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Research Report

RVLM glycine receptors mediate $GABA_A$ and $GABA_B$ independent sympathoinhibition from CVLM in rats

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ABSTRACT

The caudal ventrolateral medulla (CVLM) provides tonic inhibitory and also excitatory inputs to the rostral ventrolateral medulla (RVLM). These experiments evaluated the role of RVLM γ-amino butyric acid (GABA) receptor subtypes and glycine receptors in mediating CVLM sympathoinhibition. In Inactin anesthetized female rats, the CVLM and RVLM were functionally defined by pressor and depressor responses to microinjected GABA (500 pmol, 50 nl). Although reduced, pressor and sympathoexcitatory responses due to inhibition of the CVLM with GABA persisted following ipsilateral RVLM $GABA_A$ receptor blockade (bicuculline, BIC, 400 pmol, 100 nl; $n=12$) in rats with contralateral nucleus tractus solitarius (NTS) lesion. In the presence of either ipsilateral (+contralateral NTS lesion; $n=8$) or bilateral ($n=6$) $GABA_A$ and $GABA_B$ receptor blockade of the RVLM (400 pmol BIC+400 pmol CGP35348, 100 nl), inhibition of the CVLM still increased MAP and renal sympathetic nerve activity (RSNA). Thus neither $GABA_B$ receptors nor a contralateral CVLM to RVLM GABAergic pathway explains residual responses to CVLM blockade. The addition of strychnine (300 pmol, 100 nl) to the RVLM eliminated responses to CVLM inhibition, suggesting that a $GABA_A$ and $GABA_B$ independent sympathoinhibitory influence from CVLM to RVLM is mediated by glycine receptors. Decreases in MAP and RSNA due to activation of the CVLM with glutamate (500 pmol, 50 nl) were reversed to increases in the presence of RVLM $GABA_A$ receptor blockade ($n=7$). Thus, a sympathoexcitatory pathway from the CVLM can be activated in the presence of RVLM GABA receptor blockade, but sympathoinhibitory influences from the CVLM predominate.

1. Introduction

The rostral ventrolateral medulla (RVLM) is a critical brainstem site involved in the regulation of cardiovascular function. Excitation of the RVLM produces an increase in sympathetic outflow and arterial blood pressure while inhibition of neurons in the RVLM results in a decrease in sympathetic outflow and arterial pressure demonstrating that this region is tonically active (Dampney, 1994; Dampney et al., 2003; Guyenet, 1990). As a final common site for modulation of sympathoexcitatory drive to preganglionic sympathetic neurons in the intermediolateral cell column of the spinal cord (IML), the RVLM receives and integrates neural inputs from several areas in the central nervous system.

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Tonic activity of RVLM neurons appears to be largely dependent on synaptic inputs (Lipski et al., 1996). While stimulation of several central nervous system sites excites the RVLM, the majority of these areas have not been shown to provide tonic excitation to the RVLM under normal physiological conditions (Dampney, 1994; Dampney et al., 2003). The pontine reticular formation (Hayes et al., 1994) and the caudal pressor area (Horiuchi and Dampney, 2002; Natarajan and Morrison, 2000) are regions which have been identified as potential sources of tonic excitatory drive to the RVLM. Sympathoexcitation from the caudal pressor area has been reported to be mediated through an excitatory pathway including the CVLM (Natarajan and Morrison, 2000) or through inhibition of a GABAergic pathway between the CVLM and RVLM (Horiuchi and Dampney, 2002). Others have suggested that the CVLM (Ito and Sved, 1997; Moreira et al., 2005) and the NTS (Moreira et al., 2005) may also be important sources of tonic excitatory drive to the RVLM.

In regard to inhibitory influences, one area in particular, the caudal ventrolateral medulla (CVLM), has repeatedly been shown to have an important role in the modulation of RVLM pre-sympathetic neurons which project to the IML (Cravo et al., 1991; Cravo and Morrison, 1993; Dampney, 1994; Guyenet, 1990; Jeske et al., 1993; Schreihofer and Guyenet, 2005). GABA is the primary neurotransmitter mediating inhibition of sympathetic premotor neurons in the RVLM (Cravo et al., 1991; Cravo and Morrison, 1993; Dampney et al., 1988; Dampney, 1994; Dampney et al., 2003; Guyenet, 1990; Jeske et al., 1993; Schreihofer and Guyenet, 2005) and CVLM neurons are antidromically activated from the RVLM (Agarwal and Calaresu, 1991; Dampney, 1994; Jeske et al., 1993). Retrograde labeling from the RVLM of GABAergic CVLM neurons, which also express Fos protein in response to an increase in arterial pressure, suggests a direct GABAergic projection from the CVLM to the RVLM (Chan and Sawchenko, 1998). Using a combination of electrophysiological and anatomical techniques, Schreihofer and Guyenet (2003) demonstrated that baro-activated, pulse-modulated CVLM neurons express the GABA synthetic enzyme, GAD_{67}, and project rostrally. Taken together these studies provide convincing evidence for a tonically active monosynaptic GABAergic projection from CVLM to RVLM in the medullary baroreflex pathway.

