Potassium Currents in Developing Neurons

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ABSTRACT: In *Xenopus* spinal neurons, delayed rectifier type voltage-dependent potassium currents (I_{Kv}) are developmentally regulated. These currents play a pivotal role in maturation of the action potential from a long-duration calcium-dependent impulse to a brief sodium-dependent one. Although spinal neurons are heterogeneous, I_{Kv} undergoes a synchronized and homogenous developmental functional up-regulation across this diverse population of motor, sensory, and interneurons. This finding suggested that the diverse population of neurons expressed a common potassium channel. Thus, recent efforts have been directed towards cloning the relevant potassium channel gene. However, these molecular studies reveal an unsuspected heterogeneity in the molecular components of voltage-dependent potassium channels. Further, synchronous differentiation of I_{Kv} is achieved via heterogeneous Kv channel gene expression.

When neuronal precursors exit the cell cycle, they begin terminal differentiation. Extensive cytological and morphological differentiation occurs. A critical aspect of terminal differentiation is acquisition of electrical excitability, which underlies rapid signaling—a hallmark of the adult nervous system. Considerable evidence has accumulated over recent years indicating that excitability is acquired early by embryonic neurons and is subject to continual developmental modulation.

When neurons are synaptically interconnected, excitability is required for functional output of newly forming networks. In addition, developmental changes in excitability of individual cells will modify circuit output and resultant behaviors. Prior to the formation of synapses and establishment of sensory input, embryonic neurons are already excitable. However, it is not immediately obvious what the role of excitability is at these early stages of neuronal differentiation. Recent evidence suggests that ion channel activity, while typically associated with the rapid signaling of mature nervous systems, can also initiate intracellular signaling cascades that act over a longer time domain.[1] Further, the signaling cascades that are triggered by activity can have a profound influence on subsequent differentiation of fundamental neuronal properties such as process outgrowth, connectivity, neurotransmitter selection, and the properties of ion channels themselves.[2]

Thus, studies of excitability in embryonic neurons can be divided into two periods: one prior to synapse formation, and the second after connections are formed. The majority of our work has concerned neurons during the first period at the earliest stages of their differentiation. This is for many reasons, notably because the changes that neurons display during this period are important, and elucidating the mechanism that underlies these changes has been informative. Practical considerations have also influenced our choice of period of study. It is clear that for studies of development of electrical excitability, a key requirement of the system is electrophysiological access to neurons of interest. Primary spinal neurons of the developing *Xenopus laevis* embryo meet this essential criterion. These neurons are a

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diverse set of sensory, inter-, and motor neurons that underlie the essential swimming behavior of the embryo.[3,4] Moreover, with respect to birthdates, these neurons are a relatively synchronized population.[5,6] Secondary neurons, in contrast, are born later over a protracted period of time.[6] Primary spinal neurons can be studied during early stages prior to synapse formation both in intact preparations as well as *in vitro*. This review will briefly summarize development of electrical excitability in these neurons, since these results provide the rationale for our focus on potassium currents. Next, the development of potassium currents in these neurons will be discussed. Our recent work is aimed at identifying the molecular components of developmentally regulated potassium currents, and these studies will be summarized. The article will close with considerations of future directions.

DEVELOPMENT OF ELECTRICAL EXCITABILITY IN *XENOPUS* PRIMARY SPINAL NEURONS

Excitability is acquired by developing *Xenopus laevis* primary spinal neurons 7–8 hours after their last round of DNA synthesis[5,6] and several hours before initial signs of chemosensitivity and synapse formation.[7,8] A similar pattern has been observed for the differentiation of primary spinal neurons in culture. Typically, the neural plate is dissected from a 17 1/2–hour-old embryo. Neurons initiate neurite outgrowth 6 h after plating,[9] which parallels the time of neurite outgrowth *in vivo* (22 h). At this time, action potentials can be elicited from every cell with a neuronal morphology, indicating that neurite extension *in vitro* does not precede the acquisition of excitability. Thus, the initial appearance of electrical excitability occurs at similar times *in vivo* and *in vitro*.

