ENZYME HISTOCHEMICAL INVESTIGATIONS OF THE MAMMALIAN CAROTID BODY

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ABSTRACT

The carotid body (CB) in mammals is a small cluster of chemosensory and supporting cells located at the carotid bifurcation. It has been proposed that the chemoreceptor glomus cells release a variety of neurotransmitters that trigger upon hypoxia an action potential through the afferent fibers, thus conveying the chemosensory information to the central nervous system. By means of histochemical techniques the presence and distribution of certain metabolic enzymes was demonstrated in the CB of rats, guinea pigs and rabbits. In particular, we have revealed that the glomus cells expressed hydrolytic enzymes such as alkaline phosphatase (AP), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and ATPase as well as oxidoreductases including oxidases like monoamine oxidase (MAO) and dehydrogenases like succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PD), glutamate dehydrogenase (GDH) and NADPH dehydrogenase (NADPH-diaphorase). In addition, the sustentacular cells also contained, although in a much lesser degree, AP, BChE, LDH, G6PD, GDH and NADPH-d. Some AP and SDH activity was seen in the CB microvasculature as well. Our results provide evidence that the two types of parenchymal CB cells display a different enzyme content and that the glomus cells possess enzymatic properties necessary for the secretory process. It can also be inferred that the chemoreceptor function and the nerve impulse conduction need an intensive molecular and cation exchange, and energy supply.

Key words: carotid body, chemotransduction, chemotransmission, enzyme histochemistry, hydrolases, oxydoreductases

INTRODUCTION

The mammalian carotid body (CB), also known as the glomus caroticum, is a small paired organ, located in the vicinity of the common carotid artery bifurcation (8). Its structure is similar in mammalian species, consisting of richly perfused clusters of oxygen-sensitive, neuron-like secretory cells called type I or glomus cells, surrounded by processes of glial-like type II or sustentacular cells (1,5).

The CB is the main peripheral arterial chemoreceptor that transduces arterial oxygen levels into action potential activity on carotid sinus nerve afferents. Upon exposure to hypoxia, voltage-gated Ca^{2+} influx into type I cells initiates neurosecretion (6,8) and release of neurotransmitters. The major neurotransmitters involved in chemotransduction in the adult cat, rat and mouse CB are ATP and acetylcholine (ACh)(both considered excitatory), and dopamine (DA) commonly regarded to be inhibitory

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(8,9,15). Moreover, the co-release of ACh and ATP has been proposed to be the main mechanism mediating hypoxic chemotransmission in the mammalian CB (19,20).

In line with this, the CB was found to contain large amounts of ACh (8) and the content remains unchanged after the section of the carotid sinus nerve or the removal of the superior cervical ganglion (SCG) (see 8 and references therein). The localization of acetylcholinesterase (AChE), the ACh-degrading enzyme, has often been interpreted as an indication of sites at which cholinergic mechanisms may act. A number of researchers have studied the distribution of cholinesterases in the CB of a variety of animals (2,10) and both specific AChE and non-specific butyrylcholinesterase (BChE) activities were found in strands and plexuses around the blood vessels of the CB (3). In addition, the changing levels of ATP within CB tissues and the chemosensory excitatory effects observed upon its administration were initially considered as part of the ATP metabolic role and later as its possible role as a transmitter between glomus cells and sensory nerve endings (8,9,15). With respect to enzymatic degradation of ATP, adenosine triphosphatase (ATPase) was detected in the CB (18) and localized in the cell membranes of glomus cells, sustentacular cells and nerve fibers (14). The distribution and relative activities of certain hydrolases, dehydrogenases and diaphorases in the rat CB and SCG were also studied histochemically and cytophotometrically.

This study aims at investigating by histochemical techniques the presence and distribution of some metabolic and transmitter-degrading enzymes in the CB of various mammals with an emphasis on integrating cellular mechanisms involved in hypoxic chemotransduction and chemotransmission.

