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Analysis of G-protein-activated inward rectifying K^+ (GIRK) channel currents upon $GABA_B$ receptor activation in rat supraoptic neurons

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ABSTRACT

While magnocellular neurons in the supraoptic nucleus (SON) possess rich Gi/o-mediated mechanisms, molecular and cellular properties of G-protein-activated inwardly rectifying K^+ (GIRK) channels have been controversial. Here, properties of GIRK channels are examined by RT-PCR and whole-cell patch-clamp techniques in rat SON neurons. Patch clamp experiments showed that the selective $GABA_B$ agonist, baclofen, enhanced currents in a high K^+ condition. The baclofen-enhanced currents exhibited evident inward rectification and were blocked by the selective $GABA_B$ antagonist, CGP55845A, the IRK channel blocker, Ba^{2+}, and the selective GIRK channel blocker, tertiapin, indicating that baclofen activates GIRK channels via $GABA_B$ receptors. The GIRK currents were abolished by N-ethylmaleimide pretreatment, and prolonged by GTPγS inclusion in the patch pipette, suggesting that Gi/o proteins are involved. RT-PCR analysis revealed mRNAs for all four GIRK 1–4 channels and for both $GABA_BR1$ and $GABA_BR2$ receptors in rat SON. However, the concentration-dependency of the baclofen-induced activation of GIRK currents had an EC_{50} of 110 μM, which is about 100 times higher than that of baclofen-induced inhibition of voltage-dependent Ca^{2+} channels. Moreover, baclofen caused no significant changes in the membrane potential and the firing rate. These results suggest that although GIRK channels can be activated by $GABA_B$ receptors via the Gi/o pathway, this occurs at high agonist concentrations, and thus may not be a physiological mechanism regulating the function of

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SON neurons. This property that the membrane potential receives little influence from GIRK currents seems to be uncommon for CNS neurons possessing rich Gi/o-coupled receptors, and could be a special feature of rat SON neurons.

1. Introduction

It is known that magnocellular neurons in the supraoptic nucleus (SON) of the hypothalamus that produce and secrete vasopressin or oxytocin possess several distinct types of K^+ channels. Several types of voltage-dependent K^+ channels (delayed rectifying K^+ channels, rapidly inactivating K^+ channels, and Ca^{2+}-activated K^+ channels) and their modulation by membrane potentials and physiological ligands have been reported to play key roles in SON neurons (Bourque et al., 1985; Bourque, 1988; Cobbett et al., 1989; Muller et al., 1999; Nagatomo et al., 1995). The voltage-independent groups of K^+ channels consist of two large superfamilies, namely, the inwardly rectifying K^+ (IRK) channel group and the tandem-pore K^+ channel group. It has been reported that SON neurons possess several types of tandem-pore K^+ channels, and that these channels play roles in the maintenance of the resting membrane potential in SON neurons (Han et al., 2003a).

Much less information is available for the IRK subgroup of K^+ channels and their functions in the physiological regulation of SON neurons. In situ hybridization histochemistry revealed that multiple types of G-protein-activated IRK (GIRK) channels are present in the SON (Karschin et al., 1996; Li et al., 2001; Saenz del Burgo et al., 2008). Four distinct subclasses of GIRK channels have been cloned from mammalian tissues to date and termed GIRK1–4 (Hibino et al., 2010; Inanobe and Kurachi, 2014; Jan and Jan, 1997; Luscher and Slesinger, 2010). GIRK channels are known to be activated by G-protein-coupled receptors (GPCRs) that are coupled to the Gi/o subclass, which also inhibits voltage-dependent Ca^{2+} (VDC) channels and adenylate cyclases (Limbird, 1988; Nicoll, 1988). It is known that SON neurons possess several Gi/o-coupled receptors such as dopamine D_2, galanin, opioid, and $GABA_B$ receptors (Harayama et al., 1998; Muller et al., 1999; Papas and Bourque, 1997; Soldo and Moises, 1998; Yang et al., 1991). It has been reported that activation of D_2 receptors in SON neurons caused cation channel activation (Yang et al., 1991) and that of galanin or opioid receptors caused K^+ channel activation, however, the K^+ channels activated by galanin or opioids were not IRK channels (Muller et al., 1999; Papas and Bourque, 1997).

Even though it has also been reported that the selective $GABA_B$ receptor agonist, baclofen, activated IRK channels, and also hyperpolarized SON neurons of guinea pigs (Slugg et al., 2003), the $GABA_B$ receptor-mediated activation of GIRK channels in SON neurons are controversial. Another study conducted in guinea pig SON neurons found no effect of baclofen (Ogata, 1987). In rat SON neurons, there is a report that baclofen caused a small membrane hyperpolarization and reduced firing activity (Li and Stern, 2004). On the other hand, several studies conducted in rat SON neurons reported that $GABA_B$ receptor activation had no effect on the membrane currents under voltage-clamped conditions (Kabashima et al., 1997; Kombian et al., 1996; Mouginot et al., 1998; Wuarin and Dudek, 1993), and also that baclofen did not affect the membrane potential of cultured SON neurons (Jourdain et al., 1996). In fact, although expression of GIRK channels in the SON has been identified (Karschin et al., 1996; Li et al., 2001; Saenz del Burgo et al., 2008), there has been no report showing activation of GIRK currents in response to any ligands in rat SON neurons. This seems very different from the situation in other CNS neurons, such as neurons in the cerebellum or hippocampus, where GIRK channels play major roles in the slow component of the inhibitory postsynaptic potentials (IPSPs) (Inanobe and Kurachi, 2014; Luscher and Slesinger, 2010; North, 1989).

The purpose of the present study was to examine whether GIRK channels function in rat SON neurons. For this purpose, we focused on effects of $GABA_B$ receptor activation on GIRK channels to examine this, because we have previously found that $GABA_B$ receptors are in the postsynaptic sites of rat SON magnocellular neurons and $GABA_B$ receptor activation inhibits VDC channels in SON neurons (Harayama et al., 1998). We have also reported that $GABA_B$ receptor activation leads to the suppression of action potential discharge in rat SON neurons (Ibrahim et al., 1998). In the present study, we found, for the first time, enhanced GIRK currents upon $GABA_B$ receptor activation in rat SON neurons, however, the amplitude of enhanced currents was very small, and moreover, the enhancement requires nearly 100 times higher concentrations of baclofen than for inhibition of VDC currents.

2. Results

In order to measure IRK currents accurately, we used a high (50 mM) extracellular K^+ solution to record membrane currents in the voltage-clamp mode, because it has been reported that IRK channels favor this environment (Davila et al., 2003; Han et al., 2003b; Luscher and Slesinger, 2010; Slesinger et al., 1997; Sodickson and Bean, 1996; Takigawa and Alzheimer, 1999b). An increase in the extracellular K^+ concentration consistently elicited an increase in inward currents through K^+ channels (Fig. 1A&B). In this high K^+ solution, the selective $GABA_B$ agonist, baclofen, at a concentration of 100 μM, enhanced the inward currents reversibly. An enhancement larger than 10 pA was observed in 39 out of 79 cells (49%). Baclofen at 10 μM, a concentration that consistently inhibited voltage-dependent Ca^{2+} channels (Harayama et al., 1998), enhanced inward currents in only 5 out of 29 cells examined (17%). The average peak amplitude of the current response to baclofen recorded in the