Single Cell Analysis of Voltage-gated Potassium Channels for Electrophysiological Properties of Rat Hypothalamic Paraventricular Nucleus Neurons

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INTRODUCTION

The hypothalamic paraventricular nucleus (PVN) is an important integrating site in the regulation of neuroendocrine and the autonomic nervous system. The PVN neurons are composed of heterogeneous neurons according to their different electrophysiological properties, presence and absence of the transient outward rectification, in type I and type II, respectively. The property is known to be associated with the voltage-gated K+ (Kv) currents. However, the direct correlation between electrophysiological properties and Kv channel subunits expression in PVN neurons has not been reported yet. In the present study, we focus on the expression and the quantitative analysis of the specific Kv α subunits that are responsible for transient outward rectification in PVN neurons, which is characterized by a delay to the onset of the first action potential.

METHODS

1. Single cell RT-PCR and real-time RT-PCR
   ① Classification of PVN neurons by whole cell current-clamp recording

   ② Collection of cytoplasm of each single cell
   ③ Single cell RT-PCR for Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv4.1, Kv4.2 and Kv4.3
   ④ Gel-electrophoresis
   ⑤ Single cell real-time RT-PCR for Kv1.2, Kv1.3, Kv4.2 and Kv4.3
2. Protein expression of Kv α subunits
   - Immunohistochemistry

RESULTS

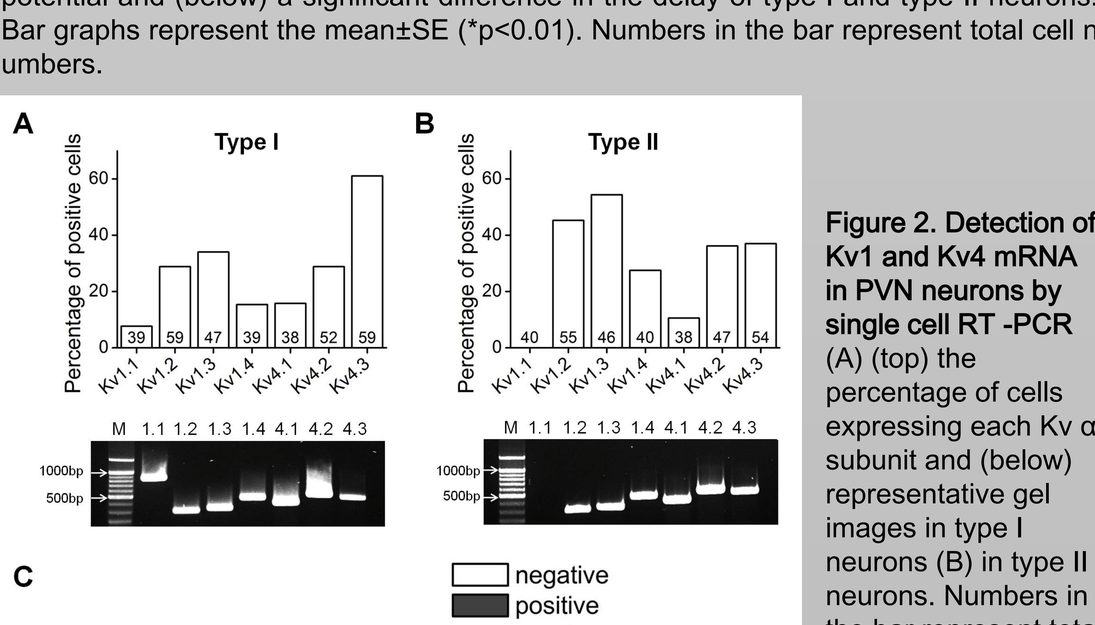

Figure 1. Classification of PVN neurons by electrophysiological properties in whole-cell current-clamp recording (A) type I neurons were characterized by a delay to the onset of the first action potential, which were not found in type II neurons. (B) the distribution of identified type I and type II neurons (C) (top) measurement of a delay (Δt) based on the duration from input of depolarizing current (-105 pA) to initiation of the first action potential and (below) a significant difference in the delay of type I and type II neurons. Bar graphs represent the mean±SE (*p<0.01). Numbers in the bar represent total cell n umbers.