MATERIAL AND METHODS

The experiments were performed on adult rabbits, guinea pigs and rats of either sex. All procedures were carried out according the ethical principles and guidelines for the care and use of experimental animals, and all efforts were made to minimize their number and suffering. Tissue samples were taken after transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The carotid bifurcation areas were dissected out and embedded in paraffin. 5 µm thick sections were cut and processed for enzyme histochemistry. Prior to incubation all sections were transferred in the buffer of the reaction medium to be used. The histochemical methods that were applied, and the substrates and specific inhibitors of the corresponding enzymatic activities are listed in Table 1. After incubation, the sections were developed and coversliped with Entellan or Kaiser's glycerol gelatin (Merck KGaA, Darmstadt, Germany). The slides were observed and

Tabl. 1. Histochemical methods for enzyme detection

Enzyme detected	Method applied	Substrate	Control incubations
AP	Burnstone (1962)	Naphtol-AS-MX-phosphate	L-tetramisol (2.5 mM)
AChE and BChE	Koelle and Friedenwald (1949)	Acetyl-thiochloride	BW284c51 (10^{-5} M) iso-OMPA (10^{-4} M)
ATPase	Mayahara et al. (1980)	p-Nitrophenyl phosphate	Ouabain (5-10 mM)
MAO	Glenner et al. (1957)	Tryptamine hydrochloride	Iproniazid (10 mM)
SDH	Nachlas et al. (1957)	Sodium succinate	Substrate-free medium
NADPH-diaphorase	Scherer-Singler et al. (1983)	β-NADPH	Substrate-free medium

photographed with a Nikon research microscope equipped with a digital camera DXM1200c.

RESULTS

Under the light microscope, several metabolic enzymes, including hydrolases and oxydoreductases were evident in the CB of rats, guinea pigs and rabbits. Specifically, a moderate bluish azo-dye staining corresponding to alkaline phosphatase (AP) activity was seen in a few glomus cells, the majority of the sustentacular cells and also in the blood vessel walls (Fig. 1A). A dark brown ATPase activity reaction product was deposited in the periphery of the glomus cells (Fig. 1B). The localization of the two cholinesterases in the CB nerve fibers was similar while their cellular distribution differed. In particular, both AChE and BChE were observed in thick, often parallel strands throughout the organ (Fig. 1C, D). Some of the positive nerve bundles were associated with blood vessels as well. On the other hand, the AChE reaction product was present in the type I cells whereas some BChE activity was visualized on the type II cell membrane.

Light microscope histochemistry also revealed the presence of certain oxidoreductases in the mammalian CB. Even after a prolonged incubation, we observed only a trace monoamine oxidase (MAO) activity in both the CB parenchymal cells. Conversely, the glomus cells displayed a strong dehydrogenase enzyme activity (Fig. 2). The enzymes exhibited in them included succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PD), glutamate dehydrogenase

