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Research Report

Activity-dependent heterogeneous populations of nitric oxide synthase neurons in the rat dorsal raphe nucleus

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Abbreviations:
5-HT, 5-hydroxytryptamine
DRN, dorsal raphe nucleus
$dm_{rostral}$, rostral dorsomedial
dm_{caudal}, caudal dorsomedial
$lat_{rostral}$, rostral lateral wing
lat_{caudal}, caudal lateral wing
$vm_{rostral}$, rostral ventromedial
vm_{caudal}, caudal ventromedial
NADPH-d, nicotinamide adenine dinucleotide phosphate diaphorase
NO, nitric oxide
NOS, nitric oxide synthase
nNOS, neuronal nitric oxide synthase

ABSTRACT

The brainstem dorsal raphe nucleus (DRN) contains an abundant distribution of nitric oxide (NO) synthase (NOS)-containing neuronal profiles in two distinct populations: faint- and intense-immunoreactive cells in midline (ventromedial and dorsomedial) and lateral wing subregions, respectively. This study tested the hypothesis that different functional dynamics underlie the topography of NOS-containing cells in the DRN rostrocaudal and mediolateral neuraxis by using a capsaicin challenge paradigm (50 mg/kg, subcutaneous). Compared with vehicle, capsaicin significantly and preferentially increased nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d, an index of constitutive NOS) reactivity in the rostral midline and caudal lateral wing subregions. Furthermore, capsaicin activated more Fos-positive cells than vehicle within all subregions of the DRN but with a caudal versus rostral predominance in activation pattern. In addition, a high proportion of capsaicin-induced Fos cells in the midline but almost none in lateral wing stained for NADPH-d. These observations suggest the existence of two functionally distinct populations of NOS neurons in the DRN. Furthermore, capsaicin increased galanin immunoreactivity with predominant staining in cell soma and fiber processes in midline and lateral wing subregions of the nucleus, respectively. The total capsaicin-induced galanin immunoreactivity was higher in rostral versus caudal DRN, and a high proportion of galanin-positive cells in the midline also contained NADPH-d and neuronal NOS, thus suggesting a potential NO–galanin interaction in these neurons. The differential pattern of Fos/NADPH-d colocalization across the nucleus suggests that midline and lateral wing NOS neurons of the DRN express their neuromodulatory actions on discrete efferent targets via different intracellular mechanisms.

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1. Introduction

The brainstem dorsal raphe nucleus (DRN) is neurochemically diverse and mediates a variety of brain functions (see Azmitia, 1999; Jacobs and Azmitia, 1992). Anatomically, the DRN is frequently defined as four major cell clusters of 5-hydroxytryptamine (serotonin, 5-HT)-containing cells running rostrocaudally through the nucleus (Agnati et al., 1982; Diaz-Cintra et al., 1981; Steinbusch, 1981). These subdivisions are: (i) dorsomedial—beneath the aqueduct; (ii) ventromedial—between the medial longitudinal fasciculi; and (iii) bilateral (lateral wing) subregions. Despite a generation of studies designed to elucidate the biological significance of the DRN and clarify the actions of its major transmitter 5-HT, many gaps remain in our understanding of how this broadly projecting brainstem nucleus (particularly, its non-5-HT domain) engages in specific physiological functions and, through its outputs, influences whole animal behavioral responses.

Observations from earlier studies suggested a homogenous functional population of DRN neurons (Chaouloff et al., 1999; DeOlmos and Heimer, 1980; Imai et al., 1986; Jacobs and Fornal, 1991; Lidov et al., 1980; Van der Kooy and Kuypers, 1979; Wilkinson and Jacobs, 1988). However, many recent reports indicate that DRN neurons maintain a rough topographic order with respect to specified efferent targets and biological function (Abrams et al., 2004; Day et al., 2004; Fite et al., 1999; Kirby et al., 2003; Kirifides et al., 2001; Lowry, 2002; Simpson et al., 1999, 2003). Likewise, approximately one-half of constituent DRN neurons express dopamine, GABA, various peptides (for example, galanin) or nitric oxide (NO) synthase (NOS). As is the case for 5-HT, many of these transmitter molecules show specific distribution patterns and exhibit unique coexistence relationships with 5-HT and/or with each other. For instance, following colchicine (an axoplasmic transport inhibitor) treatment, a large proportion of DRN 5-HT-positive neurons in the rostral–caudal midline (ventromedial and dorsomedial) subregions also contain NOS and galanin. In contrast, the lateral wing subregion shows very low incidence of such triple transmitter coexistence (Xu and Hökfelt, 1997).

