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DL-propranolol inhibits the vascular changes in the rat carotid body induced by long-term hypoxia

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Summary. Two groups of rats were exposed to hypoxia (10% O_2) for 1 and 3 weeks with or without daily injections of DL-propranolol (0.66 $mg \cdot kg^{-1}$ ip). The structure of the carotid body was analyzed by light microscopical morphometry and the catecholamine content was assayed by high performance liquid chromatography. Exposure to hypoxia induced enlargement of the carotid body due to enhanced vascularity and hypertrophy of glomic and interstitial tissues. The dopamine and norepinephrine content were increased at both 1 and 3 weeks of hypoxia and reached levels 40–50 times those of the controls. The DL-propranolol treatment abolished the vasodilatory effect of hypoxia within the first week but did not prevent the other structural changes or the rise in catecholamine content. The data suggest that 1. the vasodilation elicited by long-term hypoxia may be controlled by β-adrenoceptors and 2. the structural and biochemical events occurring in rat carotid body during long-term hypoxia do not influence each other and thus seem to be controlled by different mechanisms.

Key words: Carotid body – Long-term hypoxia – DL-propranolol – Vasodilation – Catecholamines

Introduction

Exposure of animals to hypoxia induced by high altitude or simulated altitude elicits a number of structural and biochemical alterations in the carotid body. The changes include enlargement of the organ due to hypertrophy of the glomus (type I) cells and a large increase in vascularity (Pequignot and Hellström 1983). Concomitant with the structural modifications, long-term hypoxia triggers a considerable rise in both dopamine (DA) and norepinephrine (NE) content of the carotid body (Hanbauer et al. 1981). The mechanisms underlying these structural and biochemical alterations are unknown. It is also not known whether or not these changes are linked, for example, is increased vascularity a prerequisite for type I cell hypertrophy?

Several investigators have shown that NE participates in the homeostatic adaptation to hypoxia (see Johnson et al. 1983; Kotchen et al. 1973). In a study on man, Koller et al. (1983) showed that treatment with propranolol improved the subjective tolerance to altitude and, by economizing the cardiac workload, the objective ability to withstand oxygen want. With respect to the carotid body, NE is considered as a putative neurotransmitter, but its role in the chemoreception process is obscure. NE is able to produce chemosensory excitation and to augment the chemoreceptor neural response to hypoxia (Eyzaguirre and Zapata 1984). Recently β-adrenergic antagonists such as propranolol have been found to attenuate or even to eliminate the increase in neural chemosensory drive evoked by hypoxia in cat and rabbit. It was therefore suggested that β-adrenoceptors may be an integral part of the oxygen chemoreception mechanism (Folgering et al. 1982; Lahiri et al. 1981; Milsom and Sadig 1983).

Against this background, the purpose of the present study was to examine the effects of the DL-propranolol on the structure and catecholamine content of the rat carotid body during long-term hypoxia.

Methods

Fifty male Sprague Dawley rats (275–300 g) were used for the investigation. All animals were kept in the same climatized

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room (24° C) at sea level, with a 12 h light-dark cycle. Food and water were provided ad libitum.

Forty rats were put into a plexiglass chamber supplied with a flow of air and nitrogen, adjusted to a final O_2 concentration of $10 \pm 0.5\%$ inside the chamber (Pequignot and Hellström 1983). The O_2 and CO_2 levels were monitored twice daily. The rats stayed in the hypoxic atmosphere for 1 week ($n=20$) and 3 weeks ($n=20$). Ten rats from each of these two groups received daily injections of DL-propranolol ($0.66\ mg \cdot kg^{-1}$ ip). The CO_2 level inside the cage was maintained at a level less than 1% by frequent changes of soda lime. Ten untreated animals were exposed to room air only and used as controls.

