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Localization of $Na_v1.5$ sodium channel protein in the mouse brain

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Abstract

$Na_v1.5$ or SCN5A is a member of the voltage-dependent family of sodium channels. The distribution of $Na_v1.5$ protein was investigated in the mouse brain using immunohistochemistry. Immunostaining with a $Na_v1.5$-specific antibodyrevealed that $Na_v1.5$ protein was localizedin certain distinctregions of brain including the cerebral cortex, thalamus, hypothalamus, basalganglia, cerebellum and brain stem. Notably, we found that $Na_v1.5$ protein co-localized with neurofilaments and clustered at a high density in the neuronal processes, mainly axons.These results suggest that $Na_v1.5$ protein may play a role in the physiology of the central nervous system (generation and propagation of electrical signals by axons).

Keywords

Axon; Brain; Cardiac sodium channel; $Na_v1.5$/SCN5A; Neuronal process; Seizure; Sudden death

INTRODUCTION

Voltage-dependent sodium channels are transmembrane proteins that are responsible for generating action potentials and for rapid conduction of electrical signals in excitable cells [1]. Sodium channels are classified into three types based on their sensitivity to blockade by tetrodotoxin (TTX): TTX-sensitive Na channels (TTXs), TTX-resistant Na channels (TTXr), and TTX-insensitive Na channels (TTXi) [2,3]. In the brain, six TTXs Na channels have been identified, including $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$, $Na_v1.7$. Only one TTXr channel, $Na_v1.9$, has been identified in the brain [4]. TTXr Na channels generate currents that are slower, but recover from inactivation much faster than TTXs channels [5], suggesting a possible role of TTXr Na channels in sustained firing of neurons or as pace-makers. In this study, we investigated the localization pattern of another TTXr Na channel $Na_v1.5$ in the mouse brain using immunohistochemistry.

$Na_v1.5$ or SCN5A encodes the cardiac sodium channel with 2016 amino acids and a calculated mol. wt of 227 kDa [6]. The putative structure of $Na_v1.5$ consists of four homologous domains (I-IV), each containing six transmembrane segments (S1-S6). $Na_v1.5$ mutations cause syncope, seizures, and sudden death triggered by lethal cardiac arrhythmias associated with long QT syndrome (LQTS), idiopathic ventricular fibrillation including Brugada syndrome and cardiac conduction disease [7–11]. $Na_v1.5$ was originally identified as a cardiac sodium channel. Subsequently, it was shown to be expressed in the brain at the mRNA level [2,12], and these

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findings may explain why$Na_v1.5$ mutations are associated with seizures. However, the distribution of $Na_v1.5$ in the brain at the protein level has not been investigated previously. Here, we demonstrate selective expression of $Na_v1.5$ at the protein level in regions of the mouse central nervous system.

MATERIALS AND METHODS

A $Na_v1.5$-specific polyclonal antibody was developed against a synthetic polypeptide (AC-RPQLDLQASKKLP-DLYC-Amide) corresponding to a unique portion of the less conserved N-terminus of $Na_v1.5$ as described [13]. The mouse anti-neurofilament 200 antibody (clone NE14) was from Sigma (St. Louis, MO), and fluorescein-conjugated secondary antibodies were from Jackson Immuno Research Laboratories (West Grove, PA). The tissues for Western blotting and immunohistochemical analyses were from the mouse strain C57BL.

For Western blot analysis, lysates from cells transfected with a $Na_v1.5$ expression construct (SCN5A in pcDNA3) or the protein extracts from heart, brain, kidney, liver, and skeletal muscle were separated by SDS-PAGE and probed with the anti-$Na_v1.5$ antibody. The signal was visualized with ECL Western blotting detection reagents (Amersham Pharmacia Biotech, Inc., Piscataway, NJ).

Immunohistochemistry was carried out as described previously [14]. Double immunofluorescence staining with the anti-$Na_v1.5$ antibody and the anti-neurofilament antibody was carried out as described [15]. The immunostained slides were mounted with anti-fading media, and visualized with a Zeiss Axiophot photomicroscope or a confocal laser-scanning microscope (SP2; Leica, Heidelberg, Germany).

