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Prokineticin 2 suppresses GABA-activated current in rat primary sensory neurons

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ABSTRACT

Prokineticin 2 (PK2) is a newly identified regulatory protein, which is involved in a wide range of physiological processes including pain perception in mammals. However, the precise role of PK2 in nociception is yet not fully understood. Here, we investigate the effects of PK2 on $GABA_A$ receptor function in rat trigeminal ganglion neurons using whole-cell patch clamp technique. PK2 reversibly depressed inward currents produced by $GABA_A$ receptor activation (I_{GABA}) with an IC_{50} of 0.26 ± 0.02 nM. PK2 appeared to decrease the efficacy of GABA to $GABA_A$ receptor but not the affinity. The maximum response of the GABA dose–response curve decreased to 71.2 ± 7.0% of control after pretreatment with PK2, while the threshold value and EC_{50} of curve did not alter significantly. The effects of PK2 on I_{GABA} were voltage independent. The PK2-induced inhibition of I_{GABA} was removed by intracellular dialysis of either GDP-β-S (a non-hydrolyzable GDP analog), EGTA (a Ca^{2+} chelator) or GF109203X (a selective protein kinase C inhibitor), but not by H89 (a protein kinase A inhibitor). These results suggest that PK2 down-regulates the function of the $GABA_A$ receptor via G-protein and protein kinase C dependent signal pathways in primary sensory neurons and this depression might underlie the hyperalgesia induced by PK2.

1. Introduction

Prokineticin 2 (PK2) is a secreted protein that regulates diverse biological processes including pain perception (Negri et al., 2009, 2007). Intraplantar injection of PK2 and Bv8, the frog homolog of PK2, causes a strong and localized hyperalgesia by reducing the nociceptive thresholds to thermal and mechanical stimuli (Hu et al., 2006; Mollay et al., 1999; Negri et al., 2002). Systemic injection of Bv8 into rats also induces hyperalgesia to tactile and thermal stimuli (Negri et al., 2002). Genetic studies with PK2-deficient mice confirm the critical involvement of PK2 in acute and inflammatory pain (Hu et al., 2006). PK2-deficient mice displayed significant reduction in nociception induced by thermal and chemical stimuli. In mammals, PK2 is the cognate ligand for two closely homologous G-protein-coupled receptors, PKR1 and PKR2, which are expressed in primary sensory neurons (Hu et al., 2006; Li et al., 2001; Lin et al., 2002). Calcium image studies have revealed that the majority of DRG neurons activated by PK2 (Hu et al., 2006). Mice lacking the PKR1 gene exhibited impaired pain perception to various stimuli, including noxious heat, mechanical, capsaicin, and protons (Negri et al., 2006), indicating that PKR1 is probably the dominant receptor for PK2 in the regulation of pain sensation.

The γ-aminobutyric acid (GABA) is a major inhibitory transmitter that acts not only through the $GABA_A$ but also via $GABA_B$ receptor in the central nervous system. It mediates synaptic inhibition in the axo-axonal synapse in the spinal cord, which causes a reduction of the release of excitatory transmitter from primary afferent nerve terminals, termed 'presynaptic inhibition' (Eccles, 1964; Schmidt, 1971). It has been suggested that a variety of substances modulate the GABA response through phosphorylation and dephosphorylation of the $GABA_A$ receptor–chloride channel complex (Chen and Wong, 1995; Gyenes et al., 1994; Hu and Li, 1997; Li et al., 2009; Si et al., 2004; Smith and Olsen, 1995; Yamada and Akasu, 1996). In the present study, we report that PK2 inhibited $GABA_A$ receptor-mediated responses via G-protein and protein kinase C (PKC) in freshly isolated rat primary sensory neurons.