For the structural studies rats were anesthetized intraperitoneally with pentobarbital sodium while still in the hypoxic cage and then connected to the perfusion apparatus (Hellström and Bergh 1984). The rats were perfused through the left heart ventricle for 10 min with a fixative (2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4, flow rate $20\ ml \cdot min^{-1}$). The carotid bodies were dissected out and processed for light microscopy. The tissues were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for 2 h, dehydrated in increasing concentrations of ethanol and embedded in Epon. Semithin (0.5 μm) sections were analyzed at each 25 μm level. For morphometric analyses, two computerized procedures were tested and yielded similar data. Sections were placed in a Zeiss standard microscope and the areas of tissue constituents were measured with a digitizer (Hipard TM-Houston Instruments, Houston, Texas) interfaced with a computer (Luxor ABC 806). Alternatively, sections were placed in a Zeiss microscope (Universal, Oberkochen, FRG) equipped with a projection screen and a scanning stage. The

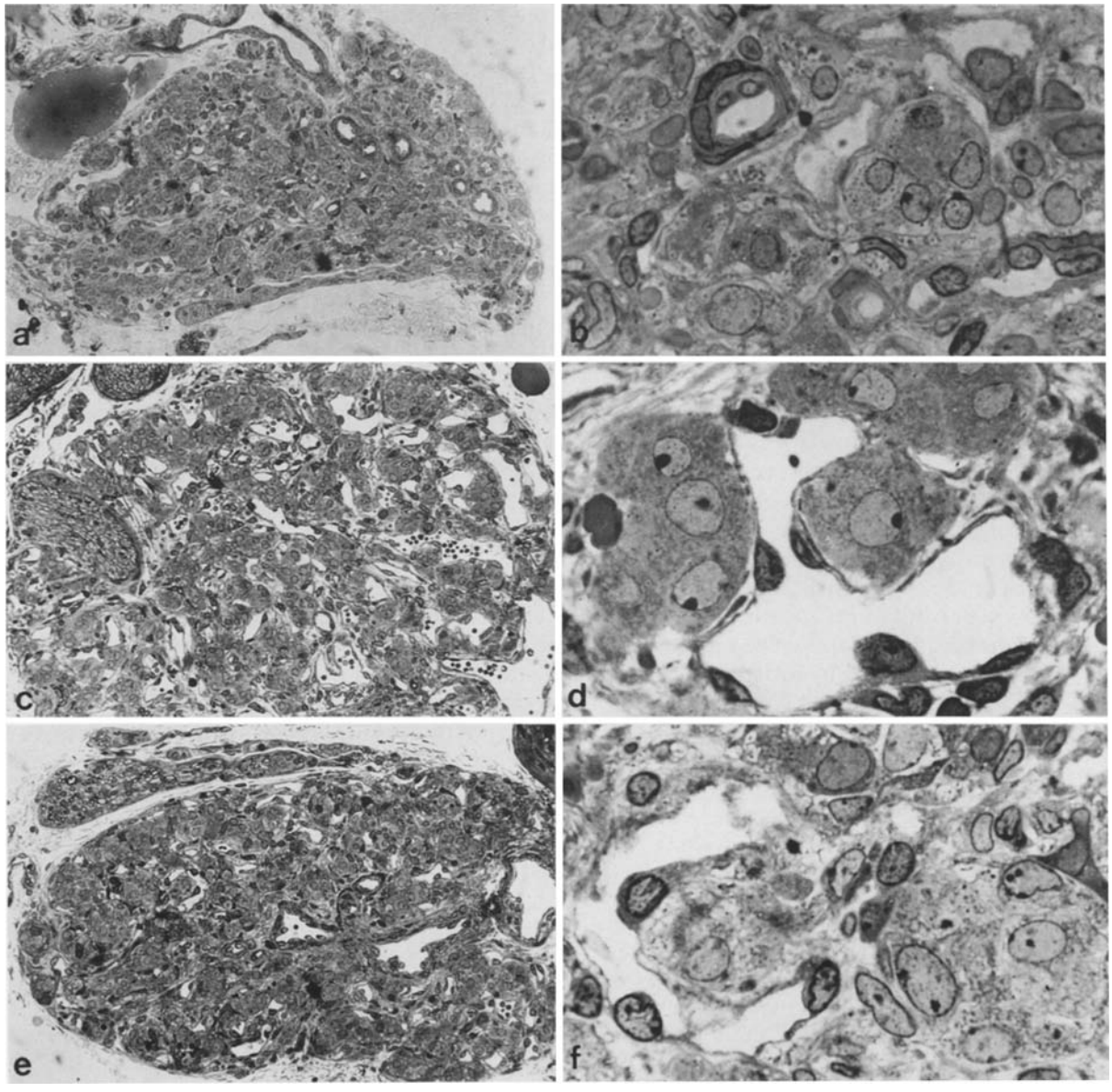

Fig. 1 a–f. Light microscopical pictures of carotid bodies from untreated and propranolol-treated rats in normoxia and long-term hypoxia. Toluidine-blue stained semithin sections. **a** normoxia, untreated, ×100; **b** detail of **a**, ×1000; **c** hypoxia 1 week, untreated. Note the marked vascularity. ×100; **d** detail of **c**, ×1000; **e** hypoxia 1 week, propranolol-treated; ×100; **f** detail of **e**, ×1000

areas were measured with an image analyzer (Quantimet 900 – Cambridge Instruments, Cambridge, UK) interfaced with a computer (LSI II 23). The volume proportions of three tissue constituents were estimated: glomic tissue (the islands containing type I and type II cells), blood vessel lumen and interstitial tissue.

For the catecholamine assay, rats were killed by cervical dislocation. The carotid bodies were dissected out and homogenized in 0.1 N perchloric acid containing 2.7 mM disodic EDTA. The DA and NE contents were determined by HPLC coupled with electrochemical detection (Pequignot et al. 1986).