RESULTS

We have developed an antibody to the $Na_v1.5$ channel. Several lines of evidence strongly indicates that this antibody is specific to $Na_v1.5$. First, the anti-$Na_v1.5$ antibody was generated using a peptide immunogen located at the less-conserved N-terminus of $Na_v1.5$ protein and database searches indicate that it is unique to $Na_v1.5$. Second, the specificity of the anti-$Na_v1.5$ antibody was confirmed by Western blot analysis with protein extracts from mouse heart, kidney, liver, brain, skeletal muscle, and HEK293 cells expressing human $Na_v1.5$. The antibody detected a band of the expected size of 227 kDa [16] strongly in the heart, weakly in the brain, but not in other tissues (Fig. 1a). These data are consistent with the results from Northern blot analysis [6]. The weak Western blotting signal from the brain suggests that our anti-$Na_v1.5$ antibody does not cross-react with major brain sodium channel proteins, indicating the specificity of our antibody. The antibody also yielded the expected 227 kDa band in the lane loaded with protein extract from the transfected cells over-expressing $Na_v1.5$ (Fig. 1b). No equivalent band was detected in the control non-transfected cells (Fig. 1b). Thirdly, as expected for a channel protein, immunofluorescence staining with HEK293 and P19CL6 cells with $Na_v1.5$ expression detected SCN5A-imunoreactivity only on the cell surface (Fig. 1c). Collectively, these data indicate that our antibody is specific to $Na_v1.5$.

Immunohistochemistry using this anti-$Na_v1.5$ antibody was performed to determine the distribution of $Na_v1.5$ in the normal mouse brain. A negative control experiment was carried out on the adjacent brain sections with the anti-$Na_v1.5$ antibody pre-absorbed with the antigen peptide. Elimination of $Na_v1.5$-immunoreactivity in the negative control experiments (compare Fig. 2a and 2b) indicated that positive staining signal was specific to $Na_v1.5$.

In the majority of cerebral cortex regions (frontal cortex, parietal cortex, temporal cortex and occipital cortex), a weak, but significant, $Na_v1.5$ immunoreactivity was observed (Fig. 2c). Within these regions, the neuronal processes originating from cerebral cortical neurons were

the structures positively stained for $Na_v1.5$, whereas little or no immunoreactivity was detected in the neuronal cell bodies or any glial components (Fig. 2c). In the forebrain, we also observed strong $Na_v1.5$ immunoreactivity in the olfactory tract (Fig. 2d), and weak staining in the olfactory tubercule (data not shown). Meanwhile, in the limbic system where $Na_v1.5$ mRNA was reported to be strongly expressed [2,12], moderate $Na_v1.5$ immunoreactivity was detected in the nerve fibers, but no or little $Na_v1.5$ immunoreactivity was detected in the cell bodies in any of the limbic nuclei (Fig. 2a). In the striatum, the anti-$Na_v1.5$ antibody detected strong staining in the nerve bundles, also known as the pencil fibers, whereas the cell bodies in the striatal nuclei showed little or no signal (Fig. 2e; Fig. 3a–c). Interestingly, $Na_v1.5$ immunoreactivity in the pencil fibers was significantly stronger within the caudate putamen (Fig. 2e, right half) than within the globus pallidus (Fig. 2e, left half). In the thalamus and hypothalamus, nerve fibers expressed $Na_v1.5$, while a low level of $Na_v1.5$ immunoreactivity was localized to the hypothalamic and thalamic nuclei (data not shown).

In the diencephalon of the adult mouse brain, $Na_v1.5$ was also localized to the nerve fiber tracts. The cerebral peduncle was intensively stained for $Na_v1.5$ (Fig. 2f).

