CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Ondine Undone

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Imagine having to remember to breathe, as in the myth of Ondine, who was cursed to think consciously of breathing even in sleep. The term "Ondine's curse" has been applied to the congenital central hypoventilation syndrome (CCHS), a condition in which children hypoventilate and retain carbon dioxide (especially in sleep), have prominent apneas, and have a severely diminished carbon dioxide chemoreflex (the increase in breathing in response to elevated carbon dioxide levels). The cause of CCHS is a mutation in *PHOX2B* that affects a single amino acid in the PHOX2B protein. When this mutation is introduced into a mouse, much of the syndrome related to breathing is recapitulated. PHOX2B is expressed in the retrotrapezoid nucleus, a small cluster of neurons lying beneath the ventral surface of the medulla that does not develop properly in mice carrying mutated *Phox2b*. This nucleus of cells has been a focus of attention as an important central chemoreceptor site; disruption of its function by many approaches substantially reduces the carbon dioxide chemoreflex (Fig. 1). The ability to improve the chemosensory function of these neurons could provide a new therapy for persons with CCHS, who now undergo lifelong ventilator support during sleep.

Recently, Kumar et al.[1] asked whether a pH-sensitive G-protein–coupled receptor (GPCR) was involved in the detection of carbon dioxide and pH in neurons of the retrotrapezoid nucleus. This GPCR is a member of a family of pH-sensitive receptors that detect carbon dioxide in insects and pH in mammalian kidney. Kumar et al. found that knockout of a GPCR protein, GPR4 (but not two other GPCR proteins), reduced the carbon dioxide chemoreflex in mice by more than 50% (Fig. 1), increased the number of apneas, and reduced the activation by carbon dioxide, in vitro, of chemosensitive neurons from the retrotrapezoid nucleus. They were then able to restore the carbon dioxide chemoreflex and reduce the frequency of apnea in these mice by restoring GPR4 activity in the neurons of the retrotrapezoid nucleus. They further elucidated the chemoreflex mechanism by knocking out the genes encoding GPR4 and TASK-2 (TASK-2 is a potassium channel previously shown to mediate the pH sensitivity of some of the neurons in the retrotrapezoid nucleus). The chemoreflex in mice with the double-knockout genotype was virtually abolished (Fig. 1), which strongly suggests that both genes are independently key to successful chemosensing.

The active sensing site of GPR4 is made up of histidine residues (Fig. 1).[2] This is of particular interest, given that the dissociation constant representing the dissociation of histidine protons varies with temperature, as do the pH of blood in cold-blooded animals in vivo and the pH of blood in humans in vitro. Thus, with changes in temperature, pH may change dramatically, but the fractional dissociation of histidine remains constant, as does the protein configuration and function. In contrast, at a constant temperature, changes in pH affect histidine dissociation, protein configuration, and in this case, breathing. Retrotrapezoid-nucleus chemosensing through histidines in GPR4 appears to be an evolutionarily old and conserved mechanism; histidine residues of GPR4 at the extracellular surface can be viewed as the pH-sensing amino acids. (TASK-2 channels sense pH through different amino acids.)

Is there a therapeutic potential that might emerge from this work? It would have been of interest to examine breathing control in these mice during sleep, the time of greatest risk of apnea and death in patients with CCHS. Gene therapy to enhance retrotrapezoid-nucleus chemosensing and therefore GPR4 function is conceptually plausible and could be very helpful to