Data were analyzed using Dunnett's test (1955) and differences were considered significant if $P<0.05$.

Results

The DL-propranolol-treated rats appeared more physically active than the untreated rats during the early stages of hypoxia. Within the first day the controls were almost completely inactive, whereas the drug-treated animals moved around.

Morphometric analysis showed that in untreated carotid bodies, long-term hypoxia elicited a tremendous increase in vascularity (Fig. 1) as previously observed (Pequignot and Hellström 1983). The volume density of the blood vessel lumen multiplied 2.2-fold after the first week of hypoxia ($P<0.05$), whereas the volume density of glomic tissue decreased ($P<0.05$) and that of the interstitial tissue remained unchanged (Fig. 2). After 3 weeks of hypoxia, the volume density of blood vessels remained high ($P<0.05$), the volume density of glomic cells had returned to basal level, whereas the proportion of the interstitial tissue decreased ($P<0.05$) (Fig. 2).

The effects of hypoxia on the structure of the carotid body were dependent on whether or not the rats received DL-propranolol (Fig. 3). In the hypoxic untreated carotid bodies the increased vascularity caused a characteristic splitting of the glomic tissue into numerous small islands separated by thick layers of interstitial tissue. Although signs of interstitial invaginations between Type I cells were observed in the hypoxic group treated with DL-propranolol, the glomic tissue still consisted of compact islands resembling those of the normoxic specimens. The most striking feature of the DL-propranolol-treated carotid bodies was their relatively poor vascularity throughout the long-term hypoxia (Fig. 1). Unlike the untreated carotid bodies, the volume density of blood vessel lumen failed to increase significantly throughout the 3 weeks of hypoxic exposure (Fig. 2). Neither the volume density of glomic cells nor that of interstitial tissue changed significantly.