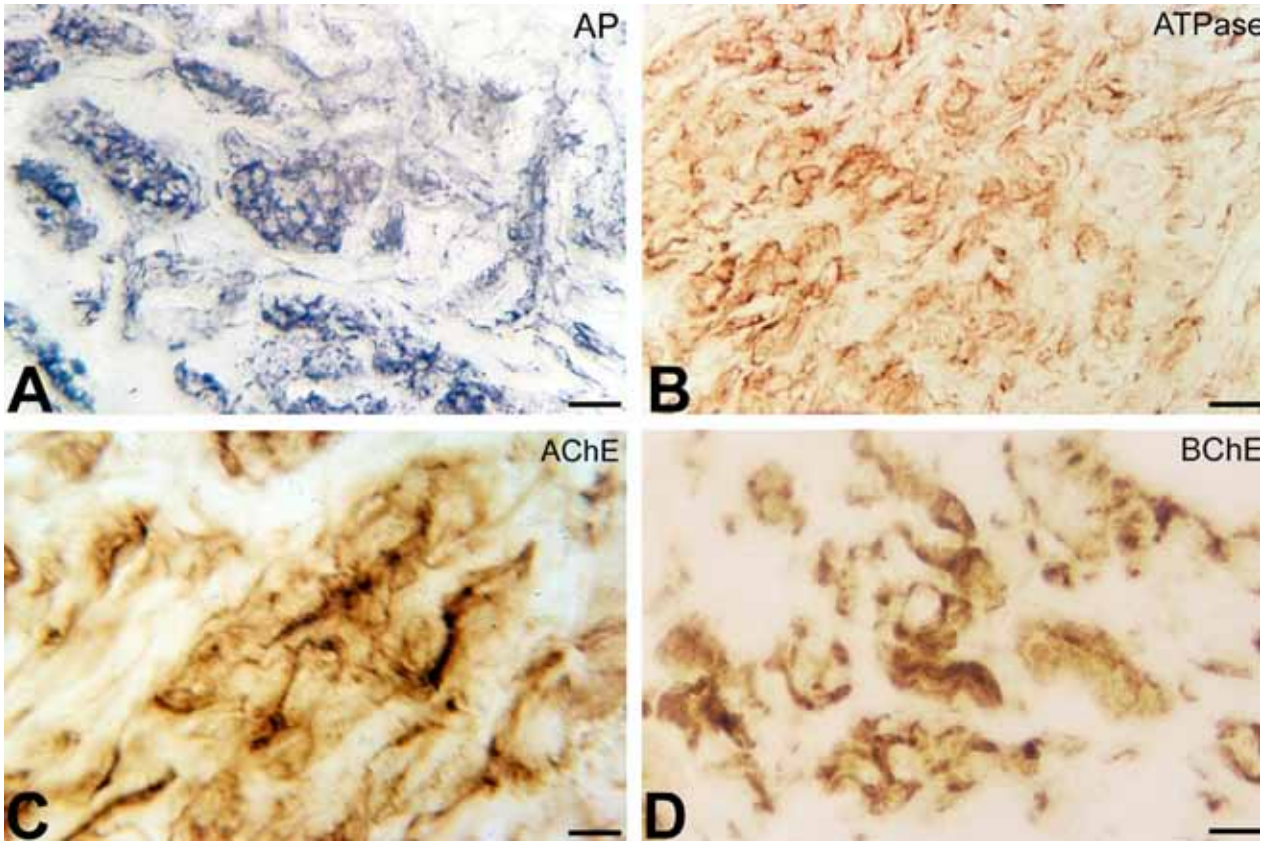

Fig. 1. Histochemical localization of hydrolytic enzymes in the rabbit carotid body. (A) Positive reaction for alkaline phosphatase (AP) can be seen in some glomus cells and the vast majority of the sustentacular cells. Precipitates are also evident in the capillary wall. (B) The dense deposits of an ATPase reaction product are observed in the periphery of the glomus cells. (C) AChE activity is present in a few glomus cells and in thick intensely-stained intraglomerular strands. (D) Strong BChE enzymatic reaction is usually associated with nerve bundles within the organ. Faint enzymatic activity is also found on the surface of sustentacular cells. Scale bars = 50 μm (A); 25 μm (B); 15 μm (C-D)

