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BRAIN RESEARCH

Research Report

Modulation by BNP of GABA receptors on ON-type rod bipolar cells is dependent on subcellular sites

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ABSTRACT

Brain natriuretic peptide (BNP) suppresses $GABA_A$ receptor-mediated current of ON-type rod-dominant bipolar cells (RBCs) in the rat retina. Here we report that such BNP-induced modulation is dependent on subcellular sites. Whole-cell currents could be induced by GABA focally applied to both dendrites/somata and axon terminals of isolated ON-type RBCs. Whilst the GABA currents induced at the axon terminal were significantly suppressed by BNP (50 nM), those at the dendrites/somata were hardly changed or slightly suppressed. Similar results were obtained when such experiments were performed in rat retinal slices. Calcium imaging showed that application of BNP (50 nM) caused a prominent increase in intracellular calcium concentrations ($[Ca^{2+}]_i$) at the axon terminal, and the increase monotonically decayed when the acting site of BNP was moved away from the axon terminal along the cell: more distant, less significant. No detectable increase in $[Ca^{2+}]_i$ was found at the dendrites. Such increase in $[Ca^{2+}]_i$ could be completely blocked by pre-incubation with anantin, an antagonist of the NP-receptor-A (NPR-A). On the other hand, caffeine, an agonist of the ryanodine receptor, caused a similar subcellular site-dependent changes in $[Ca^{2+}]_i$, thus mimicking the BNP effect. All these results suggest that BNP-induced modulation of the activity of GABA receptors may be largely restricted to the inner retina.

1. Introduction

Natriuretic peptides (NPs), which were thought to be mainly involved in osmoregulation, extracellular fluid volume regulation and cardiovascular control (Levin et al., 1998), have been recently shown to play a neuromodulatory role in the CNS (Debinski et al., 1990; Levin et al., 1998, Trachte, 2005; Yu et al., 2006). In the retina NPs and their associated receptors (NPRs) are localized to neuronal and glial elements (Palm et al., 1989; Wolfensberger et al., 1994; Haverkamp et al., 1999; Blute et al., 2000; Cao et al., 2004; Yu et al., 2006; Cao and Yang, 2007; Jin et al., 2007). In recent years several studies reported that NPs modulate the activity of ligand-gated receptors expressed on retinal neurons, including bipolar cells (BCs) and amacrine cells (Tian and Yang, 2006; Yu et al., 2006).

BCs, which are the second-order neurons in the retina, receive input from photoreceptors through the dendrites in the outer plexiform layer (OPL) and send signal to amacrine cells and ganglion cells through the axon terminals in the inner plexiform layer (IPL). The activity of these cells is further modulated by GABAergic input from horizontal and amacrine cells respectively in the outer and inner retina. BCs are classified into

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Abbreviations: $[Ca^{2+}]_i$, intracellular calcium concentrations; BC, bipolar cell; IPL, inner plexiform layer; NP, natriuretic peptide; NPR, natriuretic peptide receptor; OPL, outer plexiform layer; PTX, picrotoxin; RBC, ON-type rod-dominant bipolar cell

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two types, ON-type and OFF-type BCs, which depolarize and hyperpolarize in response to light falling in the receptive field center respectively (Dowling, 1987). In a previous work, we reported that BNP suppresses $GABA_A$ receptor-mediated currents of ON-type rod-dominant BCs (RBCs) by activating NPR-A (Yu et al., 2006). In the present work we further show, using patch clamp techniques and calcium imaging, that the BNP suppression of GABA currents induced from RBCs is dependent on subcellular sites, being most significant at the axon terminal.

2. Results

2.1. *GABA currents recorded at axon terminals and dendrites of RBCs are differentially modulated by BNP*

Fig. 1A shows a typical isolated RBC, which was characterized by bushlike dendritic trees, a long axon and a unique lobulated terminal (Dowling and Boycott, 1966; Wassle et al., 1991). Most (80%) BCs with this characteristic morphology were positive to PKC, and they were RBCs (Greferath et al., 1990). GABA sensitivity of the RBC at the axon terminal and dendrites was determined first by focal application of 100 μM GABA with a duration of 600 ms (Fig. 1A, arrows). Fig. 1B shows inward currents of the RBC, voltage clamped at −60 mV, when 100 μM GABA was focally applied to the dendrites and the axon terminal respectively. The currents induced by axon terminal puff were commonly larger in amplitudes (264.9±20.3 pA, $n=12$) than those induced by dendrite puff (154.1±23.7 pA, $n=12$). These currents were completely suppressed by 500 μM picrotoxin (PTX), a chloride channel blocker ($n=4$) (Fig. 1B). Effects of BNP on the GABA currents induced by puffs to dendrites and axon terminals of RBCs were examined. The GABA response to the axon terminal puff was reduced in size in a reversible way with bath perfusion of 50 nM BNP, whilst that to the dendrite puff was hardly changed (Fig. 1C). Such differential effects of BNP on the two different subcellular sites were consistently observed in all four RBCs tested.