2. Methods

2.1. Isolation of the trigeminal ganglion (TG) neurons

Two- to three-week old Sprague-Dawley rats were anesthetized with ether and then decapitated. The TGs were taken out and transferred immediately into Dulbecco's modified Eagle's medium (DMEM, Sigma) at pH 7.4. After the removal of the

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surrounding connective tissues, the TGs were minced with fine spring scissors and the ganglion fragments were placed in a flask containing 5 ml of DMEM in which trypsin (type II-S, Sigma) 0.5 mg/ml, collagenase (type I-A, Sigma) 1.0 mg/ml and DNase (type IV, Sigma) 0.1 mg/ml had been dissolved, and incubated at 35 °C in a shaking water bath for 30–35 min. Soybean trypsin inhibitor (type II-S, Sigma) 1.25 mg/ml was then added to stop trypsin digestion. Dissociated neurons were placed into a 35-mm Petri dish and kept for at least another 30 min before electrophysiological recording. The neurons selected for patch clamp experiment were 15–45 μm in diameter.

2.2. Electrophysiological recordings

Whole-cell patch clamp recordings were carried out at room temperature (22–24 °C) using a whole-cell patch clamp amplifier (CEZ-2400, Nihkon Kohden, Japan). The micropipettes were filled with internal solution containing (mM): KCl 140, $MgCl_2$ 2.5, HEPES 10, EGTA 11 and ATP 5; its pH was adjusted to 7.2 with KOH and osmolarity was adjusted to 310 mOsm/L with sucrose. Cells were bathed in an external solution containing (mM): NaCl 150, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 2, HEPES 10, D-glucose 10; its osmolarity was adjusted to 330 mOsm/L with sucrose and pH was adjusted to 7.4 with NaOH. The resistance of the recording pipette was in the range of 2–5 MΩ. A small patch of membrane underneath the tip of the pipette was aspirated to form a gigaseal and then a negative pressure was applied to rupture it, thus establishing a whole-cell configuration. The adjustment of capacitance compensation and series resistance compensation was done before recording the membrane currents. The holding potential was set at −60 mV, except when indicated otherwise. Membrane currents were filtered at 10 kHz (−3 dB), and the data were recorded by a pen recorder (Nihon Kohden, Japan). The gramicidin perforated-patch whole-cell recording technique was also used to preserve intracellular Cl^- levels in a few cases where indicated (Akaike, 1996).

2.3. Intracellular dialysis by using re-patch technique

For proceeding re-patch experiment the first patch is available as the control using pipette, which filled with normal internal solution. After current recording has been done, the pipette was discarded. On the same neuron the second patch is performed using another pipette that filled with normal, GDP-β-S, GF109203X, EGTA or H89 etc-containing internal solution. After 30 min, membrane current is recorded again and compared with the results of the control.

2.4. Drug application

Drugs used in the experiments include: PK2 (Zhou lab, University of California, Irvine, CA, USA), GABA (Sigma), bicuculline (Sigma) and PMA (Sigma). All drugs were dissolved daily in the external solution just before use and held in a linear array of fused silica tubes (o.d./i.d. = 500 μm/200 μm) connected to a series of independent reservoirs. The distance from the tube mouth to the cell examined was around 100 μm. The application of each drug was driven by gravity and controlled by the corresponding valve, and rapid solution exchange could be achieved within about 100 ms by shifting the tubes horizontally with a PC-controlled micromanipulator. Cells were constantly bathed in normal external solution flowing from one tube connected to a larger reservoir between drug applications. In some experiments, GDP-β-S, GF109203X, EGTA or H89 that needed to be applied intracellularly was dissolved in the internal solution.

2.5. Data analysis

Data were statistically compared using the Student's *t*-test or analysis of variance (ANOVA). Statistical analysis of concentration–response data was performed using nonlinear curve-fitting program ALLFIT. Current values are expressed as mean ± SEM.

3. Results

3.1. Depression of GABA-activated current by PK2 in rat TG neurons

Freshly isolated neurons from rat TG in the range of 15–45 μm were used in the present study. Application of GABA (10^{-4} M) to the superfusing solution for 10 s caused an inward current (I_{GABA}) in the majority of the neurons examined (94.2%, 113/120). This I_{GABA} could be blocked reversibly by bicuculline (5×10^{-5} M), a selective antagonist of $GABA_A$ receptor, indicating this current was mediated by the $GABA_A$ receptor in rat TG neurons (Fig. 1A). The I_{GABA} was repeated stably within 90 min and the change in amplitude was within 7.0%, when GABA was applied regularly for 10-s durations with 5-min intervals. Thus, we used this pattern of GABA applications in the following experiments.

