NADPH-diaphorase in the cat carotid body

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INTRODUCTION AND METHODS. Nitric oxide (NO) has recently emerged as a major modulator of carotid body (CB) chemoreception. This is evidenced by functional and morphological studies. Chemosensory activity is inhibited by NO agonists and disinhibited by antagonists in both in situ and *in vitro* preparations (1, 2). At the light microscopic level, the NADPH-diaphorase (NADPH-d)/nitric oxide synthase (NOS) seems confined to the sensory nerve fibers penetrating the CB vasculatory and chemosensory structures but is not present in the CB cells themselves (2, 3). At this level the cellular sources of NADPH-d in the CB cannot be discerned. In this study we used NADPH-d histochemistry for the localization of NADPH-d in the CB cells at the electron microscopic (EM) level. CBs were removed from anesthetized cats, fixed in 3% paraformaldehyde and 0,5% glutaraldehyde in phosphate buffer pH=7,4 for 3 h and kept in the buffer for 24 h at 4°C. The specimens were incubated with the NADPH-d reaction medium containing 2 mg nitroblue tetrazolium, 8,5 mg B-NADPH, and 10 mg sodium malate in 10 ml 50 mM Tris buffer pH=7,6 for 2 h at 37°C. Then, ultrathin sections were made and postfixed in osmium, dehydrated in ethanol, and embedded in Spurr. Controls omitted the B-NADPH substrate in media.

RESULTS AND DISCUSSION. Ultrastructural examinations of the CB showed that NOS-related NADPH-d activity is present in the chemoreceptor cells (Fig. 1A). The NADPH-d reaction product appeared as small, intense, dark particles scattered in the cytoplasm of the cells. The particles were distributed most densely or tended to aggregate in the perinuclear region, the endoplasmic reticulum channels, and in between the dense-core vesicles but not in the vesicles themselves. These particles were distinguishable from ribosomes from which they were larger and more electron dense. The particles were absent in the control specimens in which B-NADPH was omitted (Fig. 1B). The presence of NOS, which synthesizes a short-lived gaseous NO messenger, may have significant implications for the CB function. NO could not only affect the autonomic and sensory nerve endings but also be a direct participant of the chemoreceptor cell signaling process. The exact role of the nitergic pathway in the CB is unclear at present.

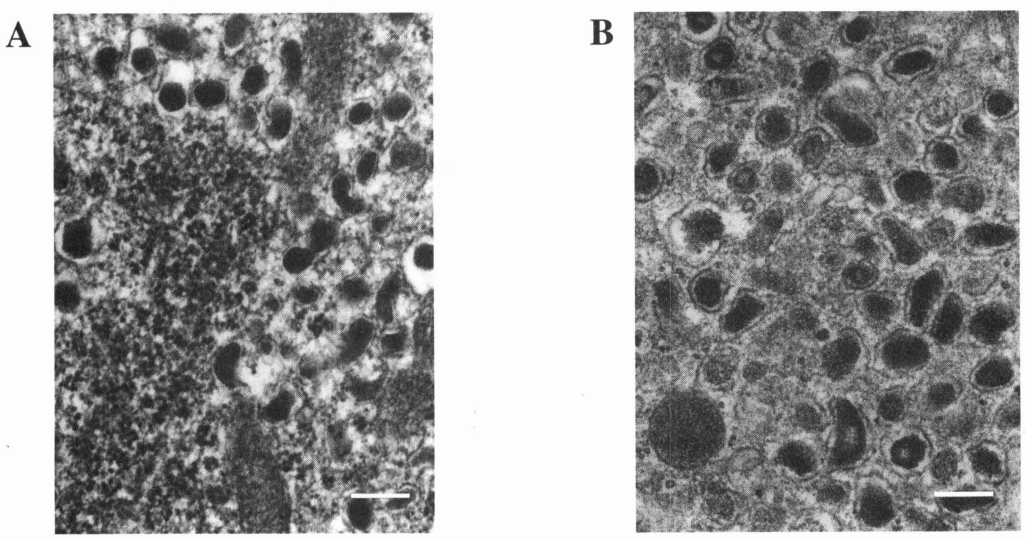

Fig. 1. EM micrographs showing cross sections through type I cells. A, the NADPH-d reaction product visible as small, dark particles partly aggregating and surrounded by the secretory dense-core vesicles in the cytoplasm. B, positive control from an experiment in which B-NADPH was omitted. No reaction product is visible. Scale bars, 200 nm.

1. Alcayaga J., Iturriaga R., Ramirez J., Readi R., Quezada C., Salinas P. (1997) Brain Res. 767:384-387.
2. Wang Z.Z., Dinger B.G., Stensaas L.J., Fidone S.J.(1995) Biol. Signals 4:109-116.
3. Prabhakar N.R., Kumar G.H., Chang C.H., Agani F.H., Haxhiu M.A. (1993) Brain Res. 625:16-22.

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