As drugs puffed to the dendrites could be easily diffused to the axon terminal and vice versa in isolated RBCs, retinal slices were instead used for studying the subcellular site dependence of the BNP effect in a more quantitative way, because drug application could be more precisely located due to diffusion barrier in these preparations. Fig. 2A shows a RBC in a retinal slice, which exhibited an oval soma and a long axon terminating in sublamina *b* at the vitreous side of the inner plexiform layer (IPL). Whole-cell responses of RBCs to GABA (100 μM) puffed to the dendrites/somata and axon terminals were recorded. As shown in Fig. 2B, the amplitude of the current to axon terminal puff, as observed in isolated RBCs, was significantly larger than that to dendrites puff (214.3±20.5 pA versus 108.9±25.5 pA, $n=10$). It was of interest that the decay kinetics of these two currents were not significantly different ($\tau=2.63\pm0.31$ for dendrites versus $\tau=3.46\pm0.34$ for terminals, $n=7$, $p>0.01$). Again, all these currents were completely suppressed by addition of 500 μM PTX (data not shown). When the retinal slice was superfused with 50 nM BNP for 2–~4 min, the GABA current to axon terminal puff was largely reduced in amplitude, with an average relative reduction of 34.7±2.1% ($n=6$). The current fully recovered after Ringer's washout (~2 min). In contrast, the GABA current induced by dendrites puff was only slightly (8.5±2.5%, $n=6$) reduced in amplitude and no statistically significant difference could be found ($p>0.01$). These results are summarized in the bar charts shown in Fig. 2C.

2.2. *Changes in $[Ca^{2+}]_i$ are dependent on subcellular sites of RBCs*

We determined changes in $[Ca^{2+}]_i$ at different subcellular sites of RBCs by calcium imaging. The experiments were carried out

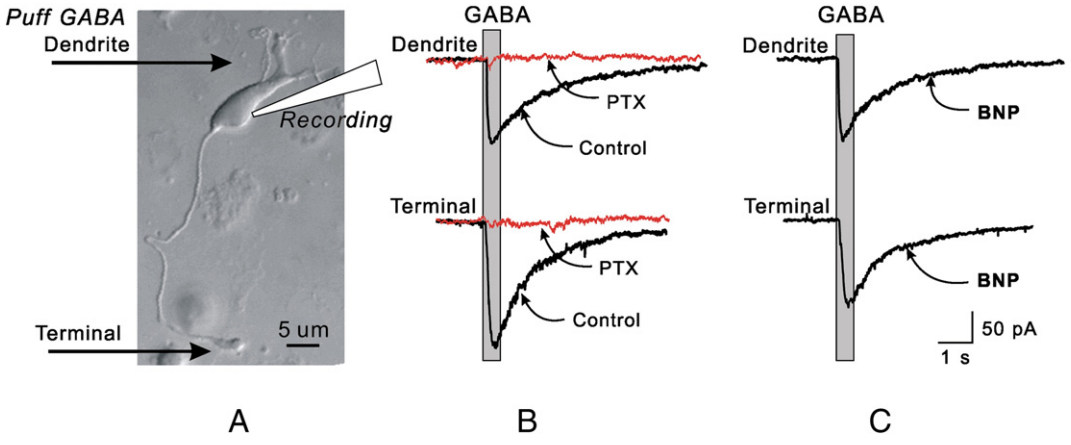

Fig. 1 – Effects of BNP on currents induced by GABA puffed to dendrites and axon terminal of an isolated RBC. (A) CCD picture of a typical RBC, which is characterized by a long axon and a unique lobulated terminal. Dendrites and terminal are indicated by arrows. Scale bar=5 μm. (B) Inward currents of the RBC shown in A, voltage clamped at −60 mV, induced by 100 μM GABA puffed to the dendrites (upper) and terminal (lower) respectively. These currents were completely suppressed by 500 μM picrotoxin (PTX). (C) In the presence of 50 nM BNP, the GABA current induced at the terminal, but not at the dendrites, was significantly suppressed.