Human carotid body neuroglobin expression in heroin subjects

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Keywords: carotid body, heroin addiction, NGB

The carotid body (CB), an arterial chemoreceptor situated at the carotid bifurcation, represents the main site for oxygen sensing. Due to its particular functions, high blood flow and metabolism, the CB represents an experimental model suitable for studying hypoxia-related processes such as intake of heroin, inducing respiratory depression with consequent hypoxia [1]. Since Neuroglobin (NGB), Hypoxia Inducible Factor 1 (HIF-1α), Vascular Endothelial Growth Factor (VEGF), and Inducible Nitric Oxide Synthase (i-NOS) involvement in hypoxia event has been demonstrated and heroin addiction, leading to respiratory depression, can induce an hypoxia condition [2], the aim of our study has been to investigate the expression of such proteins along with the apoptotic events occurrence in an experimental model as CB of opiate addicts who died of heroin intoxication, compared to control subjects who died of trauma.

CB excised at autopsy from six 27±2 years old subjects, have been processed for Mallory Trichrome staining and for immunohistochemical analysis to detect NGB, HIF-1α, VEGF, i-NOS, Bax and cleaved caspase-3 proteins and observed by means of light microscope.

Mallory Trichrome staining shows an increase of the connective compartment in heroin subjects, compared to control and a parallel reduction in parenchymal area. Immunohistochemical analyses evidence a decrease of NGB, increase of HIF-1α and VEGF levels in heroin subjects compared to controls, while i-NOS expression does not significantly modify. Bax and cleaved caspase-3, which suggests apoptotic events occurrence, are highly expressed, only in heroin subjects.

While further study is needed to clarify the role of NGB in chemoreception, these results could support the typical hypoxic condition occurring in heroin addicts. Since NGB may function as reactive oxygen or nitrogen species scavenger [3] and as apoptotic carotid body cell death protector [4], its decreased expression can suggest a key role of this globin in human because it would affect the O2-CO2 balance allowing to a decrease of CB oxygen sensitivity. Moreover NGB reduction will reduce CB type I cells protection from the oxidative-stress that occurs during heroin addiction, and NGB neuroprotective action loss increases the susceptibility to age-related changes in CB, resembling the aging modifications.

References

**Fig. 1** A Morphometric analysis of carotid bodies performed on Trichrome Mallory stained slides of control and heroin subjects. B Parenchyma and connective tissue compartment measurements expressed as % area mean (± SD). Insets show organized and irregular morphology in control and heroin subjects, respectively.

*heroin subjects vs control subjects

**Fig. 2** Immunohistochemical detection and graphic representation of NGB percentage positive area (± SD) (left panel) and HIF-1α positive nuclei (right panel) in human carotid bodies of control and heroin subjects; C(-) negative control.

*heroin subjects NGB vs control subjects NGB p<0,05
*heroin subjects HIF-1α vs control subjects HIF-1α p<0,05

**Fig. 3** Immunohistochemical detection and graphic representation of VEGF (left panel) and i-NOS (right panel) percentage positive area (± SD) in human carotid bodies of control and heroin subjects; C(-) negative control.

*heroin subjects VEGF vs control subjects VEGF p<0,01

**Fig. 4** Immunohistochemical detection of Bax (upper panel) and cleaved caspase-3 (lower panel) expression in human carotid bodies of control and heroin subjects; C(-) negative control; in table densitometric measurements of Bax and cleaved caspase-3 percentage positive areas (± SD).