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VEGF overexpression in the astroglial cells of rat brainstem following ozone exposure

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ABSTRACT

Ozone, a major photochemical pollutant, produces rapid damages in the pulmonary airway tract and in the central nervous system. This study focused on the neural mechanisms underlying the adaptive responses to an acute ozone exposure. Vascular endothelial growth factor (VEGF) is a factor associated with cellular recovery following brain injury. The aim of this study was to assess and localize the cellular expression of VEGF, since the central respiratory areas show a neuroplasticity in response to ozone. Adult rats were subjected to 0.5 ppm ozone for 3 h and then recovered for further 3 h. The expression of VEGF was evaluated by immunocytochemistry in the central respiratory areas, i.e., the nucleus tractus solitarius (NTS) and the ventrolateral medulla (VLM). The data show a VEGF overexpression at the end of ozone exposure, which persisted during the 3-h recovery. Interestingly, using confocal analysis the bulk of VEGF labeling was observed in astroglial cell bodies and branches, while neuronal labeling was hardly noticed. Moreover, VEGF colocalized with IL-6 and TNFα in astrocytes closely apposed to blood vessel walls. The vasculature area was markedly increased (+58%) during post-ozone recovery. The data show that an acute ozone exposure affects primarily glial cells in the central nervous system. The VEGF up-regulation which persists after ozone exposure may contribute to brain repair and consecutive functional adaptations.

Ozone, a highly reactive gas, is a major component of photochemical urban air pollution formed from atmospheric pollutants such as hydrocarbons or nitrogen oxides (Wright et al., 1990). This environmental chemical represents a threat for human health that affects the well being of living world. Currently, exposure to ozone becomes more and more a worldwide society issue due to progressively increased levels in the urban air. Inhalation of ozone induces rapid damages of epithelial cell membranes in the pulmonary airway tract, altering the cellular and functional homeostasis of the respiratory system, particularly breathing, and inducing lung inflammation (Schelegle et al., 2001; Shore et al., 2000). Furthermore, effects of ozone are not restricted to the respiratory functions since the central nervous system is also affected (Calderon-Garciduenas et al., 2003, 2007). A variety of neural dysfunctions, such as sleep disturbances, impaired mental performance, headache, decreased locomotor activity, neurochemical alterations and cellular degeneration, have been reported (Huitron-Resendiz et al., 1994; Rivas-Arancibia et al., 1998; Rivas-Manzano and Paz, 1999; Soulage et al., 2004).

The mechanism by which ozone causes cell injury is linked to its powerful oxidative effect, leading to the generation of reactive oxygen species from lipid peroxidation of lung cell membranes (Wright et al., 1990). In response to brain injury induced by oxidative stress, the central nervous system is able to express neuroprotective factors which play a major role in reducing the cellular and neural damages. Increased activity of antioxidant enzymes has been reported in the brain, the lung and the heart of rats exposed to ozone (Pereyra-Munoz et al., 2006; Rahman et al., 1992; Servais et al., 2005).

Vascular endothelial growth factor (VEGF) is a dimeric glycoprotein, which presents structural homology with platelet

