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Immunoblotting of T-type Ca^{2+} Channel Protein in Mouse Brain and Embryonic Heart by Using Two Antibodies against $Ca_v3.1$ Channels

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Abstract: T-type Ca^{2+} channels are particularly abundant in neuronal tissue and immature cardiac heart and mediate burst firing and pacemaker activity. Northern blot analyses have demonstrated that one subtype of T-type Ca^{2+} channels, $Ca_v3.1$ (α1G), mRNA is predominantly expressed in adult brain and immature heart. However, the protein expression of $Ca_v3.1$ channels is not clear, since the anti-$Ca_v3.1$ antibodies have not been commercially available until recently. To examine the usefulness of the new commercial antibodies against $Ca_v3.1$; sc-16259 and sc-16260, we performed Western blot analysis of adult brain and embryonic heart of mouse. sc-16259 and sc-16260 may not be suitable for detection of $Ca_v3.1$ channel protein in mouse tissue samples.

Key words: $Ca_v3.1$, T-type Ca^{2+} channel, Western blot analysis

T-type Ca^{2+} current was initially discovered in sensory neurones (Carbone et al, 1984) and subsequently founded in different cell types; cardiomyocytes (Bean, 1985; Nilius et al, 1985), pituitary GH3 cells (Matteson et al, 1986) and so on. T-type Ca^{2+} channels open at negative membrane potential that are near the resting potential and are rapidly inactivated. Those electrical properties suggest their roles on the mediation of pacemaking activity (Hagiwara et al, 1988), burst firing (White et al, 1989) and hormone secretion (Cohen, 1988). T-type Ca^{2+} current is abundant in immature cells (Xu et al, 1900; Berthier et al, 2002) and has been implicated in cell growth. Three different subtypes of pore-forming (α1) subunit of T-type Ca^{2+} channels have been identified; $Ca_v3.1$ (α1G), $Ca_v3.2$ (α1H) and $Ca_v3.3$ (α1I). The distribution of channel subtype is tissue-specific and development-stage-specific. Perez-Reyes et al. (Perez-Reyes et al, 1998) cloned $Ca_v3.1$ gene from rat brain in 1998. Northern blot analyses revealed that $Ca_v3.1$ mRNA is predominantly expressed in the brain and less abundant in the mature heart in rodent (Perez-Reyes, 1998; Klugbauer et al, 1999) and human (Monteil et al, 2000). Monteil et al. showed also that the $Ca_v3.1$ mRNA expression level is higher in fetal human heart compared with adult tissue. Cribbs et al. (Cribbs et al, 2001) demonstrated that $Ca_v3.1$ channel protein is expressed in mid-gestational mouse cardiomyocytes using their custom-made antibody. At this time, two types of antibodies against $Ca_v3.1$ channel protein (sc-16259 and sc-16260) are commercially available, however those sensitivity and specificity are uncertain.

In the present study we examined the usefulness of the antibodies in mouse brain and embryonic heart.

Material and Methods

Samples were prepared from cardiac ventricles of 18 day post coitum (dpc) embryo and from brains of 10 weeks old mouse. Tissues were homogenized in cold RIPA buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 150 mM NaCl, 0.5 mM Na_3VO_4, 50 mM NaF, 1% TritonX-100) and a protease inhibitor cocktail (P8340; Sigma, USA). Protein concentration of each sample was measured using BCA Protein Assay Reagent (Pierce Biotechnology, USA).

We performed Western blot analysis using two types of polyclonal antibodies (sc-16259 and sc-16260) obtained commercially from Santa Cruz Biotechnology, Inc. (USA). These antibodies were raised in goat against the peptides mapping near the amino terminus (sc-16259; Santa Cruz Biotechnology, Inc., USA) and near the carboxy terminus (sc-16260; Santa Cruz Biotechnology, Inc., USA) of T-type Ca^{2+} channel $Ca_v3.1$ of human origin. The data sheets show that the antibodies react $Ca_v3.1$ channel protein of mouse, rat and human.