Figure 2. Detection of Kv1 and Kv4 mRNA in PVN neurons by single cell RT -PCR (A) (top) the percentage of cells expressing each Kv α subunit and (below) representative gel images in type I neurons (B) in type II neurons. Numbers in the bar represent total cell numbers. (C) the proportion of positive and negative cells for each Kv α subunit in type I and type II neurons. A significant difference in the proportion of Kv4.3 subunit (*p<0.05, Fisher's exact test).

RESULTS

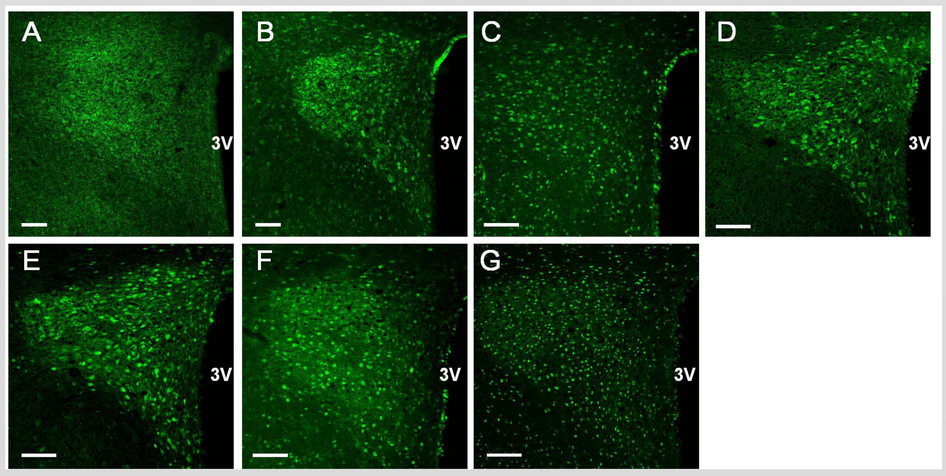

Figure 3. Immunohistochemical detection of Kv1.1 (A), Kv1.2 (B), Kv1.3 (C), Kv1.4 (D), Kv4.1 (E), Kv4.2 (F) and Kv4.3 (G) in PVN 3V, the third ventricle; scale bar, 100 μm

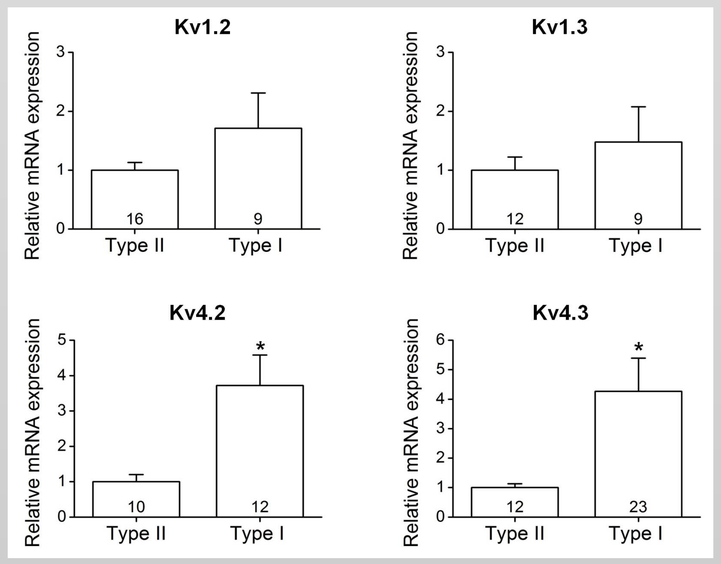

Figure 4. Relative quantification of Kv α subunits mRNA expression in type I and type II PVN neurons by single cell real-time RT-PCR.
The relative amounts of Kv4.2 and Kv4.3 mRNA in type I neurons were significantly higher compared to type II neurons. Bar graphs represent the mean±SE (*p<0.05). Numbers in the bar represent total cell numbers.