In the brain stem, intense $Na_v1.5$ immunoreacitivy was localized to some spotty regions in the lateral medulla (Fig. 2g). These signals seemed to correspond to discrete nerve tracts in the medulla. Based on a report showing that $Na_v1.5$ mRNA expression is located in the lateral paragigantocellular nuclei (LPGi) [2], the nerve tracts with $Na_v1.5$ immunoreactivity shown in this study may be assumed to be originated from LPGi.

In the cerebellum, no signal was detected in the granular layer and the Purkinje cell layer. However, weak $Na_v1.5$ immunoreactivity on some fibrous structures was observed in the cerebellar molecular layer, and strong $Na_v1.5$ immunoreactivity was detected in the white matter of the cerebellum (Fig. 2h).

It is important to note that not all the nerve tracts or nerve bundles through the mouse brain were positively stained by the anti-$Na_v1.5$ antibody, for example, a huge nerve tract connecting both hemispheres in the corpus callosum was not positively stained (data not shown). These data suggest that the distribution of $Na_v1.5$ protein is restricted to some specific nerve tracts like those in the limbic system, olfactory system, and striatum.

To characterize the localization of $Na_v1.5$ at the cellular level, we performed double immunostaining using the rabbit anti-$Na_v1.5$ antibody and the anti-neurofilament antibody. Figure 3 shows confocal microscopic images of the caudate putamen of the basal ganglion. Co-localization of $Na_v1.5$ and neurofilaments (the major intermediate filament proteins in axons) indicates that $Na_v1.5$ is mainly localized in axons (Fig. 3a–c). Furthermore, because neuro-filaments are synthesized in cell bodies and then transported into axons [17], co-localization of $Na_v1.5$ and neurofilaments may suggest that $Na_v1.5$ is synthesized in cell bodies and then transported into axons.

DISCUSSION

Increasing evidence suggests that the distinctive roles played by different brain Na channels are often dictated by their unique localization in excitable tissues. We observed the striking and distinct localization of the $Na_v1.5$ Na channels in axons of certain distinct regions of the brain. Axons are specialized for conducting electrical signals. These results are consistent with the functional role of sodium channels, i.e. generation and propagation of the action potentials in the neurons and for conducting electrical signals throughout the nervous system. Together with $Na_v1.9$, $Na_v1.5$ channels may generate the TTX-resistant sodium currents in the brain, which may play a role in sustained firing of neurons or as pace-makers.

The distribution of the cardiac sodium channel $Na_v1.5$ in the brain has not been previously characterized at the protein level. Our study represents the first detailed characterization of $Na_v1.5$ protein distribution in the mouse brain. Consistent with the distribution of $Na_v1.5$ transcripts in rat and human brain [2,12], the mouse $Na_v1.5$ protein was present in the limbic system, olfactory system, thalamus, and hypothalamus. However, our study revealed localization of $Na_v1.5$ protein in several brain regions that were not described or analyzed in previous studies. These regions include the cerebral cortex, striatum, and cerebellum. In the limbic system, Hartmann *et al.* [12] and Donahue *et al.* [2]showed strong expression of $Na_v1.5$ mRNA in the piriform cortex, septal nuclei, the diagonal band of Broca, amygdala, and habenular nuclei. We found weak or moderate presence of $Na_v1.5$ protein in the nerve fibrous structures in these regions. A possible explanation for the difference may be that $Na_v1.5$ mRNA is localized to the neuronal cell bodies, whereas the $Na_v1.5$ protein molecules are synthesized in these locations, and are transported to and accumulate in the nerve fibers. An alternative explanation is that $Na_v1.5$ mRNA in some regions may not be fully translated, as reported for the GABA A receptor mRNA and the NMDA R1 mRNA [2]. Our results imply that it is important to investigate the distribution of ion channels in the brain at the protein level.