Using Simpson's rule (Dunnill 1968), the relative changes in amount of each tissue constituent

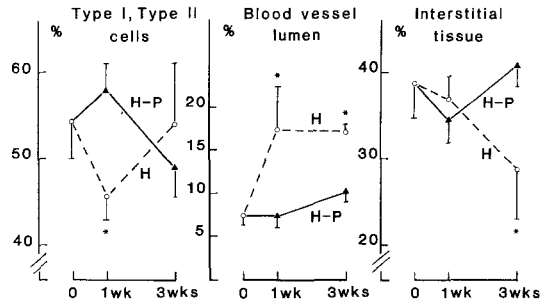

Fig. 2. Effects of DL-propranolol on the volume densities of the different tissue constituents of rat carotid body during hypoxia lasting for 1 and 3 weeks. *H*=untreated rats (*broken lines*); *H-P*=propranolol treated rats (*solid lines*). Values are means ± SE expressed as percent of the controls. * $P<0.05$ vs controls

were assessed in the untreated and DL-propranolol-treated carotid bodies (Fig. 4). The volume of each component, i.e., blood vessels, glomic and interstitial tissues, was enhanced in the controls after the first week of hypoxia ($P<0.01$). Consequently the whole carotid body was enlarged 2.5 fold ($P<0.01$). In contrast, the volume of blood vessels failed to augment after 1 week of hypoxia in DL-propranolol-treated carotid bodies. After 3 weeks of hypoxia an increased vascular volume was manifest ($P<0.01$). The DL-propranolol treatment did not prevent the rise in volumes of glomic and interstitial tissues during hypoxia ($P<0.01$). The increase in volume of interstitial tissue was even higher in DL-propranolol-treated than in untreated carotid bodies after 3 weeks of hypoxia ($P<0.01$). Regarding the enlargement of the carotid body, there was no significant difference between the untreated and DL-propranolol-treated rats.

Long-term hypoxia elicited a dramatic increase in both DA and NE contents of the carotid bodies at both one and three weeks (Table 1). Comparing DL-propranolol treated and non-treated animals there was no difference in catecholamine amounts except after 1 week hypoxia. At this time DL-propranolol caused an even higher augmentation in NE content ($P<0.05$).

Discussion

The present morphometric data provide evidence that DL-propranolol is able to inhibit or delay the vasodilation elicited by long-term hypoxia in the rat carotid body. In contrast, the increases in other tissue components and catecholamine content were not altered by chronic DL-propranolol treatment.

DL-propranolol has been characterized as a non-selective β-adrenergic antagonist able to block

