Oxygen chemoreception by carotid body cells in culture
(glomus cells/dopamine/hypoxia/sensory cells)

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ABSTRACT Chemoreceptors for oxygen reside within the carotid body, but it is not known which cells actually sense hypoxia and by what mechanisms they transduce this information into afferent signals in the carotid sinus nerve. We have developed systems for the growth of glomus cells of the carotid body in dissociated cell culture. Here we demonstrate that, as in vivo, these cells contain the putative neurotransmitters dopamine, serotonin, and norepinephrine. Oxygen tension regulates the rate of dopamine secretion from the glomus cells. Similar to chemically stimulated catecholamine secretion from other adrenergic cells this hypoxia-stimulated release requires extracellular calcium. These results are compatible with the suggestion that the glomus cells of the carotid body are chemoreceptor cells and that they signal hypoxia by regulated secretion of dopamine.

Since the work of Heymans (1) it has been known that the hypoxic ventilatory drive originates primarily within the carotid body. Subsequent work has outlined the role of this sensory organ in control of breathing at sea level and at higher altitude (1, 2) and in the sensation of dyspnea (3). However, definition of the molecular basis of oxygen transduction has been hampered by the complexity of the carotid body. It contains several cell types, including endothelial, sustentacular, and glomus cells as well as the terminals of afferent and sympathetic nerves (4, 5), but it is not known which are actually capable of sensing the level of oxygen. The glomus cells have been attractive candidates to be chemoreceptor cells because they contain dopamine, a transmitter secreted from the carotid body during hypoxia, and because they form morphological synapses with afferent nerve terminals (5, 6).

We have designed systems for the growth of glomus cells in culture in order to investigate the neurophysiology of these cells and their potential role in chemoreception (7). Here, we report evidence that carotid body glomus cells in culture synthesize and secrete putative neurotransmitters and manifest chemosensitivity in their control of dopamine secretion.

MATERIALS AND METHODS

Dissociated newborn rat carotid body cells were cultured as described (7) in 24-well plates and were grown in a humidified atmosphere of P02 151 mm Hg, Pco2 36 mm Hg in supplemented F-12 medium (pH 7.2). Nerve growth factor was not added to the medium. Enrichment for glomus cells could be achieved by the use of tyrosine-free medium (7), but successful elimination of other cells required several weeks of growth under these conditions. Since the very few cells in the carotid body was the limiting factor in the number of experiments that were possible and since there was a slow but noticeable decrement in catecholamine content in cultures >7–10 days old, we elected not to use this long-term enrichment procedure. Hypoxic conditions were identical except that the PO2 was diminished to 36 mm Hg. Since there are only a few thousand cells in the carotid body, each experiment necessitated the pooling of dissociated cells of 10–12 rats and subsequent distribution to two plates, one to be used for control and one for hypoxic conditions. Cells in both plates for each experiment were grown in culture for the same period of time. Experiments were performed after culturing of the cells for 5–7 days.

Prior to testing the cells were washed twice in Hanks' balanced salt solution supplemented with 100 µM pargyline/5 mM ascorbate/4.5 g of glucose per liter, and then exposed in 500 µl of the same solution for 1–2 hr to either PO2 151 mm Hg or PO2 36 mm Hg. The PO2 of the cells beneath 2 mm of culture fluid can be estimated to be diminished below that of the surrounding air by <5 mm Hg (8). Prior to analysis, the cells were sonicated and freeze-thawed in HPLC running buffer. This buffer consisted of 0.75 M dibasic sodium phosphate/0.1 M citric acid/1.5 mM sodium heptane sulfonate (Kodak)/14% (vol/vol) methanol. Particulate matter was removed from both the sonicated cellular extract and the supernatant by centrifugation in an Airfuge (Beckman) for 5 min. [Concentration of the catecholamines by alumina extraction (9) proved infeasible because of the very small amounts of tissue.] The supernatant from the centrifugation was separated by the use of HPLC (Beckman 420) with a C-18 column (Altex Ultrasphere ODS), and detection was by an electrochemical detector (BAS LC-3A). Elution rate was 1.5 ml/min, and the sample injection size was 200 µl. The electrochemical detector was used at 0.70 V. The identity of peaks was confirmed by exact superimposition of standard peaks with presumptive norepinephrine, dopamine, and serotonin peaks from the cellular extract, which represented cell content, or the culture fluid, which reflected the secreted catecholamines. The sensitivity of the HPLC-EC system for measuring catecholamines was ~100 pg of dopamine, 150 pg of norepinephrine, and 200 pg of serotonin.