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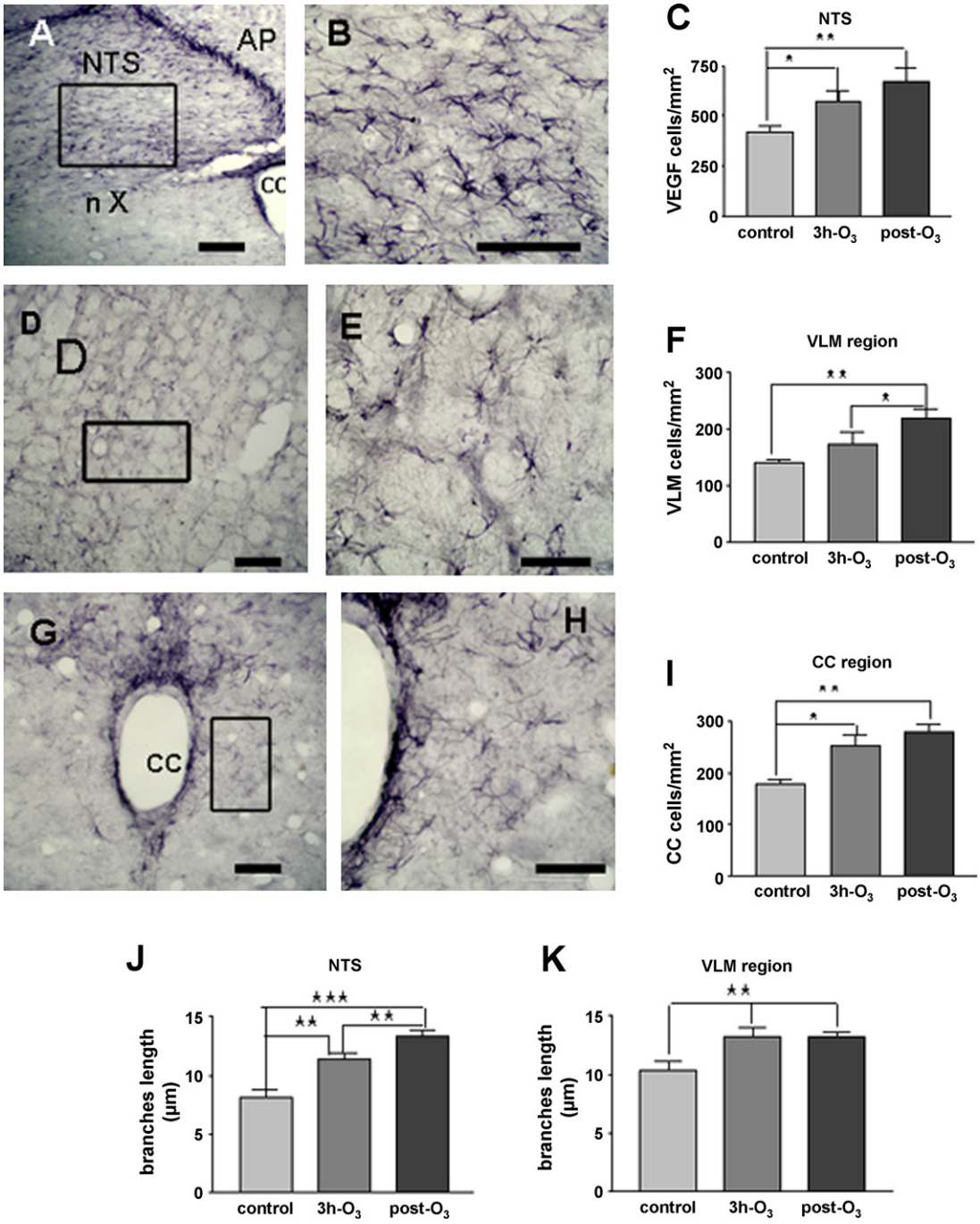

Fig. 1. Photomicrographs showing immunocytochemical detection of VEGF-immunoreactive cells in the nucleus of tractus solitarius (NTS), (A and B), the ventrolateral medulla (VLM), (D and E), the region lateral to the central canal (CC), (G and H). All pictures correspond to the control group. Enlarged frame of (A), (D) and (G) is displayed in (B), (E) and (H), respectively. Note that VEGF immunolabeling is observed in cells showing the morphology of astrocytes with numerous and ramified branches. Most neuronal cells are devoid of VEGF labeling. The density of VEGF-immunoreactive cells is higher in NTS than in area postrema (AP) or motor nucleus of vagus (nX). VEGF-immunoreactive cells were counted in a selected area indicated as a rectangle in each structure. Quantification of VEGF-immunoreactive cells was performed in the NTS (C), the VLM (F) and the region lateral to the CC (I) of the control, 3 h ozone-treated and post-ozone groups. The quantification of the number of VEGF-immunoreactive cells is depicted as the number of VEGF-IR cells/mm^2. (J and K) show data obtained from measurement of branch length (μm) in the NTS (J) and VLM (K) of the three experimental groups. Data are mean ± S.E.M. values of five independent experiments. Scale bar: 10 μm. One way ANOVA analysis and Newman–Kewls test give the following significance between groups: $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

derived growth factor. Several epitopes of the VEGF have been described, that arise by alternative mRNA splicing. Primarily, VEGF is recognized as a powerful inducible factor of angiogenesis, which interacts with growth factors having angiogenic activity in tumours. Following injury of central nervous system, VEGF can exert neuroprotective effects in inflammatory processes of neural tissues where it has been associated to other neurotrophic factors such as NGF and BDNF (Brockington et al., 2004; Sun et al., 2006). In the central nervous system of adult mice exposed prenatally to ozone, Santucci et al. (2006) found increased expression of NGF and BDNF. Therefore, we hypothesized that VEGF is a marker of brain cellular adaptive responses to ozone. The aim of the present study was to demonstrate and characterize the putative influence of an acute inhalation of