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BRAIN RESEARCH

Research Report

Respiratory responses induced by blockades of GABA and glycine receptors within the Bötzinger complex and the pre-Bötzinger complex of the rabbit

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ABSTRACT

The respiratory role of $GABA_A$, $GABA_B$ and glycine receptors within the Bötzinger complex (BötC) and the pre-Bötzinger complex (preBötC) was investigated in α-chloralose-urethane anesthetized, vagotomized, paralysed and artificially ventilated rabbits by using bilateral microinjections (30–50 nl) of GABA and glycine receptor agonists and antagonists. $GABA_A$ receptor blockade by bicuculline (5 mM) or gabazine (2 mM) within the BötC induced strong depression of respiratory activity up to apnea. The latter was reversed by hypercapnia. Glycine receptor blockade by strychnine (5 mM) within the BötC decreased the frequency and amplitude of phrenic bursts. Bicuculline microinjections into the preBötC caused decreases in respiratory frequency and the appearance of two alternating different levels of peak phrenic activity. Strychnine microinjections into the preBötC increased respiratory frequency and decreased peak phrenic amplitude. $GABA_A$, but not glycine receptor antagonism within the preBötC restored respiratory rhythmicity during apnea due to bicuculline or gabazine applied to the BötC. $GABA_B$ receptor blockade by CGP-35348 (50 mM) within the BötC and the preBötC did not affect baseline respiratory activity, though microinjections of the $GABA_B$ receptor agonist baclofen (1 mM) into the same regions altered respiratory activity. The results show that only $GABA_A$ and glycine receptors within the BötC and the preBötC mediate a potent control on both the intensity and frequency of inspiratory activity during eupneic breathing. This study is the first to provide evidence that these inhibitory receptors have a respiratory function within the BötC.

1. Introduction

The neuronal network responsible for the generation of the eupneic pattern of breathing is localized within the pons and the medulla oblongata (for reviews, see Alheid and McCrimmon, 2008; Bianchi et al., 1995; Ezure, 1990; St John, 1998; Von Euler, 1986). Several lines of evidence (Alheid and McCrimmon, 2008; Monnier et al., 2003; Mutolo et al., 2002, 2005; Smith et al., 2007) indicate that the essential circuit generating the respiratory rhythm is located within the rostral part of the ventral respiratory column (VRC), including the Bötzinger complex (BötC) and the pre-Bötzinger complex (preBötC).

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Abbreviations: BötC, Bötzinger complex; preBötC, pre-Bötzinger complex; PRG, pontine respiratory group; TE, expiratory time; TI, inspiratory time; VRC, ventral respiratory column

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The BötC contains predominantly expiratory neurons which exert extensive inhibition on medullary respiratory neurons (e.g., Bongianni et al., 1997; Ezure et al., 2003a,b; Jiang and Lipski, 1990; Shen et al., 2003; Tian et al., 1999a; for reviews, see Alheid and McCrimmon, 2008; Bianchi et al., 1995; Ezure, 1990; Von Euler, 1986). The preBötC, a region located between the BötC and the more caudal inspiratory portion of the VRC, is thought to contain the kernel structure of the respiratory network generating inspiratory rhythmic activity (Feldman and Del Negro, 2006; Rekling and Feldman, 1998; Smith et al., 1991). In adult animals, this medullary region is characterized by the presence of a mix of different types of respiratory neurons (Bongianni et al., 2008a; Connelly et al., 1992; Monnier et al., 2003; Mutolo et al., 2002, 2005; Schwarzacher et al., 1995; Solomon et al., 1999).

