in CA1 neurons the amplitudes of EPSPs at the distal dendrites are relatively larger than those at the proximal dendrites. Therefore, the amount of somatic depolarization by EPSPs is not dependent on the location of synapses. This finding indicates that dendritic Na^+ and Ca^{2+} channels do not amplify small EPSPs at the distal dendrites. It looks that these channels contribute rather to normalization of the synaptic potentials. Whether inhibitory postsynaptic potentials also show a similar gradient of efficacies might be worth examining.

One crucial question is how far can we generalize the findings obtained only from a few specific neuron types. In other words, whether the mechanisms suggested by the evidence from pyramidal neurons are applicable to other types of neurons must be investigated. The function as well as the morphology of dendrites in the CNS is highly differentiated. Regional electrical properties of these branching structures are not only dynamic but also complicated. Therefore, it is not easy to understand the detailed properties of dendrites without precise measurements. Conventional electrophysiological and pharmacological studies are still useful to analyze electrical activities of neurons, but may not be enough to obtain other information required for estimating the role of intracellular mechanisms. In this context, several studies were performed to investigate Ca^{2+} dynamics in pyramidal-cell dendrites by microfluorometric imagings *(91–94).* In addition, new kinds of optical experiments analyzing the spatiotemporal profiles of changes in membrane potential in the dendrites *(95,96),* and quantitative measurements of protein synthesis in living neurons by immunohistochemical imaging *(97)* have recently been made. These improved optical techniques combined with electrophysiological recordings could help reveal the key concepts for understanding dendrites of CNS neurons.

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