Tanaffos (2002)1(1), 9-13

Central Respiratory Drive: Molecular Basis and Genetics

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ABSTRACT

Central respiratory drive is of paramount importance in control of ventilation. The central drive is exquisitely sensitive to changes in CO_2/H^+ concentration. New data show that the fast- acting neurotransmitter acetylcholine is essential in the CO_2/H^+ ventilatory response as well as in generating the central drive. Ret gene and MASH-1 are essential in development of the parasympathetic system and thus in the central respiratory drive. Clinical states of hypoventilation most likely have a genetic defect affecting the cholinergic system.This article will review briefly the site and mechanisms of action of CO_2 centrally, the neurotransmitters involved in the process, the gene(s) involved in the process and clinical states where there are abnormalities in the system and inevitably hypoventilation results.

(Tanaffos 2002;1(1):9-13)

Keywords*:central respiratory drive, CO_2^+/H^+ , Cholinergic system.*

INTRODUCTION

Central respiratory drive encompasses interactions between varied and complex processes,which initiate the respiratory frequency and respiratory depth (tidal volume). These processes include biochemical and biophysical events and interactions between several groups of neurons. In the process of generating the central respiratory drive peripheral receptors, mainly in respiratory apparatus, as well as chemoreceptors in the aortic and carotid bodies which sense $[H^+]$,the Pco_2 and Po_2 send signals to the brain which are integrated and appropriate response generated which we call the central respiratory drive. The central rhythm is most likely generated by a central pacemaker located in the pre-Boetzinger complex of neurons. This central output is then acted upon by other stimuli and the final command determined. As in well known a number of agents depress or increase the central out put, and among them are both excitatory and inhibitory neurotransmitters and neuromodulators(1).

One of the key elements is control of respiration is the exquisite sensitivity of the system to carbon dioxide(CO_2). Small changes in CO_2 tension (PCO_2) are associated with large changes in minute ventilation(V_E). How and where CO_2 acts and the mechanisms of transduction of CO_2 signal centrally have opened up new vistas on the molecular basis of central respiratory drive and its probable genetic basis. This article will review briefly the site and

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mechanisms of action of CO_2 centrally, the neurotransmitters involved in the process, the gene(s) involved in the process and clinical states where there are abnormalities in the system and inevitably hypoventilation results. A comprehensive and up- to- date review of current knowledge on respiratory control, presented at the VIII oxford conference, has been published recently and is a valuable reference source (2), as is a special issue of Respiration Physiology(3).

CO_2/H^+ and central respiratory drive

In the hierarchy of factors affecting respiration the central effects of CO_2/H^+ are at the apex of the order. Compared to all other stimuli, central effects of CO2 are paramount and since the effects of CO_2 are primarily through changes in $[H^+]$, it is appropriate to term it the CO_2/H^+ effect.

Historically the suggestion was first made in 1905 by Haldane and priestly that the ventilatory response to CO_2 was because CO_2 made the brain more "acid". In the 1950's work of Leusen(4,5) showed that perfusing the ventricular-cisternal system of the anesthetized animal with either acid or alkaline cerebrospinal fluid had profound effects on ventilation. Acid CSF increased ventilation and alkaline CSF decreased ventilation. In the 1960's pappenheimer and colleagues showed that in the unanesthetized goat ventilation in the steady state could be expressed as a simple function of $[H^+]$ in the interstitial fluid (ISF) of the brain (7).

A number of studies since then have identified loci on the ventral surface of the medulla (VMS) as being sensitive to changes in $[H^+]$ concentration and have been termed chemosensitive areas. There are three such sites on the VMS termed caudal, intermediate and rostral chemosensitive areas (7,8).

Microinjection techniques have further confirmed the importance of these areas in respiratory control (9). In addition, other chemosensitive areas have been identified at other sites such as the pre-Boetzinger complex and retrotrapezoid nucleus (RTN). The chemosensitive areas are within 1.5 mm of the brain surface and many studies reported as within 50-200 μmm (1,10). The exact mechanism of neural stimulation by CO_2 and/or H^+ has been the subject of speculation and not completely elucidated. However, recent data show that the neurotransmitter acetylcholine is essential for the CO_2/H^+ effect.

Neurotransmitters and CO_2/H^+ central drive of ventilation

Exactly how H^+ causes neuronal discharge is unknown, but several possibilities exist.

One is that there are specific H^+ receptors, but none have been identified. Hydrogen ion might effect ionic pumps and transmembrane traffic of ions, but no convincing evidence exists that these effects are the mechanisms of neuronal central respiratory drive. A number of fast-acting neurotransmitters are increased in the brain on exposure to CO_2 and include GABA, glutamine and acetylcholine, while glutamate and aspartate fall(11,12).The interest in acetylcholine as a central neurotransmitter dates back to the work of Gesell and Hansen(13). who showed that the ventilatory response to inhaled CO_2 and acetylcholine infusion were similar. Later other studies showed that an intact cholinergic system was needed for acid solutions to cause neuronal discharge (14). Work from our laboratory showed that the stimulatory effects on ventilation of acid perfusion into the ventricular-cisternal system was totally abolished by the muscarinic receptor blocker atropine, whether acidosis was of " respiratory" or " metabolic" origin(15). Nattie et al.(16) also showed that blocking the muscarinic receptors at the ventral medullary sites markedly blunted the ventilatory response to CO_2 .Further evidence of the significance of the cholinergic system in central ventilatory drive, and not only CO_2 sensitivity, was demonstrated by the fact that infusion of atropine into the CNS in the anesthetized dog caused significant hypoventilation and hypercapnia (17,18,19). These observations on the cholinergic

system led to other studies in the isolated brain stem preparation of the neonatal rat. Our studies showed that inhibition of acetylcholine synthesis, blockade of acetylcholine release and inhibition of muscarinic receptors M_1 and M_3 all caused marked reduction and in many instances cessation of central neuronal respiratory discharge(17,18).

