BRAIN RESEARCH 1369 (2011) 36–45

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BRAIN RESEARCH

Research Report

Substance P induces the reversible formation of varicosities in the dendrites of rat brainstem neurons

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ARTICLE INFO

Article history:
Accepted 26 October 2010
Available online 31 October 2010

Keywords:
Rostral ventromedial medulla
Dendritic varicosity
Dendritic beading
Substance P
Neurokinin-1 receptor

ABSTRACT

This study investigated the ability of substance P (Sub P) to induce dendritic varicosities (DVs) or beads in neurons of the rostral ventromedial medulla (RVM) of the rat. Microinjection of 5–200 pmol Sub P in the RVM produced a concentration-dependent increase in the number of DVs in distal dendrites of RVM neurons that were immunoreactive for the neurokinin-1 receptor, but not serotonin. The effect was reversible, as DVs were essentially absent 2 and 4 h after microinjection. Fluoro-Jade B labeled neurons were not evident in the RVM 4 days after microinjection of Sub P, although such neurons were present 4 days after microinjection of a neurotoxic dose of kainate. Bath application of Sub P to brainstem slices for a period as brief as 30 s also produced DVs in neurokinin-1 immunoreactive RVM neurons. Prior exposure to L-703606 prevented the formation of DVs by Sub P, implicating the neurokinin-1 receptor, a Gq type of G protein coupled receptor, in the formation of DVs by Sub P. Finally, stabilization of microtubules by prior exposure to taxol also prevented the formation of DVs, consistent with the idea that increases in intracellular Ca^{2+} lead to the formation of DVs secondary to a disruption of the linear arrays of microtubules in dendrites. These data establish a mechanistic basis for the formation of DVs by Sub P and support further studies to test the hypothesis that the formation of DVs is a morphological mechanism by which neurons can regulate their responses to inhibitory or excitatory inputs.

1. Introduction

Internalization of the neurokinin-1 receptor (NK-1R) has been extensively used as a measure of the release or action of substance P (Sub P) in the central nervous system (Adelson et al., 2009; Allen et al., 1997; Marvizon et al., 1999, 2003). In the course of these studies, several authors have commented on changes that occur in the dendrites of these neurons. For example, the dendrites of dorsal horn neurons that exhibit internalization of NK-1R after intradermal injection of capsaicin exhibit a highly varicose or tortuous appearance (Mantyh et al., 1995b). The formation of dendritic beading or

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Abbreviations: ACSF, Artificial cerebrospinal fluid; DV, Dendritic varicosity; GPCR, G protein coupled receptor; NK-1R, Neurokinin-1 receptor; PBS, Phosphate buffered saline; RVM, Rostral ventromedial medulla; Sub P, Substance P

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doi:10.1016/j.brainres.2010.10.091

varicosities (DVs) has also been documented after microinjection of Sub P in the striatum (Mantyh et al., 1995a), and after prolonged application to cultured dorsal horn neurons (Marvizon et al., 1998).

Other than documentation of their occurrence, little is known about the mechanisms that underlie the formation of DVs induced by Sub P. Yet, DVs may have functional consequences for neuronal function. The formation of DVs is accompanied by a reduction in the diameter of the intervening segments of the dendrites (McNeil et al., 1999), which radically alters the geometry of the dendrites. In theory, such changes will reduce the passive electrical properties of dendrites because internal resistance is increased in the constricted regions between varicosities (Ellias and Stevens, 1980). Thus, synaptic potentials initiated in varicose dendrites could attenuate with distance. Dendritic varicosities could potentially function to isolate synaptic currents, reduce the electrotonic conduction of synaptic potentials along the dendrite, and effectively insulate the soma from excitatory or inhibitory inputs. The purpose of this study was to conduct an in vitro and in vivo analysis of DVs and the mechanisms that are responsible for their formation after application of Sub P. This study focused on the rostral ventromedial medulla (RVM) because this region contains high densities of NK-1R and Sub P (Ljungdahl et al., 1978; Nakaya et al., 1994; Saffroy et al., 2003). Moreover, there is strong evidence that endogenously released Sub P in the RVM may play a role in the maintenance of thermal hyperalgesia and mechanical allodynia after peripheral inflammatory injury (Hamity et al., 2010; Pacharinsak et al., 2008).

