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The unique organization of filamentous actin in the medullary canal of the medulla oblongata

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ABSTRACT

In the central canal, F-actin is predominantly localized in the apical region, forming a ring-like structure around the circumference of the lumen. However, an exception is found in the medulla oblongata, where the apical F-actin becomes interrupted in the ventral aspect of the canal. To clarify the precise localization of F-actin, the fluorescence signals for F-actin were converted to the peroxidase/DAB reaction products in this study by a phalloidin-based ultrastructural technique, which demonstrated that F-actin is located mainly in the microvilli and terminal webs in the ependymocytes. It is because the ventrally oriented ependymocytes do not possess well-developed microvilli or terminal web that led to a discontinuous labeling of F-actin in the medullary canal. Since spinal motions can change the shape and size of the central canal, we next examined the cytoskeletons in the medullary canal in both rats and monkeys, because these two kinds of animals show different kinematics at the atlanto-occipital articulation. Our results first demonstrated that the apical F-actin in the medullary canal is differently organized in the animals with different head-neck kinemics, which suggests that the mechanic stretching of spinal motions is capable of inducing F-actin reorganization and the subsequent cell-shape changes in the central canal.

1. Introduction

In many mammals, the central canal of spinal cord communicates rostrally with the fourth ventricle and is patent caudally throughout to the filum terminale (Murthy and Deshpande, 1980; Réthelyi et al., 2004; Del Bigio, 2010). Under normal conditions, the lining of the central canal consists of a single layer of cuboidal to columnar ependymal cells, which play important roles in the brain homeostasis (Del Bigio, 2010). The integrity of the ependyma is highly dependent on the unique organization of filamentous actin (F-actin), and disturbance of F-actin has been shown to result in partial or complete denudation of ependymocytes from the lining (Mestres and Garfia, 1980; Koshiba, 1987).

Phalloidin is a highly specific probe for F-actin because it recognizes F-actin but not free actin monomers (Wulf et al., 1979). By using this probe, we have documented that F-actin in the central canal is organized into a ring-like structure in the apical region in the adult rats, which is thickest at the lower cervical level but becomes narrower at the upper thoracic level (Li et al., 2007). To reveal the structural nature of the F-actin ring, we converted the fluorescence signals for F-actin to the peroxidase/DAB reaction products visible under the electron microscope by use of a phalloidin-based FITC-anti-FITC method (Li et al., 2009). By this technique, we demonstrated that the F-actin ring is located predominantly in the microvilli and terminal webs in the apical region of ependymocytes (Li et al., 2009).

A variety of evidence has shown that F-actin enriched structures can adapt to altered gravity, hydrostatic pressure, or flow rate by the reorganization of actin filaments (Tilney and Cardell, 1970; Maunsbach et al., 1987; Gabrion et al., 1996; Mani-Ponset et al., 1997; Davet et al., 1998; Doughty, 1998). This seems to be true for the F-actin ring in the central canal, since we have found that the regionally differing organization of F-actin along the central canal did not appear until 21 days after birth and could not be developed if the intervertebral movements of the preweaning rats were restrained continuously for one week (Li et al., 2008).

Different from other spinal levels, the medullary canal is characterized by a ventrally interrupted F-actin network in the adult rats. Our studies showed that the rats aged less than 21 days had a continuous F-actin ring around the luminal surface of the medullary