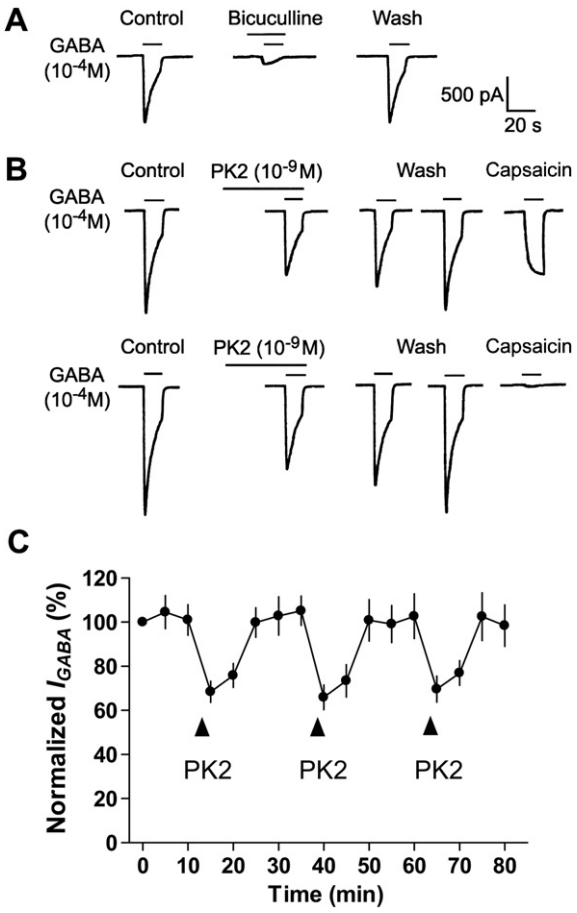

Fig. 1. Prokineticin 2 depresses the $GABA_A$ receptor currents. (A) The inward current evoked by 10^{-4} M GABA could be blocked by the $GABA_A$ receptor antagonist bicuculline (5×10^{-5} M) in a trigeminal ganglion (TG) neuron voltage clamped at −60 mV (B) PK2 exerts an inhibitory effect on GABA-activated currents (I_{GABA}) in a capsaicin-sensitive neuron from rat TGs with conventional whole-cell recording (upper row). The diameter of the neuron is 24 μm. PK2 also inhibits I_{GABA} in a capsaicin-insensitive neuron with gramicidin perforated-patch recording (lower row). The diameter of the neuron is 31 μm. I_{GABA} was elicited by application of GABA (10^{-4} M) for 10 s durations. PK2 (10^{-9} M) was pre-applied to external solution for 2 min. (C) The depression of I_{GABA} produced by repeated applications of PK2. Data were obtained from a total of 7 cells. Arrows indicate the pre-applications of PK2 (10^{-9} M). GABA (10^{-4} M) was applied regularly with 5-min intervals.

Thirteen TG neurons sensitive to GABA (10^{-4} M) were pretreated with PK2 (10^{-9} M) for 2 min. We observed that the majority (69.2%, 9/13) of I_{GABA} recorded were depressed obviously by PK2 and the amplitude of I_{GABA} decreased to 68.2 ± 7.3% of control in the 9 neurons ($P < 0.01$, paired *t*-test) (Fig. 1B). PK2 had no effect on the I_{GABA} in the other four neurons examined (30.8%, 4/13) and the I_{GABA} change in amplitude was 4.1 ± 0.9% ($P > 0.05$, paired *t*-test). In the present study, we established a cut-off value for the effect of PK2, which was the I_{GABA} change in amplitude exceeding 10.0%. When pooling all the data from the 13 neurons examined, pre-application of PK2 was found to decrease the I_{GABA} by 23.3 ± 3.5% ($P < 0.05$, paired *t*-test). The effect of PK2 may be independent on size of cell examined. The PK2 showed inhibitory effect on I_{GABA} of the nine TG neurons in the whole range of 15–45 μm, and the four neurons with no response to PK2 were 18, 25, 33 and 42 μm, respectively. Inhibition of PK2 on I_{GABA} was TRPV1 independent and observed in both capsaicin-sensitive and capsaicin-insensitive TG neurons (Fig. 1B).