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Co-localization of L-type voltage dependent calcium channel alpha 1D subunit ($Ca_v1.3$) and calbindin (CB) in the mouse central nervous system

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HIGHLIGHTS

- $Ca_v1.3$ was localized in neurons of the CNS.
- Calbindin was co-localized with $Ca_v1.3$ in some of the neurons.
- The possible implications of $Ca_v1.3$ and CB co-localization were discussed.

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ABSTRACT

Previous study has shown that the co-localization of calbindin (CB) with L-type voltage dependent Ca^{2+} channel (VDCC) alpha 1C subunit ($Ca_v1.2$) in the rat insulinoma 1046-38 (RIN) beta cells may play an important regulatory role in Ca^{2+} influx and exocytosis of insulin granules. In the present study, L-type voltage dependent Ca^{2+} channel (VDCC) and calbindin (CB) were demonstrated in different regions of the mouse central nervous system (CNS). Double labeling immunofluorescence staining showed a co-localization of $Ca_v1.3$ and CB. The co-localization of $Ca_v1.3$ and CB in certain brain regions such as the hippocampus suggests their important roles in neuroplasticity. The relative high percentages of co-localization of $Ca_v1.3$ with CB in the laminae II of the dorsal horn of the spinal cord indicate that the regulation mechanism of nociceptive transmission may be related with both VDCC and Ca^{2+} binding protein.

1. Introduction

A number of cellular events, such as neurotransmitter release, hormone secretion, neuronal differentiation, migration and apoptosis, are controlled by alterations of intracellular calcium (Ca^{2+}) concentration [3,5,9]. Intracellular Ca^{2+} may be regulated by intracellular Ca^{2+} stores and Ca^{2+} regulating factors (such as Ca^{2+} channels, Ca^{2+} pumps and Ca^{2+} binding proteins). The intracellular Ca^{2+} stores, such as endoplasmic reticulum and mitochondria release Ca^{2+} to cytoplasm or uptake and store Ca^{2+} in the intracellular space [11,16,29]. The membrane Ca^{2+} channels (including voltage dependent, ligand gated and other plasma membrane ion channels) and Ca^{2+} pumps (including Ca^{2+}ATPase and Na^+/Ca^{2+} exchanger) [22,23,31,40], regulate the influx and extrusion of Ca^{2+} respectively. The Ca^{2+} binding proteins, including calbindin (CB), calretinin, parvalbumin and calmodulin, etc., act as Ca^{2+} buffer by binding Ca^{2+}, also regulate the intracellular Ca^{2+} homeostasis [2,14]. Studies indicate that both Ca^{2+} channels and Ca^{2+} binding proteins may play important role in controlling cell activity and plasticity [17]. Neurons that express only one kind of these Ca^{2+} regulators and those with a co-expression are very likely different in Ca^{2+} control ability, Ca^{2+} overload toxicity tolerance and functional complexity. From this point of view, the investigation of Ca^{2+} regulating factor expression patterns and co-localization of these factors will be important for understanding the functional activity of different groups of neurons.

Among the voltage dependent Ca^{2+} channels (VDCCs), L-type VDCC mediates long-lasting Ca^{2+} currents in response to depolarization in excitable cells. Brain L-type VDCC consists of five

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subunits: α1, α2, β, γ and δ [18,32,36]. The α1 subunits are responsible for the formation of ion-conducting pore and contain the binding sites for L-type VDCC antagonists (especially for the dihydropyridine class) [7]. Two different brain L-type VDCC α1 subunits have been identified: α1C (Ca_v 1.2) and α1D (Ca_v 1.3) [19,34].

CB is a high affinity Ca^{2+} binding protein, which is expressed in mammalian kidney, brain and pancreas [8,12,24]. In brain, CB has been reported to act as a Ca^{2+} buffer to modulate intracellular Ca^{2+} transients and evoked Ca^{2+} signals in most neuronal groups [4,6,27,38]. In the nervous system, it has been suggested that neuronal CB, by buffering Ca^{2+}, can regulate intracellular responses to physiological stimuli and protect neurons against Ca^{2+} mediated neurotoxicity [1,10,15,27].

Previous study indicated that CB was co-localized with $Ca_v1.2$ in the rat HC-13 CaBP40 cells, which played an important regulating role in Ca^{2+} influx via L-type VDCC and exocytosis of insulin granules [28]. In the presence of CB, the Ca^{2+} channels enhanced sensitivity to Ca^{2+} dependent inactivation. Further study indicated a functional interaction between CB and $Ca_v1.2$ in insulinoma 1046-38 (RIN) beta cells [20]. Combined with our previous study showing co-localization of CB with different L-type VDCCs in hippocampal interneurons [39], it suggests that CB may also modulate Ca^{2+} influx through L-type VDCCs in neurons and help to keep neuronal intracellular Ca^{2+} homeostasis.

In the present study, we aimed to investigated the co-localization of CB and α1D subunit ($Ca_v1.3$) of L-type VDCC in the mouse central nervous system (CNS) in order to provide neuroanatomical basis for further functional study of possible interaction between Ca^{2+} channel and Ca^{2+} binding protein in regulation of Ca^{2+} homeostasis in neurons in the CNS.

2. Materials and methods

A total of 10 mature adult Swiss mice (6-month-old; 5 male and 5 female respectively) were used for this study. The experiment was performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and was approved by the Xi'an Jiaotong University College of Medicine Animal Care and Use Committee.

