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Research Report

Possible contribution of pannexin channel to ATP-induced currents *in vitro* in vasopressin neurons isolated from the rat supraoptic nucleus

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ABSTRACT

Release of arginine vasopressin (AVP) from magnocellular neurosecretory cells (MNCs) of the supraoptic nucleus (SON) is controlled by the electrical activity of these neurons. ATP plays a crucial role in the regulation of SON MNCs by activating the purinergic P2X and P2Y receptors. Recent reports of interaction between P2X receptors and pannexin channels have provided new insights into the physiology of the central nervous system; however, the function of pannexin channels has not been assessed in AVP neurons. In the present study, we examined the possible contribution of the pannexin channel in ATP-induced responses in SON AVP neurons. We used the whole-cell patch-clamp technique in isolated rat SON MNCs that express an AVP-enhanced green fluorescent protein transgene. The ATP-induced current was inhibited in a concentration-dependent manner by pannexin channel blockers carbenoxolone and mefloquine, whereas the connexin channel blockers flufenamic acid and lanthanum had no effect. Multi-cell reverse transcriptase-polymerase chain reaction experiments confirmed the existence of pannexin-1 mRNA in AVP neurons. The involvement of the ATP-activated transient receptor potential vanilloid and acid-sensing ion channels was excluded. These results suggest that pannexin channels in SON AVP neurons are involved in the regulatory mechanisms of neuronal activity.

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Abbreviations: ASIC, acid-sensing ion channels; AVP, arginine vasopressin; CBX, carbenoxolone; eGFP, enhanced green fluorescent protein; FFA, flufenamic acid; HBS, HEPES buffer solution; KHB, Krebs–Henseleit solution; MFQ, mefloquine; MNC, magnocellular neurosecretory cell; RT-PCR, reverse transcription-polymerase chain reaction; SON, supraoptic nucleus; TRPV, transient receptor potential vanilloid

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1. Introduction

Extracellular ATP, which acts as gliotransmitter and neurotransmitter in the central nervous system (CNS), activates the ionotropic P2X (P2X1-7) and metabotropic P2Y (P2Y1, 2, 4, 6, 11, 12, 13, and 14) receptors on the cell surface (Abbracchio et al., 2006; Day et al., 1993; Halassa and Haydon, 2010; Hamilton and Attwell, 2010; Housley et al., 2009; North, 2002; Sladek, 2000). Previous studies have shown that multiple P2X and P2Y, including subunits P2X2, 3, 4, 2/3, 7, and P2Y1, are functionally expressed in hypothalamic supraoptic nucleus (SON) magnocellular neurosecretory cells (MNCs) (Gomes et al., 2009; Hiruma and Bourque, 1995; Shibuya et al., 1999; Song et al., 2007).

Studies of the interaction between some types of P2X receptors and the large nonselective conductance pannexin channel have provided insight into the physiology of the CNS (Evans et al., 1996; MacVicar and Thompson, 2010). Recent reports provided evidence that the pannexin channel is likely the permeation pore induced by the P2X receptor activation (Iglesias et al., 2008; Locovei et al., 2007; Pelegrin and Surprenant, 2006).

Members of the pannexin gene family (pannexin-1, pannexin-2, and pannexin-3) are homologous to the innexins, which are invertebrate gap junction proteins (Bruzzone et al., 2003). No significant sequence similarity has been found between pannexins/innexins and connexins, the prototypical gap junction proteins (Yen and Saier, 2007). The most-studied pannexin family member in the CNS is pannexin-1, a voltage-sensitive ~500-pS channel (MacVicar and Thompson, 2010). Neither pannexin-2 nor pannexin-3 form functional homomeric channels (Barbe et al., 2006; Bruzzone et al., 2003). Although pannexin-1 mRNA are widely expressed in the brain including in SON MNCs (Bruzzone et al., 2003; Ray et al., 2005), their functional interaction with P2X activation in SON arginine vasopressin (AVP) neurons has not been assessed. Therefore, we examined whether ATP-induced currents are mediated by pannexin channels in SON AVP neurons. In the present study, we observed transient ATP-induced activation of pannexin channels and P2X receptors in SON AVP neurons. Extracellular ATP-induced currents were recorded with the whole-cell patch-clamp technique from dissociated SON MNCs expressing the AVP-enhanced green fluorescent protein (eGFP) transgene (Ueta et al., 2005). Pannexin channel antagonists carbenoxolone (CBX) and mefloquine (MFQ) inhibited the peak ATP-induced current in a concentration-dependent manner, whereas the connexin channel antagonists flufenamic acid (FFA) and lanthanum (La^{3+}) exerted no significant effect. We ruled out the contribution of the ATP-activated transient receptor potential vanilloid (TRPV) and acid-sensing ion channels (ASICs), which are structurally similar to P2X (Gonzales et al., 2009; Phelps et al., 2010). Multi-cell reverse transcription-polymerase chain reaction (RT-PCR) experiments confirmed that pannexin-1 gene was expressed in AVP-eGFP neurons. These results suggest that pannexin channels play an important role in regulating neuronal excitability in SON AVP neurons.

