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INCREASED RELEASE OF [^{3}H]DOPAMINE DURING LOW O_2 STIMULATION OF RABBIT CAROTID BODY IN VITRO

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SUMMARY

Rabbit carotid bodies synthesized [^{3}H]dopamine (DA) during a 3-h incubation period in modified Tyrode's solution containing 40 μM [^{3}H]tyrosine. Following this loading period, the carotid bodies were exposed for one additional hour to unlabelled Tyrode's solution equilibrated with either 10% oxygen in nitrogen or with 100% oxygen. The carotid bodies exposed to low O_2 released 81% more [^{3}H]dopamine during this one-hour period than the carotid bodies exposed only to pure oxygen. These data suggest that hypoxia induces release of DA from the carotid body.

It is now well documented that the mammalian carotid body contains the biogenic amines dopamine, norepinephrine and 5-hydroxytryptamine [9,12,29], and that of these, dopamine (DA) is present in the tissue in the highest concentration [12,15]. There is also convincing evidence that these substances are contained primarily within the glomus, or Type I, cells of the carotid body [6,7]. Electrophysiological and pharmacological studies in the cat [23,24,28], dog [4] and rabbit (Monti-Bloch and Eyzaguirre, pers. comm.) have shown that exogenous DA alters chemoreceptor activity recorded from the carotid nerve, and it has been suggested that during natural stimulation of the carotid body release of endogenous DA from the glomus cells may function to modulate the activity of chemoreceptor nerve fibers which terminate upon these cells [2,14,28]. However, previous studies of the effects of natural stimulation on the DA stores of the carotid body have produced conflicting results, and there are no studies which have attempted to directly demonstrate either increased release or turnover of carotid body DA during natural stimula-

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tion. On the basis of ultrastructural and fluorescence studies, Hoffman and Birrell [18], Blumcke et al. [5] and Hervonen et al. [17] suggested that hypoxic stimulation of the carotid body depletes the glomus cells of their catecholamines, whereas with similar techniques Al-Lami and Murray [1], Chen et al. [8] and Verna [26] could find no evidence for catecholamine depletion, even under conditions of severe anoxia. Using biochemical determinations of endogenous catecholamines, Zapata et al. [29] found no change in cat carotid body after severe hypoxia, while Mills and Slotkin [22] observed a significant decrease in carotid body DA with less severe hypoxia in the same species. More recently, Hellstrom et al. [16] also observed a decrease in rat carotid body DA levels with hypoxia. However, these studies have been criticized [20] as inconclusive because catecholamine synthesis is known to be depressed in other tissues with severe hypoxia [11,13,19,25,27]. This fact, coupled with the acknowledged high oxygen dependence of the carotid body [10,21], means that a reduction in tissue DA levels with hypoxia need not reflect increased release of DA, but might arise from reduced synthesis as well. Thus, a more direct approach to the question of DA release during chemoreceptor stimulation is required. In the present study, we have employed [^{3}H]tyrosine as precursor of DA and have measured directly [^{3}H]DA released from the carotid body in vitro, and we report here that low O_2 (10% O_2 in N_2) results in an increased release of DA.

Carotid bodies were removed from adult New Zealand rabbits and quickly cleaned of surrounding connective tissue in a chamber filled with an oxygenated modified Tyrode's solution [3]: in mM, NaCl, 111.23; Na-glutamate, 41.99; KCl, 4.69; $CaCl_2$, 2.16; $MgCl_2$, 0.49; Hepes, 4.99; HCl, 2–4; glucose, 5.5. The tissue was then transferred for preincubation to oxygenated Tyrode's at 37°C containing 40 μm [^{3}H]tyrosine (2 Ci/mM; Amersham), 1 mM ascorbic acid and 100 μM 6-methyltetrahydrobiopterin (Calbiochem). Following a preincubation period of 3 h to allow for synthesis of adequate stores of labelled catecholamines, the tissue samples were divided into experimental and control groups. The experimental group was transferred for incubation to a vial containing unlabelled Tyrode's solution at 37°C equilibrated with 10% O_2 in N_2. The control tissue samples were transferred to a similar vial with Tyrode's solution equilibrated with 100% O_2. Both vials also contained enzymatic inhibitors of DA catabolism: the COMT inhibitor propylgallate (100 μM; Sigma) and the MAO inhibitor harmaline (5.7 μM; Sigma). After one hour, the labelled dopamine in the superfusates was analyzed as described below. In all, 8 animals, or 16 carotid bodies were used in the present series of experiments.