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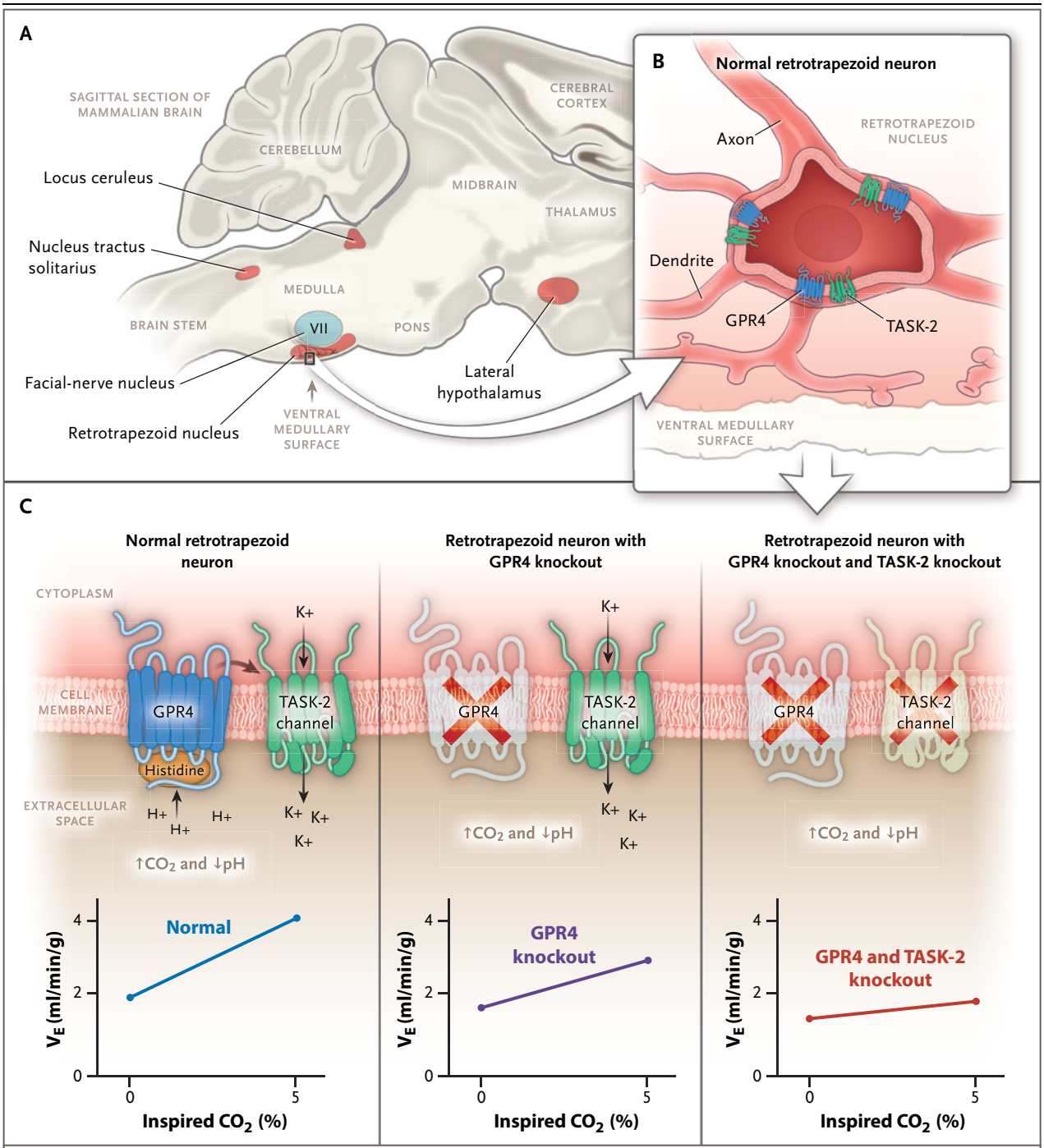


Figure 1. The Drive to Breathe.

The retrotrapezoid nucleus central chemoreceptor neurons detect pH and carbon dioxide (CO_2) through histidine residues in GPR4 and TASK-2. Panel A shows a sagittal section of the mammalian hindbrain and highlights the location of some putative central chemoreceptor sites (in red), with emphasis on the retrotrapezoid nucleus. Panel B is a schematic drawing of a neuron of the retrotrapezoid nucleus with GPR4 receptors and TASK-2 channels, located on the cell membrane and identified in a study by Kumar et al.,[1] as detectors of CO_2 and pH. In Panel C, the cell membranes of a normal retrotrapezoid neuron, of a retrotrapezoid neuron with knockout of GPR4, and of a retrotrapezoid neuron with knockout of both GPR4 and TASK-2 are shown. In the normal ventilatory CO_2 chemoreflex, increases in inspired CO_2 lead to increases in ventilation (V_E) through stimulation of chemoreceptors. In mice with knockout of GPR4 only, the CO_2 chemoreflex is reduced by approximately 50%; in mice with knockout of both GPR4 and TASK-2, the CO_2 chemoreflex is nearly absent.

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these patients, although there would be substantive technical challenges to realizing such an approach.

Are these findings relevant to other disorders with altered central chemoreception, such as sleep apnea and chronic obstructive pulmonary disease? Here, the picture is a bit less clear. In addition to the retrotrapezoid nucleus, there are other sites of the central nervous system that participate in central chemoreception (Fig. 1). For example, acute chemogenetic silencing of medullary serotonergic neurons[3] also reduces the carbon dioxide chemoreflex by approximately 50%. Furthermore, mice that are deficient in orexin[4] have a reduced carbon dioxide chemoreflex during wakefulness. It remains unclear how the neural network involved in chemoreception is organized, what sensing mechanisms are in operation at other sites, and which sites participate in sleep when the carbon dioxide chemoreflex response is normally suppressed (and especially suppressed in patients with CCHS). Nevertheless, the firm identification of the protein and amino acid that detects carbon dioxide and pH in the neurons of the retrotrapezoid nucleus is an exciting finding that moves us one step closer to a potential therapy for disordered breathing in CCHS and may prove relevant to the modification of central chemoreception in other disease states.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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DOI: 10.1056/NEJMcibr1507734
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