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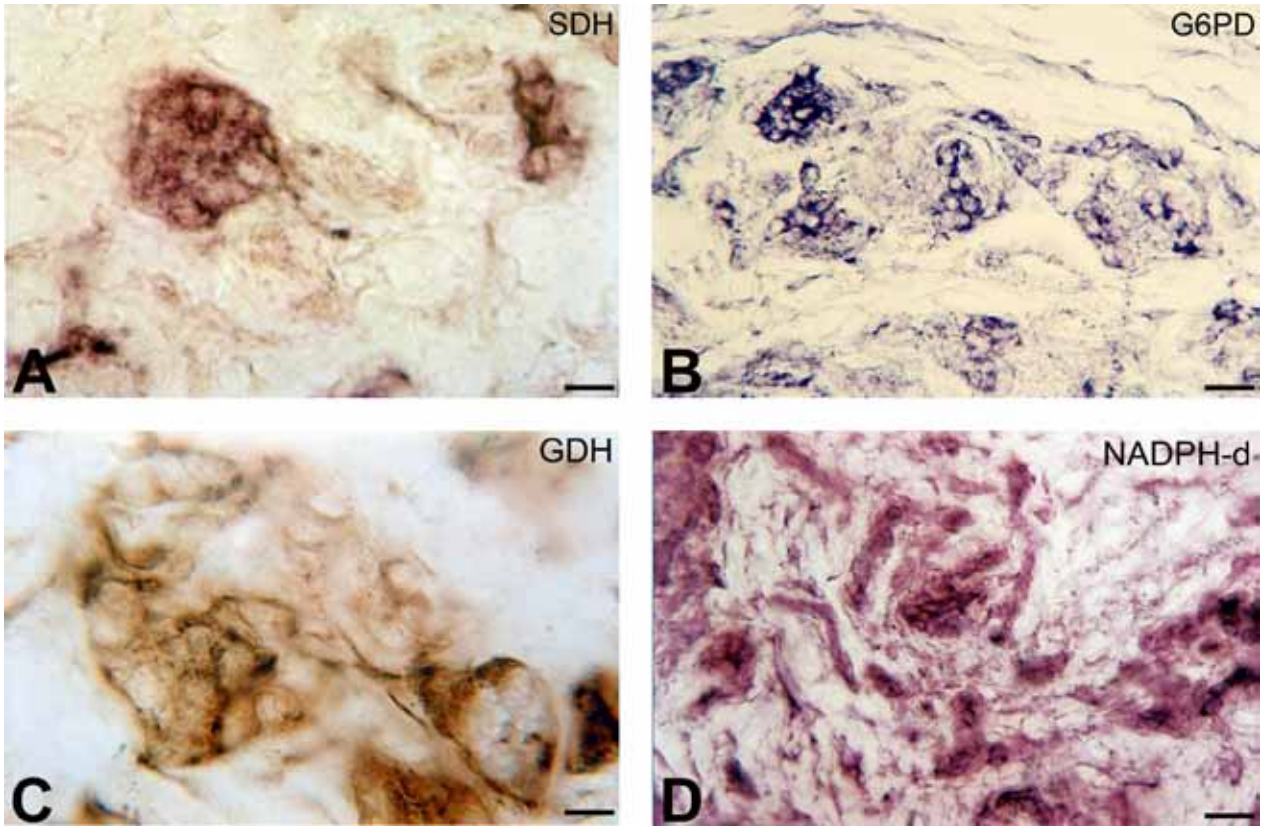

Fig. 2. Histochemical localization of oxidoreductases in the rabbit carotid body. (A) Succinate dehydrogenase (SDH) reaction product is observed in the glomus cells and is also associated with the CB microvasculature. (B) Dense precipitates of glucose-6-phosphate dehydrogenase (G6PD) activity outline the glomus cells. (C) Glutamate dehydrogenase (GDH) histochemical staining of both the glomus and sustentacular cells. (D) The NADPH-diaphorase reaction product is located in the periphery of the glomus cells, although the most intense reaction is restricted to the sensory nerve fibers penetrating the CB vasculatory and chemosensory structures. Scale bars = 25 μm (A); 50 μm (B, D); 15 μm (C)

(GDH) and NADH dehydrogenase (DH). The sustentacular cells also displayed a faint staining for LDH, G6PD, GDH and NADPH-d. Some SDH activity was seen in the CB microvasculature as well.

DISCUSSION

Our results provide evidence that the two types of parenchymal CB cells display a different enzyme content. On the one hand, the glomus cells possess enzymatic properties necessary for the generation of action potential and for the secretory process of neurotransmitters in the chemoreceptor organ. In particular, the type I cells respond to a natural stimulation by releasing ACh, ATP and DA, which cause the type II to contract, thereby stimulating the nerve endings enclosed by them. Indeed, recent data have implicated a chemotransduction role of ACh, ATP, catecholamines such as DA and nitric oxide acting via the corresponding receptors in the adult cat, rat and mouse CB (8,9,15). In addition, it is evident that the glomus cell, which is considered the chemoreceptor cell of the organ, is the most active cation-exchange zone in the CB. It is well-known that oxygen-sensing in the CB occurs in neuroectoderm-derived type I glomus cells where hypoxia elicits a complex chemotransduction cascade involving membrane depolarization, Ca^{2+} entry and the release of excitatory neurotransmitters. It can also be inferred that the chemoreceptor function and the nerve impulse conduction need an intensive molecular and cation exchange, and energy supply. On the other hand, the presence of AP activity, which is used to test pluripotency and detect undifferentiated stem cells, in the type II cells is a strong indicator of their neurogenic nature (16). Last but not least, it seems

likely that a cholinergic pathway controls the blood vessels of the CB.

Taken together, the data of our study suggest that the different morphologic features of the two main cell populations in the CB coincide with a diverse cell metabolism due to a wide-ranged enzymatic equipment.

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