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Research Report

Expression of alpha-7 and alpha-4 nicotinic acetylcholine receptors by GABAergic neurons of rostral ventral medulla and caudal pons

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ABSTRACT

Rostral ventral medulla (RVM) contains significant numbers of local GABAergic neurons which may subserve respiratory chemosensory and baroreceptor reflexes. Nicotinic mechanisms stimulate release of GABA in certain brainstem neurons. Whether the GABAergic neurons at RVM express nicotinic cholinergic receptors (nAChRs) is not known. We used glutamic acid decarboxylase 67-kDa isoform (GAD67) and parvalbumin (PV) as anatomical markers to identify the GABAergic neurons of the RVM and caudal pons and performed double labeling to evaluate the expression of alpha-7 and alpha-4 nAChRs by GAD67 and PV-imnunoreactive (ir) cells at these sites. GAD67-ir cells were found at the ventrolateral pontomedullary border in areas adjacent to the A5 noradrenergic cell group and overlapping the facial nucleus lateral subnuclei and para-facial zones. Of 205 GAD67-ir cells labeled at these sites, 74% exhibited immunoreactivity for alpha-7 nAChRs. Alpha-4 immunoreactivity was also present in 35% of GAD67-ir cells at these sites. The PV-ir cells of RVM and caudal pons were found medial to the facial nucleus and lateral to the pyramid in a column distinct from the GAD67-ir cells. Virtually all the PV-ir cells demonstrated immunoreactivity for alpha-4 nAChR (95%) and alpha-7 (93%) subunits of nAChRs. Differential expression of GAD67 and PV by neurons at the pontomedullary border implies that PV may not be a valid marker for GABAergic neurons. The expression of alpha-4 and alpha-7 nAChRs by GAD67-ir cells suggests nicotinic cholinergic modulation of GABAergic signaling at these ventrolateral pontomedullary sites.

1. Introduction

GABAergic neurons of the rostral ventral medulla (RVM) are known to be involved in the control of a wide range of autonomic functions including cardiorespiratory regulation. These cells provide the major inhibitory input to the sympathetic preganglionic neurons which mediate the baroreceptor reflex response to elevation of blood pressure (Coote and Macleod, 1974; Coote et al., 1981; Morrison and Gebber, 1984; Stornetta et al., 2004). The GABAergic neurons at RVM are also a

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component of the central respiratory chemosensory network (Zhang et al., 2003) and activation of $GABA_A$ receptors in RVM attenuates CO_2 chemosensitivity (Curran et al., 2001). Despite their importance, the neurochemical mechanisms regulating these GABAergic neurons have not been fully elucidated. Activation of presynaptic nicotinic cholinergic receptors increases GABA release and enhances inhibitory postsynaptic current in brainstem neurons (Bertolino et al., 1997; Zhu and Chiappinelli, 1999; Neff et al., 2003; Endo et al., 2005). GABAergic inhibition of preganglionic cardiac vagal neurons is enhanced by endogenous cholinergic activity and nicotine and is blocked by an antagonist of alpha-4 nicotinic receptors (Wang et al., 2003). In addition, prenatal exposure to nicotine modulates the GABAergic inhibition to preganglionic cardiac vagal neurons associated with hypercapnia and hypoxia (Neff et al., 2003, 2004; Huang et al., 2006). These findings suggest a relationship between nicotinic and GABAergic neurotransmission in the regulation of preganglionic autonomic neurons. However, they do not provide evidence for the presence of nicotinic cholinergic receptor (nAChR) proteins on GABAergic neurons projecting to the central autonomic and cardiorespiratory network. The present study was, therefore, designed to provide direct immunohistochemical evidence for the presence of alpha-7 and alpha-4 nAChRs on GABAergic neurons of the RVM and caudal pons.

2. Results

2.1. *Expression of nAChRs by GAD67-immunoreactive cells of RVM and caudal pons*

We focused mainly on the distribution of GABAergic neurons and terminals in the RVM and caudal pons. Distribution of GABAergic neurons in other brainstem regions has been previously described (Stornetta et al., 2004; Fong et al., 2005). In the present study, GAD67-ir cells were clustered in ventrolateral pontomedullary regions at sites adjacent to the A5 noradrenaline cells. These GAD67-ir cells overlapped the facial nucleus lateral subnuclei, lateral superior olive (LSO) and para-facial zone in a column that extended from bregma −11.00 mm to bregma −9.68 mm in pons (Paxinos and Watson, 1986). GAD67-ir cells exhibiting staining of much less intensity were also seen in raphe nuclei (RN), gigantocellular reticular nucleus ventral (GiV), inferior olive (IO), lateral paragigantocellular nucleus (LPGi), gigantocellular nucleus alpha (GiA), rostral ventrolateral reticular nucleus (RVL) and parapyramidal nucleus (PPy). Dense GAD67-ir terminal labeling was found throughout the medulla and caudal pons. At the RVM, GAD67-ir terminals were present in regions that corresponded mainly to LPGi, GiA, PPy and RVL. More rostrally, pontine GAD67-ir

