Parvalbumin-immunoreactive neurons in the rat ventral respiratory column receive close appositions from galanin-immunoreactive axons

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The neurochemistry of neurons in the ventral respiratory column (VRC) correlates with their function (Feldman *et al.*, 2003; Alheid & McCrimmon, 2008). Galanin (GAL) influences breathing when injected into the VRC (Abbott *et al.*, 2009) and occurs in about 40% of chemosensitive neurons in the retrotrapezoid nucleus (RTN), which lies ventral and caudal to the facial nucleus (Stornetta *et al.*, 2009). Two-thirds of large VRC neurons with spinally-projecting axons contain the calcium binding protein, parvalbumin (Parv; Alheid *et al*., 2002). In the VRC, the neurokinin 1 receptor (NK1R) occurs in small neurons of the PreBötzinger (PreBöt) group and in larger bulbospinal neuron that occur caudal to the PreBöt neurons (Guyenet *et al.*, 2002). In this study, we used peroxidase immunohistochemistry to investigate whether GAL-immunoreactive axons innervate Parv-immunoreactive VRC neurons and to characterize the neurochemistry of a new group of GAL-immunoreactive neurons that we identified in the caudal ventral respiratory group (cVRG).

Male and female Sprague-Dawley rats were anesthetized with pentobarbitone (60 mg/kg i.p.) and perfused transcardially with phosphate-buffered 4% formaldehyde. Each perfused medulla was cut on a cryostat at 30 μm into 4 series of sections. In some series of sections, GAL-immunoreactivity was visualized alone using a black peroxidase reaction product. A second set of sections was stained to reveal Parv with a brown peroxidase reaction product and GAL with a black peroxidase reaction product. In a third set of sections, NK1R-immunoreactivity was detected with a black reaction product; and GAL, with a brown product.

We identified two groups of GAL-immunoreactive somata in the ventral medulla. The rostral group, which are the RTN neurons, lay medial and ventral to the caudal end of the facial nucleus. A second GAL-positive group was located more caudally, immediately ventral and slightly lateral to the column of Parv neurons. In a 1:4 series of sections, there were on average 56 ± 12 neurons (n=8) in this caudal GAL-containing group. The location of the second group suggested that these might be PreBöt neurons and therefore might contain NK1R. Some of the caudal GAL-immunoreactive neurons were NK1R-immunoreactive as well as GAL-immunoreactive. However, these NK1R-positive neurons were too big to be PreBöt neurons. In the VRC, occasional black GAL-immunoreactive axons closely apposed some large brown Parv-immunoreactive cell bodies.

These observations indicate that GAL occurs in a population of bulbospinal neurons in cVRG as well as in some RTN neurons. The data also suggest that GAL may influence respiration at two sites within central respiratory circuitry. RTN neurons may release GAL onto Parv-immunoreactive VRC neurons, which innervate spinal motor neurons that control respiratory pump muscles. Bulbospinal cVRG neurons may also release GAL directly onto spinal motor neurons innervating pump muscles.

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