The labelled dopamine was separated by high voltage paper electrophoresis on Whatman 54 at 32 V/cm for 10^4 V-h using a high ionic strength buffer (acetic acid, 0.23 M; formic acid, 0.25 M; NH_4OH, 0.25 M; pH = 4.5). Prior to electrophoresis, the superfusate samples were acidified to pH = 3.6 with acetic acid, and ascorbic acid was added to reach a final concentration of 10 mM; the samples were dried in a vacuum centrifuge at 0°C and resuspended in 200 μl of a carrier mixture (pH = 3.6). The electrophoretic channels were fed through

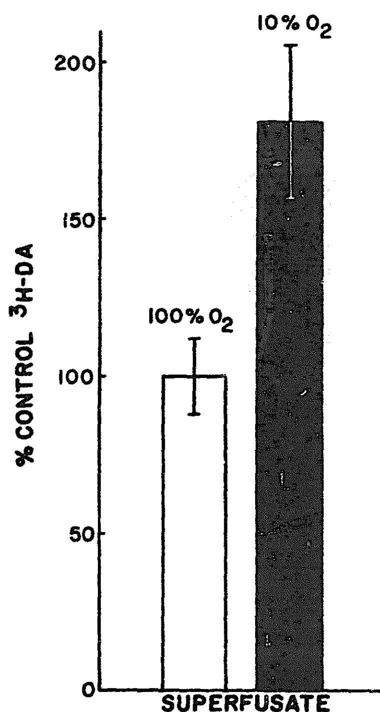

Fig. 1. Increased release of [^{3}H]DA from rabbit carotid bodies exposed to low O_2. Carotid bodies were preloaded with [^{3}H]DA by incubation for 3 h in an oxygenated modified Tyrode's solution containing 40 μM [^{3}H]tyrosine. The tissue samples were then divided into experimental and control groups and transferred to vials containing unlabelled Tyrode's which had been equilibrated with 10% O_2 in nitrogen (experimental) and with 100% O_2 (control). After one hour, the superfusates from these vials were analyzed for their content of [^{3}H]DA. The carotid body samples exposed to low O_2 (black bar) released 81% ($P < 0.02$) more [^{3}H]DA than the control carotid bodies (clear bar) when compared on a tissue weight basis. Values given are ± S.E.

a radiochromatogram scanner (Packard Model 7201), and the DA peaks were combusted in a sample oxidizer (Packard Model 306) and counted in a scintillation counter (Packard Model 3385).

When [^{3}H]DA levels in the superfusates were compared between control (100% O_2) and experimental (10% O_2 in N_2) carotid bodies, it was found that on a tissue weight basis the vials with tissue samples exposed to low O_2 contained 81% more [^{3}H]DA (Fig. 1). These data would suggest, therefore, that hypoxia brings about an increased release of [^{3}H]DA from the carotid body. This observation is consistent with the view that reduction in carotid body catecholamines with hypoxia, observed by other workers in previous studies, results at least in part from the release of endogenous DA stores. Whether there is, in addition to release, a depression of DA synthesis during hypoxia cannot be determined from the present data, but experiments are now in progress to resolve this point. The release of DA from the carotid body, presumably from the glomus cells, may function to modulate the activity of afferent fibers which terminate upon these cells. However, the precise mode of action of DA in carotid body chemoreception remains to be elucidated, and there is now

evidence that the effe ; of exogenous DA on the chemosensory discharges of the carotid nerve may be species dependent. Thus, in the cat, DA inhibits the chemosensory discharges [23,24,28], but appears to be stimulatory in the dog [4] and rabbit (Monti-Bloch and Eyzaguirre, pers. comm.). A comparative study of the effects of hypoxia on the release of DA in these different species is now in progress in our laboratory.

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