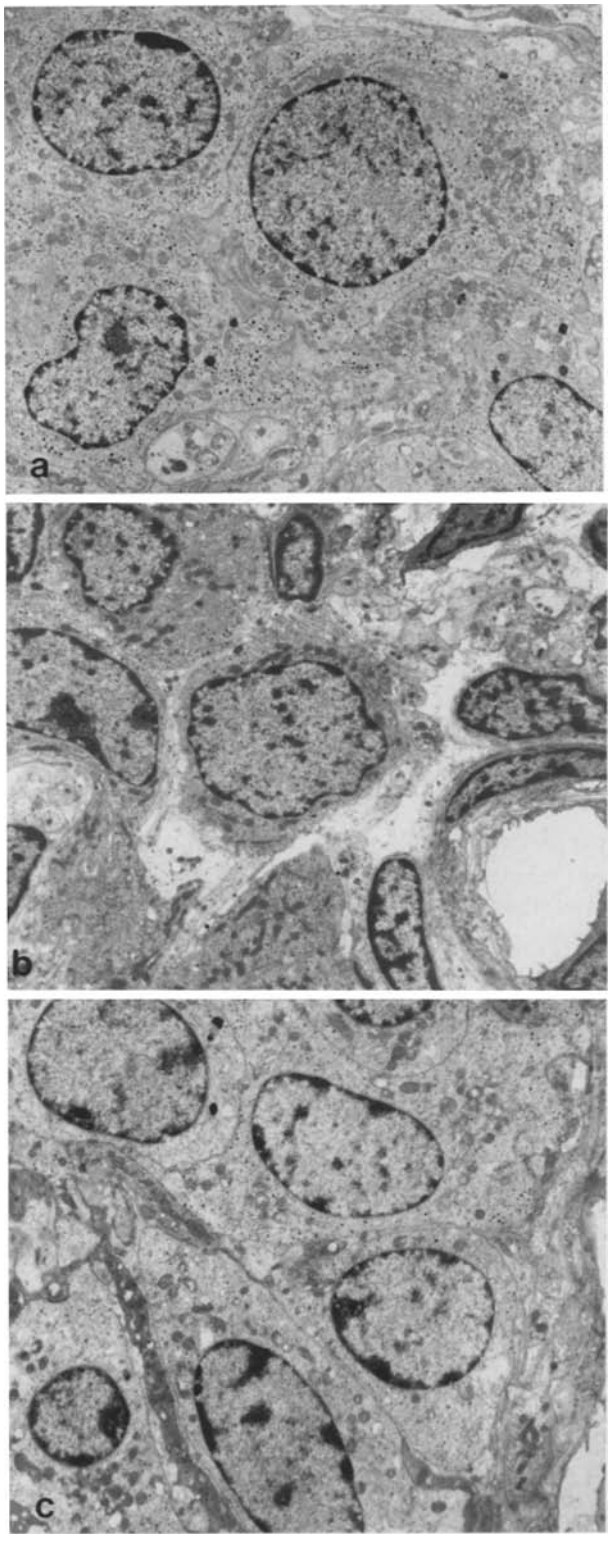

Fig. 3a–c. Electron microscopical pictures of rat carotid bodies in normoxia and after 1 week hypoxia with or without DL-propranolol treatment. ×4700. **a** normoxia; **b** hypoxia 1 week; **c** hypoxia with DL-propranolol 1 week

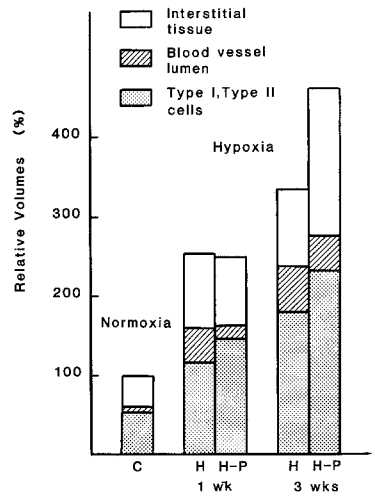

Fig. 4. Mean volumes of tissue constituents (estimated according to Dunnill 1968) in carotid bodies of untreated and propranol-oltreated rats during hypoxia lasting for 1 and 3 weeks. The volumes are expressed as percentages of the untreated control level. *C*: normoxia – untreated rats; *H*: hypoxia – untreated rats; *H-P*: hypoxia – propranolol-treated rats

Table 1. Effects of DL-propranolol on catecholamine content, dopamine and norepinephrine, of the carotid body during hypoxia lasting for 1 and 3 weeks

Exposure to hypoxia	Treatment	Dopamine pmol/cb	Norepinephrine pmol/cb
0	no drug (6)	4.50± 0.33	4.50± 0.65
1 week	no drug (6)	59.1 ± 7.2*	25.7 ± 3.3*
	propranolol (5)	63.2 ± 5.1*	46.1 ±10.8*
	P	NS	0.05
3 weeks	no drug (5)	212 ±33*	166 ±18*
	propranolol (5)	178 ±28*	124 ±35*
	P	NS	NS

Values are expressed as mean±SE. Number of observations within parentheses. *P* refers to the comparison between untreated and propranolol-treated rats.
* $P<0.01$ hypoxic vs normoxic rats

both β-$_1$ – and β-$_2$ receptors. Thus, the present data suggest that the vasodilation in the carotid body produced by long-term hypoxia is regulated through an adrenergic action on β-adrenoceptors. Although these receptors are probably localized on the capillaries themselves, other locations cannot be excluded. In this case propranolol may indirectly affect the changes in capillary diameter. Evidence supporting this hypothesis is the presence of β-adrenoceptors on the sympathetic nerve endings which facilitate NE release by a positive feedback mechanism (Adler – Graschinsky and Langer 1975). However, we have shown that the vasodilatory effects of long-term hypoxia were only atten-

uated, but not abolished, in the sympathectomized rat carotid body (Pequignot and Hellström 1983).