When action potentials are first detected at the neural tube stage (22 h), they are calcium dependent and of long duration. During the following day *in vivo* and *in vitro*, the impulse matures to a brief sodium-dependent spike.[9,10] This program is expressed in a cell-autonomous manner, since a neuron developing in the absence of other cells also exhibits a transient period of long-duration impulses.[11]

Calcium-dependent action potentials are spontaneously expressed in embryonic amphibian spinal neurons, and their pharmacological removal disrupts the subsequent elaboration of other phenotypes, suggesting their developmental significance.[12] The transient elevations of intracellular calcium, as assessed by calcium ion imaging with fluorescent indicators, occur most frequently at early stages of differentiation, when potassium currents are small and neurons fire long, calcium-dependent action potentials. With further development the incidence of spontaneous calcium transients declines in parallel with the conversion of the action potential to brief sodium-dependent impulses. Furthermore, early blockade of calcium influx for brief periods shows that perturbations applied during the period of greatest spontaneous calcium influx have the most profound effects on subsequent development. These data suggest that transient elevations of intracellular calcium may trigger specific sequences of differentiation.

More recently, it has been shown that the differentiation cues imparted by spontaneous transient elevations of calcium are frequency encoded.[13,14] Suppression of calcium-dependent action potentials prevents normal maturation of several key neuronal properties: expression of the neurotransmitter GABA, neurite outgrowth, and the kinetics of voltage-dependent potassium current. These properties will be referred to here as calcium-dependent phenotypes. Calcium-dependent action potentials were suppressed and then reimposed at

desired frequencies. Calcium-dependent phenotypes were best restored when impulses were reimposed at their natural frequency.[14,15]

DEVELOPMENTAL REGULATION OF VOLTAGE-DEPENDENT POTASSIUM CURRENTS IN *XENOPUS* SPINAL NEURONS

Analysis of the voltage dependent currents that underlie the action potential indicates that the transition from a long-duration calcium-dependent to a sodium-dependent brief impulse is due primarily to the maturation of a delayed rectifier potassium current.[16,17] During the first day *in vitro*, calcium current shows very little change, and sodium current doubles in density during this period. Delayed rectifier potassium current, however, triples in density, and its kinetics are accelerated.[17]

Quantitative analysis using mathematical reconstruction of action potentials also indicates that the developmental changes noted for potassium current are most significant.[18] Further, changes in potassium current are not the only theoretical way to bring about the developmental change in the impulse. In fact, in mathematical reconstructions, the action potential duration is most sensitive to changes in calcium current. Thus, in addition to understanding how potassium current density is regulated during development, it will be important to determine why this current is the major determinant of impulse duration.

RNA synthesis inhibition prevents development of the mature delayed rectifier and arrests the normal maturation of the action potential. Further, a critical period of transcription is required for the differentiation of this potassium current.[19] This critical period is specific to the delayed rectifier, since the two inward currents do not show sensitivity to inhibitors of transcription when applied during the same period,[20] and a potassium A current recovers and matures following removal of transcriptional block.[19]

The appearance of neuronal morphology also requires new RNA and protein synthesis[19,21,22] Application of RNA or protein synthesis inhibitors applied before 3 or 5h *in vitro*, respectively, completely suppresses the appearance of morphologically identifiable neurons, defining a sensitive period of transcription required for the differentiation of this phenotype. It does not appear to contain a critical period, however, since acute application of a reversible inhibitor permits neurite extension after the appropriate delay, the duration of inhibition—3h.[19]

In sum, properties of the delayed rectifier potassium current undergo significant developmental changes during the first 48 hours of development of primary spinal neurons.

IDENTIFICATION OF THE MOLECULAR COMPONENTS OF DEVELOPMENTALLY REGULATED POTASSIUM CURRENTS

The properties of I_{Kv} determine the phenotype of the action potential in young and mature neurons.[17,18] Accordingly, a major focus of recent work has been to identify the molecular components of developmentally regulated potassium currents. These efforts have taken advantage of the advances in molecular cloning of potassium channel genes.