Mutations in $Na_v1.5$ cause cardiac arrhythmias and sudden cardiac death [7–11]. It has been recognized that the nervous system plays a role in the genesis of cardiac arrhythmias. Although it is speculative and remains to be investigated, it is possible that mutant $Na_v1.5$ proteins expressed in certain brain regions of patients with cardiac arrhythmias may play a role in generating the trigger to provoke arrhythmias in these patients. Of particular interest is the expression and localization of $Na_v1.5$ in the brainstem, which is known to be involved in regulating cardiac activities through autonomic motor neurons.

CONCLUSION

This study represents the first detailed characterization of $Na_v1.5$ protein distribution in the mouse brain. $Na_v1.5$ protein is widely distributed throughout the central nervous system, including the cerebral cortex, thalamus, hypothalamus, striatum, cerebellum and brainstem. At the cellular level, $Na_v1.5$ protein is mainly localized to the axons. Our results are consistent with the hypothesis that $Na_v1.5$ protein may play an important role in regulating neuronal excitability.

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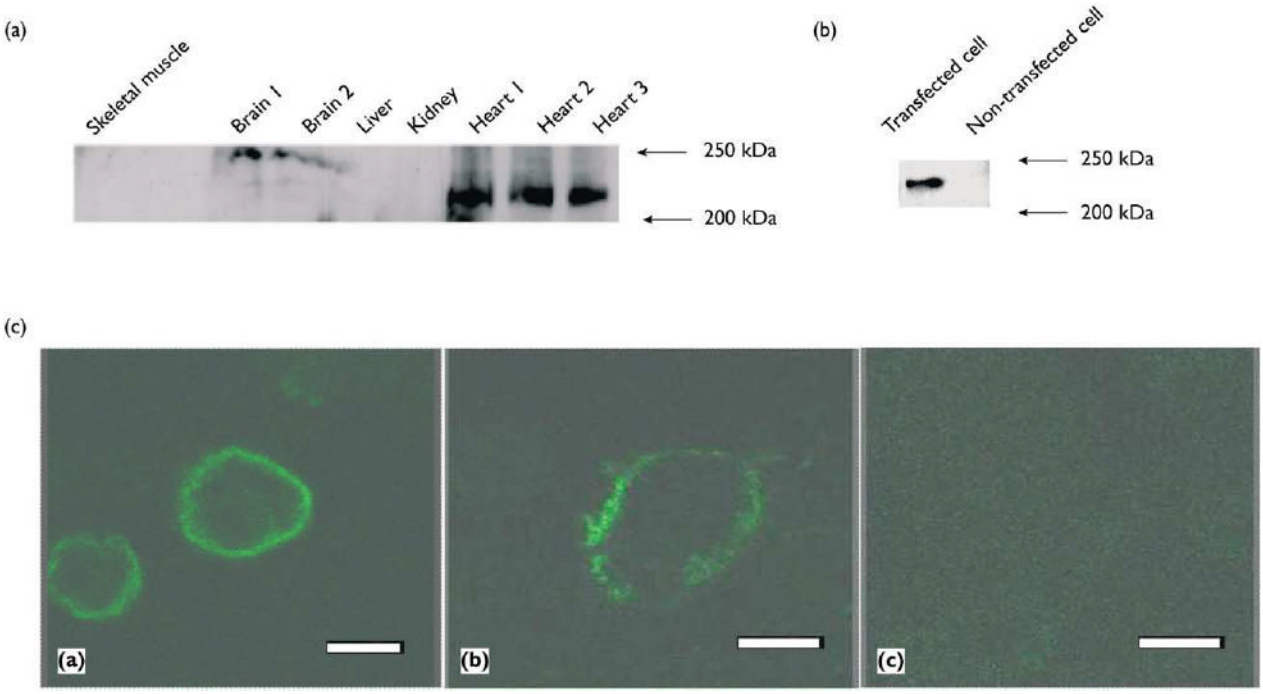

Fig. 1.