In the nervous system, NO modulates the activity of other neurotransmitters, acts as a cellular communicator in plasticity and regulates blood flow to different brain regions (Kim and Rivier, 2000; Lopez-Figueroa et al., 1998; McLeod et al., 2001; Munzel et al., 1997). In formaldehyde-fixed tissues, nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) reduces nitroblue tetrazolium to dark blue-black, insoluble formazan (Kuonen et al., 1988). Neuronal NADPH-d is a NOS (Hope et al., 1991) and selectively reveals constitutive NOS cells (Nakos and Gossrau, 1994). The presence of neuronal (n)NOS and NADPH-d in many DRN neurons (Dun et al., 1994; Léger et al., 1998a; Onstott et al., 1993; Simpson et al., 2003; Sugaya and McKinney, 1994; Tagliaferro et al., 2001; Vincent, 1994; Wang et al., 1995; Wotherspoon et al., 1994; Xu and Hökfelt, 1997) suggests a significant NO involvement in DRN function. For example, NO produced in the DRN regulates sleep cycles (Burlet et al., 1998; Monti et al., 2001), interacts with 5-HT cells (Tagliaferro et al., 2001), impairs escape performance and enhances conditioned fear in rats exposed to an uncontrollable stressor (Grahn et al., 2000). Hence, the endogenous DRN NOS system is subject to activity-related and stimulus-dependent changes. However, much remains to be elucidated regarding the physiological significance of NOS distribution within specific DRN subdivisions for local neural activity and its influence on the efferent target sites.

For instance, a high proportion of the midline DRN NADPH-d-positive neurons contain 5-HT and project to the cortex; on the other hand, the lateral wing NADPH-d-positive neurons do not contain 5-HT and project to subcortical (thalamic) regions (Simpson et al., 2003). An important physiological implication of the different specific efferent projections between midline and lateral wing DRN NOS neurons is that the differential spatial distribution pattern might reflect ontogenetic and/or functional distinction. As yet, the potential physiological significance of the topographical ordering of NOS within the DRN neuraxis has not been addressed in any detail. Furthermore, the differential NOS, 5-HT and galanin coexistence/distribution pattern in DRN subregions (Simpson et al., 2003; Xu and Hökfelt, 1997) raises questions about how the transmitters, particularly, the non-serotonergic NO and galanin systems, might interact under specific stimulus conditions.

Therefore, the general rationale for the present study was to better understand the contribution of non-serotonergic cellular components to DRN function by determining the effect of acute systemic capsaicin injection on NO-producing neuron profiles. The rationale for adopting this approach derives from the documented nitrergic regulation of capsaicin effects within other regions of the nervous system (Okere et al., 1999, 2000a,b; Sakurada et al., 1996a,b; Wu et al., 1998). In addition, capsaicin enhances galaninergic activity in many brain areas (Giuliani et al., 1989; Jimenez-Andrade et al., 2004; Kar et al., 1990; Kar and Quirion, 1994; Noguchi et al., 1993; Papka and Traurig, 1989; Skofitsch and Jacobowitz, 1985; Wendland et al., 2003; Xu et al., 1997). However, not much is known of capsaicin's impact on DRN function. Three specific questions that, taken together, constitute the rationale for the present study were addressed. First, what is the effect of acute capsaicin injection on NADPH-d and Fos (a marker of intracellular activation) expression on cells within mediolateral and rostrocaudal domains of the DRN? Second, what proportion of Fos cells stain for NADPH-d following capsaicin treatment? Third, what is the effect of capsaicin on galanin immunoreactivity and does capsaicin alter galanin synthesis in DRN nNOS-containing neurons?

2. Results

Fig. 1 illustrates the initial findings that (1) the midline versus lateral wing differential NOS staining is demonstrable by NADPH-d histochemical reaction (A, A′, A″), bright-field (B, B′, B″) or fluorescent (C,C′,C″) nNOS immunohistochemistry; (2) nNOS and NADPH-d coexist in cellular profiles in ventromedial (D) and lateral wing (E) subregions, thus agreeing with the results of previous studies (Simpson et al., 2003; Xu and