RESULTS

Glomus cells in vivo contain several putative neurotransmitters, including norepinephrine, dopamine, and serotonin (5, 10). As shown in Fig. 1, measurement of the extracted contents of cultured glomus cells by HPLC combined with electrochemical detection (HPLC-EC) documented their synthesis of norepinephrine, dopamine, and serotonin. Dopamine and serotonin secretory rates were great enough to be detected in the culture fluid after 1 hr of incubation, as shown in Fig. 1. Secreted norepinephrine was not detected, but very low levels might have been obscured by interference from other oxidizable compounds. Interestingly, carotid body cells cultured for <4 days synthesized dopamine but did not secrete measurable quantities into the medium.

In each of the six experiments using the cultured carotid body cells, hypoxia caused an increase in dopamine secretion. The mean dopamine secretion increased from 0.092 ± 0.029 ng per carotid body per hr to 0.211 ± 0.091 ng per

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carotid body per hr (mean ± SD; n = 6 independent experiments; P < 0.01, Student's t test). As shown in Fig. 2, this enhanced secretion was accomplished without significant diminution in cellular content of dopamine. This suggests first of all that hypoxia did not injure the cell and thereby cause a nonspecific leak of dopamine. In addition, the increase in secretion without diminution in content suggests that hypoxia also stimulated enhanced synthesis of dopamine, an effect of hypoxia that has been noted using explanted rabbit carotid bodies (11).

Decrease in extracellular calcium diminishes hypoxia-stimulated release of dopamine from explanted carotid bodies (6). Since calcium influx is integral to many secretory processes that involve vesicular release (12), it was of interest to evaluate the effect of calcium on glomus cell function. Omission of calcium from the medium, with magnesium concentration left unaltered, did not significantly affect baseline dopamine secretion from the cultured carotid body cells (not shown). However, as shown in Fig. 3, the oxygen-sensitive component was abolished. Under the same conditions but with the addition of calcium to the medium, hypoxia induced a significant increase in the mean dopamine secretory rate from 0.040 ± 0.020 ng per carotid body per hr to 0.077 ± 0.023 ng per carotid body per hr (mean ± SD; n = 4; P < 0.05). Extracellular calcium might, of course, be involved not only in secretion but also at other steps in the sensory transduction mechanism.

**DISCUSSION**

The hypoxic stimulus to ventilatory drive originates predominantly within the carotid body. As arterial $P_{O_2}$ decreases, the discharge rate of the carotid sinus sensory nerve increases. It has been suggested, however, that it may not be the nerve itself that is chemosensory, but rather other cells of the carotid body, because a cut carotid sinus nerve must regenerate to the carotid body to manifest chemosensitivity (15).
In this paper, we have demonstrated that glomus cells grown in dissociated cell culture synthesize dopamine, serotonin, and norepinephrine and develop (or reestablish) secretory capability for dopamine and serotonin. Catecholamine secretion from these cells has an unusual property, in contrast, for example, to that of epinephrine secretion from cultured adrenal cells, in that it is modulated by ambient $O_2$ levels, suggesting that the glomus cells register $O_2$ concentration and transmit this information by regulated and calcium-dependent release of dopamine. Alternatively, another intrinsic carotid body cell might serve as the $O_2$ transducer and signal the glomus cell to release dopamine, and this intercellular communication might be calcium dependent. What neurotransmitter or neuromodulator role dopamine plays in carotid body function remains debated, but in these experiments dopamine secretion serves as a marker of an oxygen-regulated function. The molecular means by which $P_{O_2}$ level is measured remains unknown, but it has been suggested that it involves cell-surface components (16) as well as components of the mitochondrial respiratory chain (17). Hopefully, the retention of the oxygen-sensitive response in the relatively well-controlled environment of cell culture might facilitate characterization of some of the processes of oxygen chemoreception.

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