Characterization of the anti-$Na_v1.5$ antibody. (a) Western blot analysis of mouse membrane proteins from the heart, kidney, liver, brain, and skeletal muscle tissues with the anti-$Na_v1.5$ antibody.The positions of mol w markers are indicated. (b) Western blot analysis with protein extracts from HEK293 cells expressing $Na_v1.5$ (transfected cell), and control cells that were not transfected with the $Na_v1.5$ expression construct (non-transfected cell).(c) Cellular localization of wild type human SCN5A protein on plasma membrane (green signal). HEK293 cells (left) and P19CL6 cells (middle) transfected with the human SCN5A expression construct were immunostained with the anti-SCN5A antibody. No detectable immuno£uorescence staining was observed with the anti-SCN5A antibody pre-absorbed with the peptide antigen (right). Bar = 5 μm.

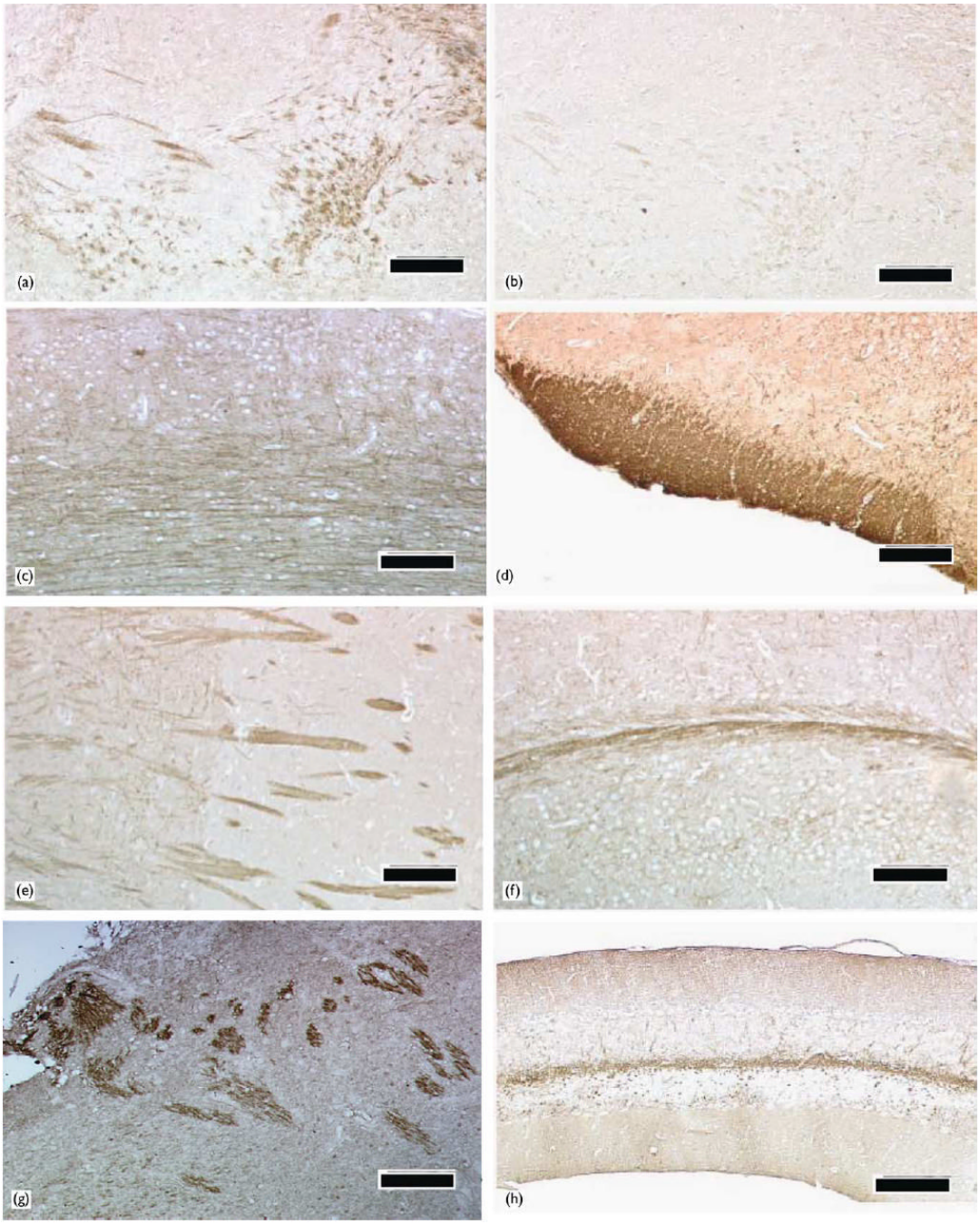

Fig. 2.