Thus the importance of cholinergic system in central respiratory drive and its primary role in the central response to CO_2 / H^+ was established. Then the issue of acetylcholine synthesis and responsible genes took center stage.

Acetylcholine synthesis and genes relevant to its synthesis

The strong evidence in support of acetylcholine as the key neurotransmitter in generating central respiratory drive and in signal transduction for the CO_2/H^+ central effect has brought to the forefront examination of the steps involved in its synthesis. Acetylcholine is formed by combining acetyl-CoA from the krebs' cycle with choline. This chemical reaction is mediated by the enzyme choline acetyltransferase (CHAT). In the absence of CHAT, acetylcholine cannot be formed. In the early 1990's, a gene was discovered on chromosome band 10q11.2, which among its several functions was associated with development of neural crest and the autonomic system(20,21)and essential for formation of CHAT . This gene, the Ret gene, when abnormal was associated with development of neuroendocrine tumors and hypoventilation(22).

Genetic basis of respiratory drive

The Ret gene encodes for tyrosine kinase receptor and is essential for cell growth. Null mutation in the gene in mice results in loss of aspects of sympathetic and parasympathetic system, renal dysgenesis and death (23,24,25). In our studies we evaluated the ventilatory response to CO_2 in knock-out mice for the Ret gene. The homozygotes had essentially no ventilatory response to CO_2 and died within 24 hours of birth (26). The heterozygotes had a diminished ventilatory response to CO_2 compared to normals and developed normally in the postnatal period. These findings emphasize the importance of the cholinergic system in respiratory control and the fact that abnormalities in this system are associated with respiratory depression and can be lethal. In addition to the Ret gene, other studies have shown that MASH-1 is also important in activating tyrosine kinase and heterozygote mice (MASH-1 +/-) have a weak CO_2 ventilatory response(27).

Clinical relevance to states of hypoventilation

Disordered breathing, hypoventilation and diminished sensitivity to CO_2 are present in many clinical states and are often apparent early in life and can be fatal.

Neuropathological studies in sudden infant death syndrome(SIDS) victims have shown abnormalities in the arcuate nucleus and in expression of muscarinic receptors(28). The same abnormalities have also been reported in central congential hypoventilation syndrome (CCHS)(29).

Familial factors are important in both CCHS& SIDS and they may overlap between the two syndromes. In another congential disease affecting the parasympathetic system, Hirschsprung's disease, there is a 20% incidence of congential hypoventilation. In CCHS patients some 50% have Hirschsprung's disease(30). All these clinical entities underline the importance of the cholinergic system in central drive of ventilation and the fact that hypoventilation and CO_2 retention result in a poorly developed or malfunctioning parasympathetic system .

The conditions cited above usually affect infants and children; however, a genetic expression of this biological abnormality may be the basis of hypoventilation and hypercapnia in the adult population with various forms of lung disease. In normal individuals the slope of the CO_2 ventilatory response is approximately 2-3 L/mm rise in PCO_2. However, in about 15% of the population there is a blunted response, where the slope of the CO_2

ventilatory response is about 1L/mm rise in PCO_2. These individuals are likely to develop CO_2 retention when additional problems arise affecting the respiratory system such as obesity, COPD or acute asthma. Many family members of patients with lung disease and CO_2 retention also have a depressed CO_2-ventilatory response, but have no clinical problems, because their respiratory system is structurally normal. One beneficial effect of depressed CO_2 ventilatory response is seen in endurance athletes of international rank whose ventilatory response to CO_2 has a slope of around 1. The reasons for the latter are probably a combination of genetic and training factors(31).

SUMMARY

Central respiratory drive is of paramount importance in control of ventilation. The central drive is exquisitely sensitive to changes in CO_2/H^+ concentration in the chemosensitive areas in the medulla, particularly close to the ventral surface. New data show that the fast- acting neurotransmitter acetylcholine is essential in the CO_2/H^+ ventilatory response as well as in generating the central drive. Genetic abnormalities in development of the parasympathetic system and neural crest cause hypoventilation in man and the knock-out mice model for the gene essential in development of neural crest have lost their ventilatory response to CO_2 and die within 24-48 hours of birth.Ret gene and MASH-1 are essential in development of the parasympathetic system and thus in the central respiratory drive. Clinical states of hypoventilation most likely have a genetic defect affecting the cholinergic system.

ACKNOWLEDGMENTS

Most of the work from our laboratory has been supported by grants from the National Institutes of Health (NIH) and the shoolman fund of the pulmonary & Critical Care Unit. I am most grateful to my colleague, Melvin D. Burton, M.D., for his work in the laboratory on the cholinergic system and to Kathleen Sweeney Laing for her secretarial assistance.

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