There are at least three potential sources of catecholamines able to interact with β-adrenoceptors in the carotid body. Apart from the sympathetic endings of the ganglioglomerular nerve, NE could originate from pools including glomus cells and the blood stream. NE stored in glomus cells increased during long-term hypoxia (Hanbauer et al. 1981). Furthermore, the overall sympathoadrenal activity is enhanced during long-term hypoxia, causing release of catecholamines into systemic circulation and increased plasma catecholamine levels (Johnson et al. 1983; Kotchen et al. 1973).

The suggestion that NE may induce vasodilation in long-term hypoxic carotid bodies may seem surprising because NE is generally thought to cause vasoconstriction of the carotid body blood vessels. In fact, a vasoconstricting effect of NE with subsequent ischaemia of the chemoreceptor has been hypothetized to explain the excitation of chemoreceptor activity in response to acute noradrenergic stimuli such as NE infusion or stimulation of the ganglioglomerular nerve fibers (see Eyzaguirre and Zapata 1984). However, Acker and O'Regan (1981) reported that electrical sympathetic stimulation did not influence the local blood flow in the cat carotid body. Regarding other parts of the body, long-term hypoxia was found to alter the vasoactive properties of NE in pulmonary blood vessels. In normoxic rats NE induced vasoconstriction in most cases, an effect which was progressively reversed to vasodilation within 2 weeks of hypoxia, suggesting an enhanced β-adrenoceptor activity (Porcelli and Bergman 1983). With respect to the carotid body, future studies will reveal any effect of long-term hypoxia on vasoactive characteristics of NE.

Propranolol has diverse effects (Gerber and Nies 1985) and the present data do not exclude the possibility that propranolol might have a nonspecific toxic effect on blood vessels not related to β-adrenoceptors. This, however, seems unlikely because the dose of DL-propranolol used in this work was relatively moderate. In vitro studies indicated that membrane-stabilizing activity for propranolol only occurred at a concentration about 100 fold higher than that eliciting maximal β-adrenergic blockade (Matthews and Baker 1982). Furthermore, the present finding that the inhibitory effects of DL-propranolol on carotid body vascularity were diminished after 3 weeks of hypoxia supports the view of a specific action on β-adrenoceptors. It seems likely that the tremendous increase in NE content of the carotid body (about 28-fold) during the latest stage of hypoxia competitively antagonized the action of DL-propranolol at β-adrenoceptors.

Despite its inhibitory effect on vascularity, DL-propranolol failed to prevent the hypertrophy of the carotid body and its glomic cells during long-term hypoxia. Similarly, the rise in catecholamine content also occurred in DL-propranolol-treated carotid bodies. This indicates that vasodilation, inferring an increased blood flow, is not a prerequisite for triggering the other structural and biochemical changes. Therefore the alterations elicited by long-term hypoxia in the carotid body appear to be influenced by different mechanisms. One possibility is that hypoxia may affect the structure and biochemistry of glomus cells through an increase in circulating corticosteroid levels. Endogenous glucocorticoid secretion is increased in hypoxia (Sutton et al. 1977) and exogenous administration of glucocorticoids has been reported to increase the catecholamine content of the carotid body (Hellström and Koslow 1976).

In conclusion, DL-propranolol appears to be an interesting tool able to inhibit the vascular changes in the rat carotid body induced by long-term hypoxia without preventing other structural and biochemical changes. This finding suggests that β-adrenoceptors are probably involved in the vasodilatory process observed in long-term hypoxia. In addition the changes in structure and biochemistry of the carotid body appear to be controlled by different mechanisms. It would be of interest to find other pharmacological tools which could differentiate the events involved in carotid body adaptation to hypoxia, since these may help to a better understanding of the chemoreceptor mechanisms.

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