Voltage-dependent potassium (Kv) channels are thought to be tetramers of pore-forming α-subunits[23,24]; Kv α-subunits genes belong to four major subfamilies (Kv1, Kv2, Kv3, Kv4[25–28]) with an additional fifth family that has been found in Aplysia (Kv5)[29]. More

recently, Kv6–Kv9 subfamilies have been reported.[30–32] Subunits from these subfamilies do not form functional channels when expressed by themselves. However, they affect the properties of channels when coexpressed with members of major subfamilies.

An initial way to determine the functional properties of channels encoded by the Kv genes is to express them heterologously (e.g., in *Xenopus* oocytes) and electrophysiologically characterize the resultant currents. Although the properties of homomultimeric channels are more often studied, the function of heteropolymeric channels formed from different type of Kv clones has also been examined.[33–35] The data indicate that the formation of heteropolymers yields potassium channels that are functionally distinct from those formed by association of identical subunits. Interestingly, although different Kv1 gene products can form heteropolymers, a Kv1 gene product does not form a functional channel with, for example, a Kv2, Kv3, or Kv4 gene product, suggesting that each potassium channel gene family functions independently.[36]

The biophysical properties of I_{Kv}[17] suggested that genes encoding noninactivating potassium current would contribute to the endogenous I_{Kv}. Indeed, members of the Kv1 and Kv2 subfamilies are expressed in primary spinal neurons.[37–40] However, Kv1.1 and Kv2.2 mRNAs are detected in different neuronal subpopulations. Further, heterologous expression of Kv1.1 and Kv2.2 in oocytes leads to expression of rather different sustained Kv currents (e.g., Ref. 39) in contrast to the uniform I_{Kv} of diverse spinal neurons. On the basis of whole-cell recording of voltage-dependent currents, neurons would be considered to be a homogeneous population, because subtle or no differences are noted in the whole-cell currents recorded at a given stage of development.[17] In fact, a simple scenario to account for synchronized developmental regulation of I_{Kv} is that the heterogeneous population of spinal neurons expresses a common potassium channel gene that is functionally up-regulated in a similar manner throughout the population.

Neurons in culture are heterogeneous and include motor, sensory, and interneurons.[8,9,41] Heterogeneity is revealed by the different chemosensitivity profiles that neurons acquire as they differentiate.[8] Further, a subset acquire GABA-like immunoreactivity.[2]

Our initial analyses of Kv channel gene expression were raising the possibility, however, of molecular heterogeneity in the endogenous population of functional Kv channels. This suspected molecular diversity has been recently confirmed at a functional level by overexpression of a dominant negative Kv1 subunit.[42] This subunit is expected to suppress current carried by Kv1 channels but not that of non-Kv1 channels (e.g., Kv2). Following overexpression of the dominant negative Kv1 subunit, I_{Kv} was suppressed in some, but not all, neurons. Despite the uniformity in the properties of I_{Kv} of neurons in culture, only a subset of neurons in culture. This result indicates that Kv1 channels are not expressed in all neurons. Moreover, synchronous differentiation of I_{Kv} is achieved via heterogeneous Kv channel gene expression. Examination of the contribution of Kv2 channels is currently being examined by overexpression of a dominant negative Kv2 subunit.[43]

FUTURE DIRECTIONS

A limited number of studies have examined primary neurons at stages subsequent to synapse formation, because analysis at these later stages is complicated by poor extended survival under standard culture conditions. Further, the overexpression strategies that have proved useful for study of the early period of differentiation of electrical excitability are

not likely to be applicable to analyses at later stages since injected RNA and resultant exogenous proteins have half-lives that limit their duration of action. A system that holds promise for study of this later period of differentiation of electrical excitability is the zebrafish (*Danio rerio*). The swimming behavior of the early zebrafish embryo is mediated by the same set of primary neurons that do so in *Xenopus*. Large-scale mutagenesis is feasible in *Danio rerio,*[44,45] and thousands of embryonic genetic mutants have been isolated.[46,47] Zebrafish start displaying behaviors, which allow evaluation of the function of the nervous system and muscle and isolation of genetic motility mutants, as early as one day after fertilization.[48] Moreover, it is possible to record from zebrafish primary neurons in intact preparations[49] and examine directly the electrical membrane properties of primary neurons in these motility mutants.

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