Regional distribution of $Na_v1.5$ protein in the mouse brain. (a) Some nerve fibers in the limbic system express $Na_v1.5$ protein, whereas neuronal and glial cell bodies were not stained by the anti-$Na_v1.5$ antibody. (b) An adjacent section to the section used in (a) was stained with the anti-$Na_v1.5$ antibody pre-absorbed with the immunogen peptide. (c) In the cerebral cortex, the neuronal processes, mainly axons, were positive for $Na_v1.5$ protein, but little or no signal was observed in the cell bodies. (d) The olfactory tract had strong $Na_v1.5$ signal. (e) In the striatum, $Na_v1.5$ protein was mainly localized to the nerve fibers (the pencil fibers). (f) The cerebral peduncle was stained by the anti- $Na_v1.5$ antibody. (g) In the brain stem, some nerve fibers in the lateral medulla were moderately positive for $Na_v1.5$. (h) In the cerebellum, the white matter

consisting of nerve fibers was strongly stained for $Na_v1.5$, but there was no signal in the granular layer and the Purkinje cell layer. Bars = 100 μm.

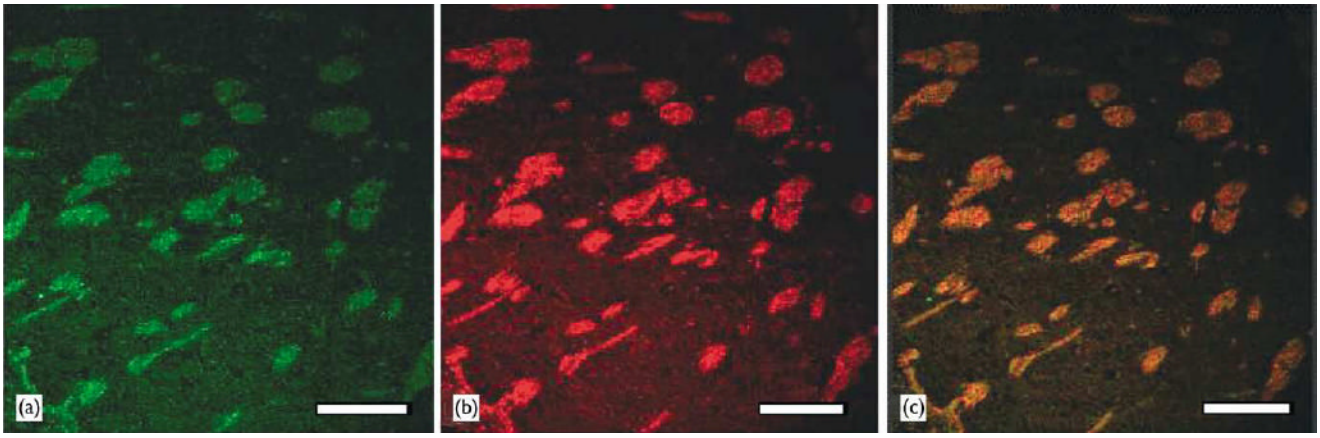

Fig. 3.

Co-localization of $Na_v1.5$ and neurofilaments.Confocal microscopic images of caudate putamen immunostained with both the anti-$Na_v1.5$ antibody and the monoclonal anti-neurofilament 200 antibody. (a) immuno£uorescent image for $Na_v1.5$ protein, green; (b) immuno£uorescent image for neurofilaments, red; (c) overlay of image A and image B. Bar = 200 μ m.