In mammals, it has been reported that the functional role of GABA and glycine receptors becomes increasingly important during maturation and that synaptic inhibition mediated by GABA and glycine plays a major role in respiratory rhythm generation in the adult (for reviews, see Alheid and McCrimmon, 2008; Ballanyi et al., 1999; Bianchi et al., 1995; Haji et al., 2000). Nevertheless, the outcomes of some studies do not seem to entirely support this view (Busselberg et al., 2001, 2003; Markstahler et al., 2002). GABA and glycine receptors have been found to be expressed in the preBötC of adult rats (Liu et al., 2001, 2002). In addition, recent studies performed on neonatal mouse medullary slices have shown that neuronal glycinergic mechanisms within the preBötC are important in the generation and maintenance of the respiratory rhythm (Winter et al., 2009) and that glycinergic pacemaker neurons are present in the preBötC (Morgado-Valle et al., 2010). In particular, studies with specific antagonists both in *in vivo* and *in situ* arterially perfused preparations have shown the importance of $GABA_A$ or glycine receptors (Busselberg et al., 2001; Dutschmann and Paton, 2002; Hayashi and Lipski, 1992; Schmid et al., 1991a,b; St John and Paton, 2002). Interestingly, blockade of fast inhibitory transmission within the preBötC led to varied results (Chitravanshi and Sapru, 2002; Monnier et al., 2003; Pierrefiche et al., 1998). Noticeably, no information is available, to our knowledge, on the specific role of the inhibitory mechanisms within the BötC. $GABA_B$ receptors are functional in the respiratory network (e.g. Alheid and McCrimmon, 2008; Ballanyi et al., 1999; Haji et al., 2000; Hayashi and Lipski, 1992; Schmid et al., 1989; Zhang et al., 2002), but their role within the BötC and the preBötC remains to be investigated, especially in adult animals.

These considerations prompted us to investigate the respiratory role of inhibitory amino acid receptors within the BötC and the preBötC in anesthetized, vagotomized, paralysed and artificially ventilated rabbits making use of microinjections of $GABA_A$, $GABA_B$ and glycine receptor antagonists. Preliminary accounts of this work have been published in abstract form (Bongianni et al., 2008b, 2009).

2. Results

2.1. *Blockade of GABA and glycine receptors within the BötC*

Bilateral microinjections of 5 mM bicuculline (150–250 pmol; $n=5$) into the BötC induced marked depressant effects on inspiratory activity up to apnea (Fig. 1). The onset of the effects was rapid (about 1 min from the completion of the injections). These responses were characterized by progressive reductions in respiratory frequency and peak amplitude of phrenic nerve activity. Apnea ensued within 5 min and lasted 10–15 min. Rhythmic activity recovered gradually and almost completely within 90 min. Since it has been reported that bicuculline, in addition to its effects on $GABA_A$ receptors, affects calcium-dependent K^+ channels (Debarbieux et al., 1998), the specific $GABA_A$ receptor antagonist gabazine (2 mM) was also used (60–100 pmol; $n=3$). Gabazine caused similar strong depression

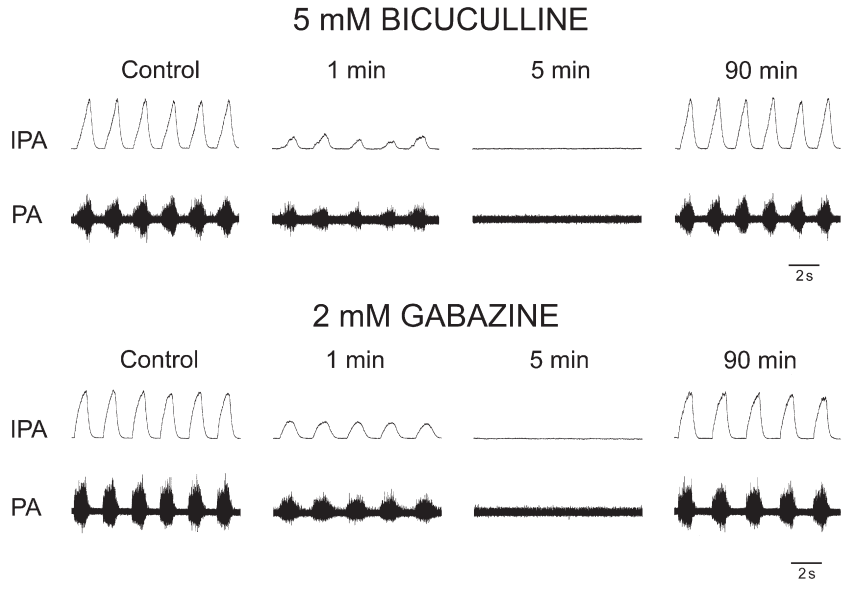

Fig. 1 – Respiratory responses induced by $GABA_A$ receptor blockade within the BötC. Bilateral microinjections of bicuculline (5 mM) or gabazine (2 mM) into the BötC caused strong depressant effects on inspiratory activity up to apnea. Traces are integrated phrenic nerve activity (IPA) and raw phrenic nerve activity (PA) under control conditions and at different times after the completion of the injections.