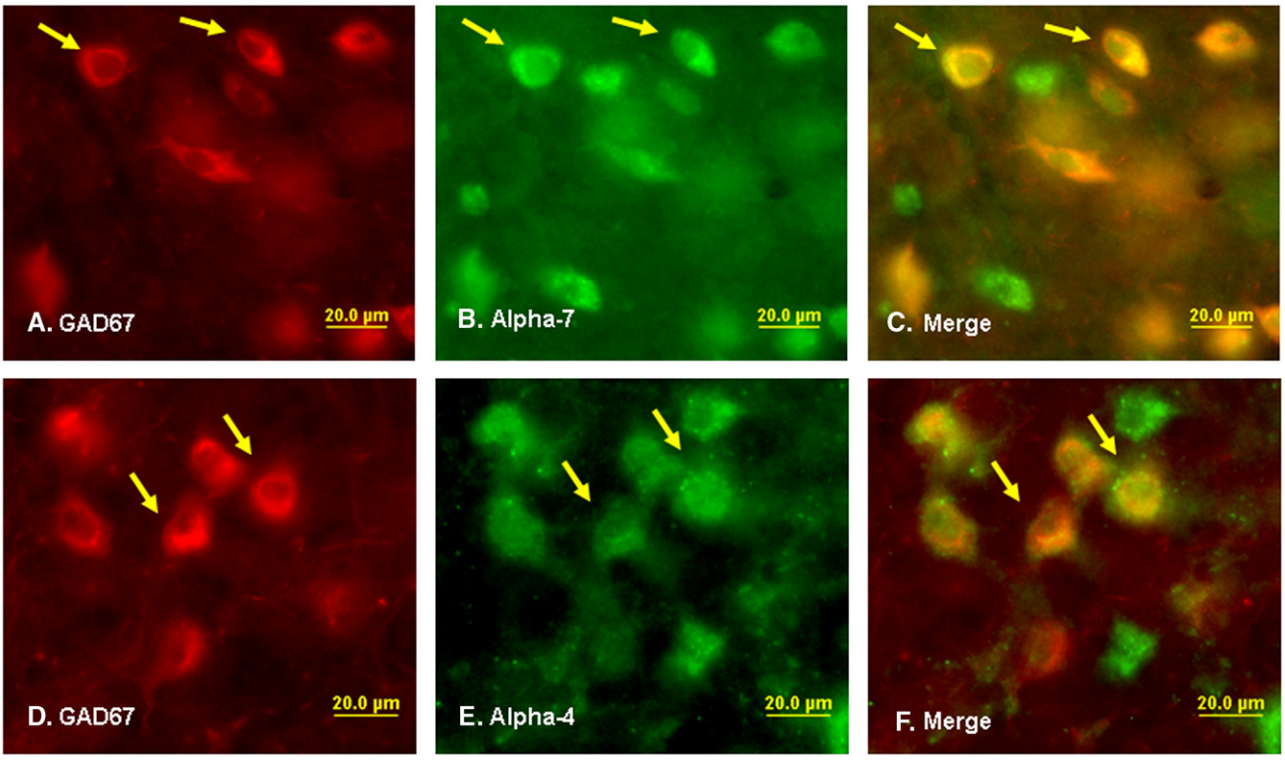

Fig. 1 – Expression of nicotinic acetylcholine receptors (nAChRs) by glutamic acid decarboxylase 67-kDa isoform (GAD67)-immunoreactive (ir) cells of the rostral ventrolateral medulla (RVLM). (A–C) GAD67 and alpha-7 subunit immunoreactivity in RVLM (bregma −11.00 mm). (A) GAD-ir cells, (B) same field showing alpha-7 nAChR expressing cells, (C) the merge image of panels A and B. (D–F) GAD67 and alpha-4 subunit immunoreactivity in RVLM (bregma −11.00 mm). (D) GAD-ir cells, (E) same field showing alpha-4 nAChR expressing cells, (F) the merge image of panels D and E. Arrows show representative neurons co-expressing GAD67 with alpha-7 and/or alpha-4 nAChRs.