Protein lysate of the same protein amount (1000μg) was incubated with the anti-$Ca_v3.1$ antibody (sc-16259 and sc-16260) and accreted with the ProteinG-agarose (Immunopure Plus Immobilized ProteinG; Pierce Biotechnology, USA). Immunoprecipitants were washed with RIPA buffer and suspended in electrophoresis sample buffer and boiled for 5 minutes to elute the bound protein. Samples were electrophoresed on 6% standard SDS-polyacrylamide gels. Proteins in the gels

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were transferred to polyvinylidene difluoride membranes. The membranes were incubated with the primary antibodies (sc-16259 and sc-16260) (1:200) for overnight at 4°C and subsequently incubated with the second anti-gout IgG antibodies (Santa Cruz Biotechnology, Inc., USA) (1:2000) for 1 hour at 30°C without blocking by non-fat milk. The blots were visualized by the enhanced chemiluminescence (Supersignal West Dura Extended Duration Substrate; Pierce Biotechnology, USA). To estimate the molecular weight of sample proteins, we used a mixture of two molecular markers, Prestained SDS-PAGE standards, high range (Bio-Rad, USA) and MagicMark Western standard (Invitrogen, USA).

Results

Figure 1 showed the results of Western blot analysis using an anti-$Ca_v3.1$ antibody, sc-16259. Although the antibody is expected to produce a band at 271–297 kD, no band was detectable on an upper level than the 200 kD marker in 18 dpc heart. Moreover, we did not detect $Ca_v3.1$ protein in adult brain as a positive control. The other three immune precipitate samples also did not show visible bands (data were not shown). We attempted the detection of $Ca_v3.1$ protein using another anti-$Ca_v3.1$ antibody, sc-16260, however, no bands were observed in 18 dpc hearts and brains (data were not shown).

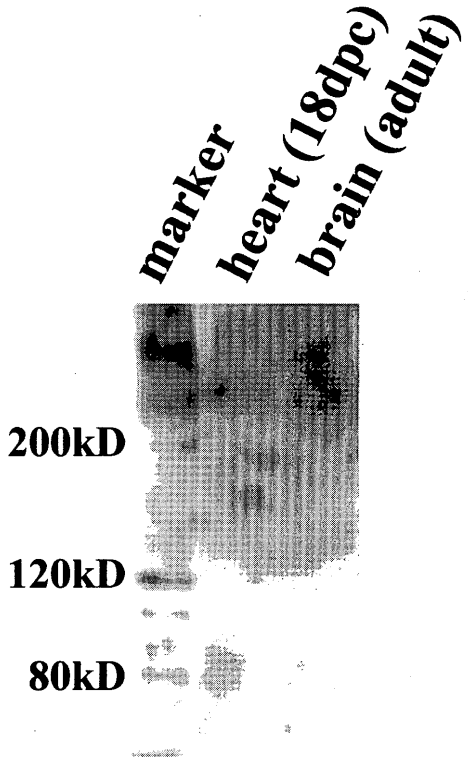

Fig. 1 Western blot analysis of $Ca_v3.1$ channel protein in mouse tissue. The left lane shows the molecular weight marker. Middle lane is from 18 dpc mouse heart and right lane is from adult mouse brain.

Discussion

In our mouse tissue samples, the anti-$Ca_v3.1$ antibodies, sc-16259 and sc-16260, did not work for detection of $Ca_v3.1$ channel protein by Western blot analysis.

T-type Ca^{2+} current have been described in various neurons (Carbone et al, 1984) and immature cardiac myocytes (Xu et al, 1902; Cribbs et al, 2001). Northern blot analysis demonstrated that $Ca_v3.1$ mRNA is predominantly expressed in the brain (Perez-Reyeset al, 1998). Additional experiments indicated that $Ca_v3.1$ mRNA is expressed at a higher level during fetal life in the heart (Monteil et al, 2000).

The expression of channel protein using specific antibodies has been demonstrated by only one research group. Cribbs et al. (Cribbs et al, 2001) provided evidence that the $Ca_v3.1$ channel protein is expressed in 14 dpc mouse cardiac myocardium by immunohistochemistry using their own antibody. However, sc-16259 and sc-16260 did not detect the $Ca_v3.1$ channel protein in adult brain and embryonic heart in our experiments. The antibodies might be less sensitive to target protein. Since the identification of channel protein using specific antibody is useful tool to prove the expression channel protein, more effective and specific antibodies will be required.

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