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# Immunohistochemical evidence for species-specific coexistence of catecholamines, serotonin, acetylcholine and nitric oxide in glomus cells of rat and guinea pig aortic bodies

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## Summary

The aortic bodies are small paraganglia distributed along the vagus nerve and its branches in the vicinity of the aortic arch which, like the carotid bodies, act as arterial chemoreceptors. In the rat carotid body, corelease of ATP and acetylcholine (ACh) from glomus cells is considered to be the main mechanism mediating fast hypoxic chemotransmission while dopamine, serotonin, and nitric oxide (NO) exert modulating effects. The present study was aimed at determination of the endogenous sources of serotonin, ACh and NO within rat and guinea pig aortic bodies by immunohistochemical double- and triple-labeling approaches, utilizing antibodies to serotonin, the NO and ACh synthesizing enzymes neuronal NO synthase (nNOS) and choline acetyltransferase (ChAT), respectively, as well as to the vesicular acetylcholine transporter (VAChT). Additional marker antibodies were directed against the rate-limiting enzyme of catecholamine synthesis, i.e. tyrosine hydroxylase (TH), and the vesicular protein, synaptophysin (SYN). In both species, all aortic body glomus cells were immunoreactive to serotonin and cholinergic markers. In the rat, all glomus cells were additionally catecholaminergic, as indicated by TH-immunoreactivity, whereas this applied only to a subgroup of guinea pig glomus cells. On the other hand, all guinea pig glomus cells were nNOS-immunoreactive, whereas only nerve fibers but not glomus cells exhibited nNOS-immunoreactivity in the rat. These data support the concept that the chemoexcitatory transmitters ACh and serotonin are involved in

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hypoxic excitation of aortic chemoreceptor terminals in both species. The production of the inhibitory modulators, dopamine and NO, however, appears to be species-specifically regulated.


## Introduction

The aortic bodies are small paraganglia distributed along the vagus nerve and its branches in the vicinity of the aortic arch (Coleridge et al., 1970; McDonald and Blewett, 1981; Easton and Howe, 1983). Structurally, they for the most part resemble the carotid body but still differ from this cervical paraganglion in several aspects. They (1) show a approximately 2-fold higher volume density of the specific glomus ( = type I) cells while other tissue components, e.g. glia-like sustentacular ( = type II) cells are similar in quality (cat; Kummer and Addicks, 1986), (2) lack a sympathetic innervation while sensory nerve terminals are indistinguishable from those of the carotid body (Hansen, 1981; Kummer and Neuhuber, 1989), and (3) contain only large, fenestrated capillaries whereas bypassing capillaries and intraglomic arterioles, as reported for the carotid body (McDonald and Larue, 1983), are lacking (Kummer and Neuhuber, 1989). Functionally, aortic bodies also closely resemble the carotid body but still show some distinctive features. Like the carotid body, they act as arterial oxygen sensors (Diamond and Howe, 1956) but whereas the carotid body is more suited for monitoring blood gas changes due to respiration, appropriate stimuli for excitation of aortic bodies are decreases in oxygen supply due to anemia or hypotension (Hatcher et al., 1978; Lahiri et al., 1980; Szlyk et al., 1984) and, accordingly, they are primarily related to circulatory reflexes (Comroe and Mortimer, 1964; Lahiri et al., 1980).

The molecular mechanisms underlying oxygen sensing and subsequent signal transduction here have not yet been fully resolved. The currently most favored concept postulates that lowered oxygen directly or indirectly inhibits potassium channels in the glomus cell membrane, leading to depolarization and release of a transmitter that excites closely related sensory nerve terminals of vagal (aortic bodies) or glossopharyngeal (carotid body) afferent neurons (Lopez-Barneo, 2003). These nerve terminals, in turn, may also release classical transmitters and neuropeptides, thereby modulating the process via feedback mechanisms. In the rat carotid body, corelease of ATP and acetylcholine (ACh) from glomus cells is considered to be the main mechanism mediating fast hypoxic chemotransmission (Nurse and Zhang, 1999, 2001; Zhang et al., 2000), while dopamine, serotonin, and nitric oxide (NO) exert modulatory effects (Gozal et al., 1996; Zhang et al., 2003; Carroll et al., 2005; Jacono et al., 2005). These modulators are also released from glomus cells, but, in addition, NO synthesizing nerve fibers innervate the carotid body (Prabhakar et al., 1993; Wang et al., 1993; Höhler et al., 1994). The mechanisms operating in aortic bodies have been much less analyzed, largely due to their small size, irregular distribution and relative inaccessibility. In the chicken aortic body, serotonin is localized within and released in response to hypoxia from the glomus cells (Ito et al., 1997, 1999). Pronounced excitatory effects on aortic chemoreceptors have been found to be exerted by cholinergic and serotonin receptor agonists in the dog, cat, and rat (Gernandt, 1946; Comroe and Mortimer, 1964; Brophy et al., 1999; Jones, 2000), and the ACh cleaving enzyme, ACh-esterase, has been histochemically detected in rabbit aortic bodies (Papka, 1975) These data indicate that ACh and serotonin are endogenously present in mammalian aortic bodies, too. Their cellular localization, however, is not known.

The present study has been undertaken to unravel the endogenous sources of serotonin, ACh and NO within the rat and guinea pig aortic bodies using an immunohistochemical approach. In the rat, aortic body glomus cells contain dopamine and can be visualized with antisera directed against the rate-limiting enzyme of catecholamine synthesis, tyrosine hydroxylase (TH), and against the synaptic vesicle protein, synaptophysin (SYN) (Kummer and Neuhuber, 1989). In this study we first validated the expected occurrence of catecholamines/serotonin in glomus cells in the guinea pig by aldehyde-induced fluorescence in whole-mount preparations of vagal branches originating in the aortic arch region. Then, TH- and SYN-antibodies were used in double- or triple-labeling experiments for cellular identification in both rat and guinea-pig. Serotonin was directly demonstrated by an appropriate antiserum, while NO- and ACh-generating cells were identified by antisera against their synthesizing enzymes, neuronal NO synthase (nNOS) and choline acetyltransferase (ChAT), respectively. In rat tissue, cholinergic cells were identified with an