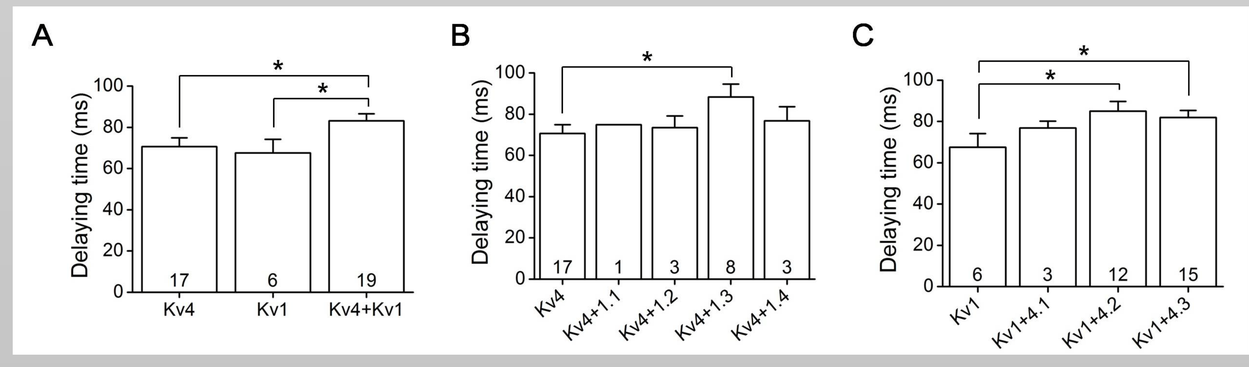

Figure 5. Relation between delay time and Kv channel expression in type I PVN neurons.
(A) PVN neurons expressing Kv1 and Kv4 together demonstrated longer delay to the onset of the first action potential than the cells expressing Kv1 or Kv4 only (*p<0.05). (B) co-expression of Kv4.x with Kv1.3 significantly extended the delay time in type I cells compared to cells expressing Kv4.x only (*p<0.05). (C) co-expression of Kv1.x with Kv4.2 or Kv4.3 significantly extended the delay time in type I cells compared to cells expressing Kv1.x only (*p<0.05). Numbers in the bar represent total cell numbers.

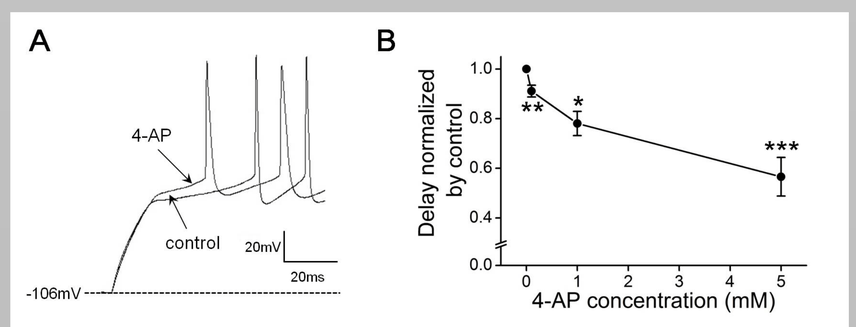

Figure 6. Effects of Kv channel blockers on delay to the onset of the first action potential in type I PVN neurons (A) a delay in type I neuron was attenuated by 5mM 4-AP, A-type K+ channel blocker. (B) reduction in delay by 0.1mM(**p<0.01), 1mM (*p<0.05) and 5mM (***p<0.005) 4-AP.

CONCLUSION

- Single cell RT-PCR and immunohistochemical analysis revealed that a variety of Kv α subunits are expressed in the hypothalamic PVN neurons.
- Kv1.2, Kv1.3, Kv1.4, Kv4.1, Kv4.2 and Kv4.3 mRNA were detected in both type I and type II PVN neurons, but Kv1.1 was detected only in type I neurons. Among them, only Kv4.3 showed a significant difference in the proportion of positive and negative cells between type I and type II neurons.
- Single cell real-time PCR analysis revealed that Kv4.2 and Kv4.3 are expressed in higher density at single cell level in type I than type II neurons.
- The analysis on the relation of delay with Kv channel expression suggests that Kv1.3, Kv4.2 and Kv4.3 are the potential candidates in determining a distinct electrophysiological property in PVN neurons.