2. Results

2.1. *Microinjection of Sub P produces DVs in vivo*

The first set of experiments established that microinjection of Sub P, but not saline produced DVs in NK-1R immunoreactive RVM neurons. The total number of DVs was determined 10–240 min after microinjection of saline or Sub P (5–200 pmol in 0.5 μl). Counts were made in serial sections through the RVM. As illustrated in Fig. 1A and B, the DVs were approximately 2 μm in diameter, and were often regularly spaced along a dendrite giving the appearance of beads on a string. Dendritic varicosities were more common on distal dendrites, than on proximal dendrites. Dendritic varicosities were infrequently observed after microinjection of saline (Fig. 1C). Fig. 2 illustrates the total number of DVs counted in serial sections through the RVM after microinjection of Sub P. The dose–effect curve for Sub P was U-shaped, with the maximum number of DVs observed after microinjection of 100 pmol Sub P (Fig. 2A). Dendritic varicosities persisted for up to 60 min after microinjection, but were essentially absent by 120 min (Fig. 2B). Substance P did not produce DVs in distal or proximal

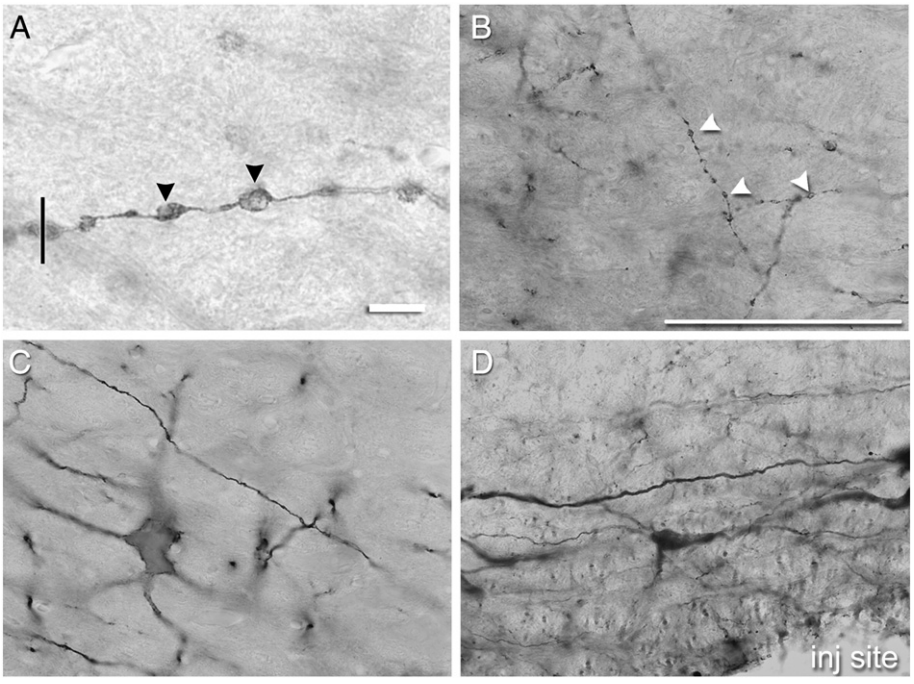

Fig. 1 – (A) Representative photomicrograph of dendritic varicosities (DVs) in neurokinin-1 receptor expressing neurons of the rostral ventromedial medulla (RVM) demonstrating the clarity with which DVs could be viewed with a 60X N.A. 1.4 objective and 10X eyepiece objective. The vertical line drawn perpendicular to the dendrite's trajectory illustrates how the diameter of a DV was determined. Note that the segments between the DVs are constricted. (B) Representative photomicrographs of DVs in neurokinin-1 receptor expressing neurons of the rostral ventromedial medulla 1 h after microinjection of 100 pmol substance P. (C) Few DVs were observed in neurokinin-1 receptor expressing neurons in the RVM after microinjection of saline. (D) Substance P did not produce DVs in serotonergic neurons in the RVM. Scale bar is 10 μm in panel A and 100 μm in panels B–D. Arrowheads indicate DVs. Where not visible in panels B–D, the microinjection site was within 250–500 μm.