2. Results

2.1. ATP-induced inward currents

We first applied 100 μM ATP to dissociated AVP-eGFP SON neurons cultured for 24 h and identified by their green fluorescence. ATP rapidly induced a desensitizing inward current when the holding potential was −70 mV, consistent with a previous report (Shibuya et al., 1999). Peak amplitude of currents induced by first and second application of ATP were 34.7±5.1 pA/pF and 21.0±3.8 pA/pF, respectively (n=16, Fig. 1A). We next evaluated the sensitivity of ATP-activated channels to P2X receptors antagonist pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS). Pretreatment with the 50 μM PPADS abolished the ATP-induced current (1.48±0.5 pA/pF, $P<0.01$; n=3, Fig. 1B), which indicate that P2X receptors are largely involved in the ATP-induced currents in SON AVP neurons, also in agreement with previous reports (Hiruma and Bourque, 1995; Shibuya et al., 1999).

2.2. Blockade of ATP-induced current by pannexin channel antagonists

To assess whether pannexin channels participate in ATP-induced current response in AVP neurons, we exposed cells to four compounds that affect gap junction channels, (carbenoxolone; CBX, mefloquine; MFQ, flufenamic acid; FFA, and lanthanum; La^{3+}), and measured current amplitudes induced by 100 μM ATP. The effect of pannexin channel blocker CBX was concentration-dependent (Fig. 2A–D): peak amplitude of second application of ATP-induced currents in pA/pF in a 30-s pretreatment with 100 μM or 10 μM CBX was significantly smaller than that in the control (100 μM CBX: 6.19±1.6 pA/pF, n=7, $P<0.01$ versus control; 10 μM CBX: 12.2±2.4 pA/pF, n=9, $P<0.05$ versus control), whereas a 30-s pretreatment with 1 μM CBX produced no significant effects (1 μM CBX: 23.1±5.7 pA/pF, n=6, $P>0.05$ versus control). Similarly, another pannexin channel blocker MFQ inhibited ATP-induced currents in a concentration-dependent manner (Fig. 3A–D). Peak amplitude of second application of ATP-induced currents in pA/pF in a 30-s pretreatment with 10 μM MFQ was significantly smaller than that in the control (10 μM MFQ: 10.2±1.8 pA/pF, n=6, $P<0.01$ versus control), whereas a 30-s pretreatment with 1 μM and 0.1 μM MFQ produced no significant effects (1 μM MFQ: 22.6±4.7 pA/pF, n=7, $P>0.05$ versus control; 0.1 μM MFQ: 23.7±7.5 pA/pF, n=5, $P>0.05$ versus control). In contrast, the ATP-induced current was not significantly inhibited by connexin channel blocker FFA at concentration of 300 μM (24.8±4.0 pA/pF, n=5, $P>0.05$ versus control) (Fig. 4A, C), which is considered sufficient to produce the maximum effect (Bruzzone et al., 2005). Similarly, the ATP-induced current were not modulated by another connexin channel blocker La^{3+} at a concentration of 10 μM (24.6±5.8 pA/pF, n=5, $P>0.05$ versus control) (Fig. 4B, C). These results suggest that ATP-induced currents in SON AVP neurons may be due, in part, to CBX-sensitive and MFQ-sensitive channels. In each experiment, there were no statistical differences of the peak amplitude evoked by first application of ATP compared to control.