2.1. Tissue preparation

Following deep anesthesia with chloral hydrate (0.4 g/kg), mice were perfused transcardially with 10 ml of saline initially, followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB) plus 15% picric acid (final pH 7.4) for 15 min. After perfusion, the brain and spinal cord of mice were removed and put into the same perfusion solution for a 2 h post-fixation, and then transferred in 30% sucrose in 0.1 M PB and kept overnight. Coronal sections in brain and spinal cord at 40 μm thickness were cut in a cryostat (HM505E, Microm, Zeiss, Germany). Serial sections were transferred to a 24-well tissue culture dish for immunofluorescence reaction.

2.2. Double labeling immunofluorescence staining

To identify co-localization of $Ca_v1.3$ and CB in different region of the mouse brain, double labeling immunofluorescence staining was carried out. For this study, brain (including cerebra, olfactory bulb and cerebellum) and spinal cord sections were washed in 0.1 M phosphate-buffered saline (PBS) containing 0.1% Triton-X 100 and treated with 0.1 M PBS (pH 7.4) containing 0.3% H_2O_2 to eliminate the endogenous peroxidase activity. After 2 h incubation in 4% normal goat serum containing 0.1% Triton-X 100, the sections were placed overnight in primary mouse anti-$Ca_v1.3$ monoclonal antibody ($Ca_v1.3$, 1:200; Abcam) and primary rabbit anti-CB-D28k (1:500; Santa Cruz Biotechnology, Inc.). Sections were then washed in PBS and placed for 1 h in rhodamine-conjugated goat anti- mouse IgG together with fluorescein isothiocynate (FITC)-conjugated goat anti- rabbit IgG (Chemicon International, Inc., CA, USA), diluted 1:100 in PBS/Triton X-100. After the reaction, sections were washed again, mounted, dried, and coverslipped with FluorSave™ Reagent (Calbiochem-Novabiochem, CA) to retard fading. Fluorescent signals were detected using a Leica confocal laser microscope (TCS4D; Leica, Heidelberg, Germany).

2.3. Data analysis

For quantitative analysis, one section with double immunofluorescence labeling from ten animals each was randomly selected to count the cell number. In areas with low density of $Ca_v1.3$+ neurons, such as the stratum oriens and stratum radium of CA1, all the $Ca_v1.3$+ and the double labelled neurons were counted. In the area with high density of neurons such as the stratum pyramidale of CA1, we randomly chose 5 small areas to count cell number to get mean density, and then got the total number by mean density X the total areas counted. As most neurons were $Ca_v1.3$ positive, each of $Ca_v1.3$ and CB double-labeled neurons were counted under confocal microscope and indicated as the percentage to fluorescence labeled $Ca_v1.3$ neurons in each subfield of the brain and spinal cord. The results were expressed as mean ± S.M.E.

3. Results

In the present study, no obvious difference for the distribution pattern of $Ca_v1.3$, CB and their co-localization in the CNS were observed between male and female mice. In the hippocampus, $Ca_v1.3$ immunopositive product was localized in the principle neuron, including the pyramidal neuron of the CA1-3 areas and the granule cell of the dentate gyrus (DG) (Fig. 1A–D); and interneurons in the stratum oriens, stratum radium, and stratum lacunosum moleculare of CA1-3 areas and DG (including molecular layer and hilus) (Fig. 1A–D). $Ca_v1.3$ immunopositive product was mainly deposited in the soma. In CA1 area, most CB immunopositive neurons were located in the inner border of the stratum pyramidale with relative weak immunoreactivity intensity (Fig. 1A1 and B1) and the other CB immunopositive neurons with relative strong immunostaining (Fig. 1A1, B1, A2 and B2) were $Ca_v1.3$ immunopositive. In the stratum lucidum of CA3 area, CB immunopositive mossy fibers were demonstrated (Fig. 1A1 and C1). In the stratum lacunosum moleculare, $Ca_v1.3$ and CB co-localized interneurons were observed (Fig. 1C2). In DG, most of the CB immunopositive granule cells were also $Ca_v1.3$ immunopositive (Figs. 1D1, D2 and 3). In cortical areas, such as primary motor cortex (Fig. 1E–E2) and piriform cortex (Fig. 2A–A2), some CB immunopositive neurons were also $Ca_v1.3$ immunopositive (Fig. 3).

In the olfactory bulb, $Ca_v1.3$ was strongly expressed in neurons of the mitral cell layer; but moderately expressed in neurons of the granular cell layer and glomerular layer. In the external plexiform layer, $Ca_v1.3$ immunopositive product was mainly localized in the processes of neurons (Fig. 2B). In the olfactory bulb, CB immunopositive neurons appeared in the glomerular layer (Fig. 2B1). No CB and $Ca_v1.3$ double-labeled neuron was demonstrated in olfactory bulb (Figs. 2B2 and 3).

In the cerebellum, $Ca_v1.3$ immunopositive product was localized in the cell body of Purkinje cells, whereas moderate $Ca_v1.3$ immunopositive products were shown in the granular cell layer and the molecular layer (Fig. 2C). Strong CB immunopositive product was localized in the cell body and tree-like processes of the Purkinje cells (Fig. 2C1). Almost all $Ca_v1.3$ immunopositive Purkinje cells were CB immunopositive (Figs. 2C2 and 3).