Inhibitory GABAergic projections from the CVLM to the RVLM that are tonically active and independent of the baroreflex have also been described (Cravo et al., 1991; Cravo and Morrison, 1993; Dampney et al., 1988; Schreihofer and Guyenet, 2003, 2005). Even at low arterial pressures, when arterial baroreceptor input is minimal, iontophoretic application of bicuculline results in increased firing of spinally projecting RVLM neurons (Sun and Guyenet, 1985). Following acute NTS lesion in rabbits (Dampney et al., 1988) and chronic NTS lesion or SAD in rats (Schreihofer et al., 2005), microinjection of bicuculline into the RVLM results in large sympathoexcitatory responses. In acutely (Cravo and Morrison, 1993) and chronically (Schreihofer et al., 2005) sino-aortic denervated (SAD) rats, neuronal blockade of the CVLM results in increased arterial pressure and sympathetic nerve activity. Thus, there is a major baroreflex independent GABAergic inhibitory influence from the CVLM to the RVLM.

There is strong evidence demonstrating that arterial baroreflex initiated inhibition of the RVLM by the CVLM is mediated through $GABA_A$ receptors (Dampney, 1994; Dampney et al., 2003; Guyenet, 1990; Sun and Guyenet, 1987). However, $GABA_B$ receptor-like immuno-reactivity has been demonstrated in the region of the RVLM of rats (Margeta-Mitrovic et al., 1999) and cats (Ohshita et al., 2004). Several studies suggest that $GABA_B$ receptors participate in GABAergic inhibition of the RVLM (Amano and Kubo, 1993; Avanzino et al., 1994; Chu et al., 1998; Li and Guyenet, 1995, 1996; Lin and Dun, 1998) and may be tonically activated (Amano and Kubo, 1993; Avanzino et al., 1994; Chu et al., 1998). Since arterial baroreflex responses appear to be mediated solely by $GABA_A$ receptors in the RVLM (Dampney, 1994; Dampney et al., 2003; Guyenet, 1990), we considered that a possible role for $GABA_B$ receptors might include mediation of baroreflex independent inhibitory influences from the CVLM. In addition, although all reports do not agree (Amano and Kubo, 1993), there is evidence for tonic glycinergic inhibition of sympathetic outflow in the RVLM (Blessing, 1988; Chan and Sawchenko, 1998) and a role for glycine in the RVLM was evaluated.

We observed that ipsilateral RVLM $GABA_A$ receptor blockade in contralateral NTS lesioned rats did not eliminate pressor and sympathoexcitatory responses to inhibition of the CVLM. The goal of subsequent experiments was to evaluate potential mechanisms for the remaining response to CVLM inhibition. Neither RVLM $GABA_B$ receptors nor a contralateral CVLM to RVLM pathway accounted for the remaining inhibition from the CVLM. The major new finding is that, although the predominant inhibitory influence from the CVLM is mediated by $GABA_A$ receptors in the RVLM, in the presence of both $GABA_A$ and $GABA_B$ receptor blockade, glycine receptors in the RVLM provide the remaining inhibitory influence originating from the CVLM.

2. Results

Preliminary experiments were performed in right NTS lesioned rats, to determine a treatment regimen to provide effective block of GABA receptors in the left RVLM. Efficacy of receptor blockade was evaluated by testing for elimination of baroreflex sympathoinhibitory responses following injection of BIC in the left RVLM (Protocols 1 and 2) or loss of MAP and RSNA responses to microinjection of GABA (1 mM) into the left RVLM following administration of BIC + CGP35348 (Protocol 2). The concentrations of BIC (4 mM) and CGP35348 (4 mM) used in this study were based on those previously reported in the literature (Amano and Kubo, 1993; Smith and Barron, 1990; Sved and Tsukamoto, 1992). In the current experiments, a single microinjection of either BIC or BIC + CGP35348 was inadequate for maintenance of a complete GABA receptor blockade over a 10 min period. However, a single injection (400 pmol, 100 nl) of the antagonist(s) followed by supplemental injection (200 pmol, 50 nl) at 5 min intervals was found to reliably establish and maintain GABA receptor blockade for 1 h. Using this regimen, supplemental injections of the GABA antagonists did not produce any additional effects on MAP or RSNA.