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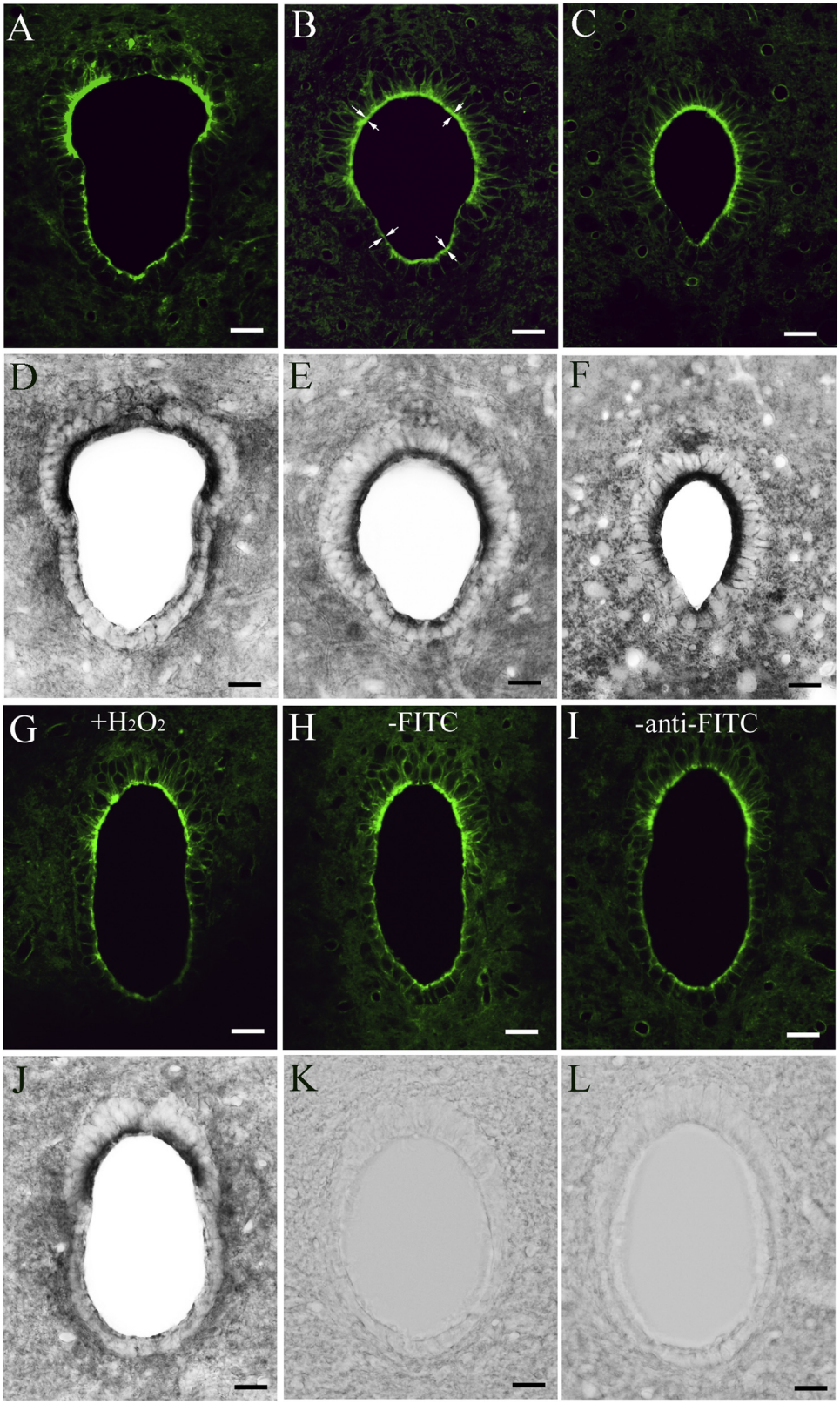

Fig. 1. Conversion from the fluorescence signals for F-actin to the peroxidase/DAB products by the phalloidin-based FITC-anti-FITC method. Scale bars: 20 μm. Images in A–C are fluorescence microscopic images of F-actin in the medullary canals at the rostral (A), middle (B) and caudal (C) levels, respectively. All the figures are arranged dorsally upwards. FITC-conjugated phalloidin staining reveals a ring-like network in the apical surface of the central canal. The lumen of the medullary canal is elongated ventrally, and the apical F-actin layer is obviously weakened in the ventral region. The distance between the two arrowheads in B is defined as the thickness of the apical F-actin layer (B). The same sections of panels A–C are subsequently incubated with biotinylated anti-FITC antibody, followed by detection with the ABC method (D–F). The overall distribution of reaction products for F-actin agrees well with that obtained by FITC-conjugated phalloidin staining and confocal microscopy, and the peroxidase/DAB reaction products are found predominantly in the apical region of the central canal (D–F). Image G shows that fluorescence signals for F-actin are still detectable after pretreatment with 3% H_2O_2 in 0.1 M PB. The same section of panel G is then reacted with biotinylated anti-FITC antibody, followed by detection using the ABC method (J). No discernable differences in the distribution of F-actin are found in the sections pretreated with and without H_2O_2. The specificity of the conversion from fluorescence signals to DAB products is confirmed by omitting FITC-conjugated phalloidin (H) or by omitting anti-FITC antibody (I) in the immunostaining procedure.

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