Respiratory Physiology & Neurobiology 164 (2008) 12–17

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Neurochemical phenotypes of cardiorespiratory neurons

Paul M. Pilowsky*

Australian School of Advanced Medicine, Dow-Corning Building, Level 1, 3 Innovation Road, Macquarie University, Sydney 2109, NSW, Australia

ARTICLE INFO

Article history:
Accepted 21 July 2008

Keywords:
Cardiorespiratory integration
Rat
Reflex
Peptide
Respiration
Blood pressure
Baroreceptor

ABSTRACT

Interactions between the cardiovascular and respiratory systems have been known for many years but the functional significance of the interactions is still widely debated. Here I discuss the possible role of metabotropic receptors in regulating cardiorespiratory neurons in the brainstem and spinal cord. It is clear that, although much has been discovered, cardiorespiratory regulation is certainly one area that still has a long way to go before its secrets are fully divulged and their function in controlling circulatory and respiratory function is revealed.

1. Introduction

The principal function of the central cardiorespiratory system is to regulate the distribution of oxygen throughout the body in a timely and appropriate fashion. This is achieved by coordinated activity of the circulatory and ventilatory systems (Adrian et al., 1932; Koizumi et al., 1971; Darnall and Guyenet, 1990; Zhou and Gilbey, 1992; Pilowsky et al., 1994; Miyawaki et al., 1995; Sun et al., 1997; Oshima et al., 2006). In addition, carbon dioxide is regulated within specific limits so as to ensure a normal acid–base balance on a breath to breath basis. Generally speaking, receptors in the carotid body report the state of oxygenation while central chemoreceptors are mostly responsible for the detection of acidity. A considerable amount of work on the generation of respiratory, or ventilatory, behaviours has been conducted on almost every species imaginable. Perhaps the best studied of these, solely in terms of neurochemistry, is the rat, so that I will focus mainly on work reported from this animal here.

Before we consider the issue of phenotype we should first consider what defines cardiorespiratory neurons physiologically. The usual definition of a 'cardiovascular neuron' is a neuron that is positively or negatively sensitive to baroreceptor input. Hence, this sensitivity may be manifested as an increase or decrease in activity when baroreceptor afferent neurons are excited. Unfortunately, this is a very narrow definition that excludes many neurons that may affect barosensitive or other cardiovascular output neurons from time to time.

Respiratory neurons are similarly difficult to define satisfactorily. Many studies have delineated the distribution (Monnier et al., 2003), pharmacology (McCrimmon et al., 1993; Padley et al., 2007), chemical content (Stasinopoulos et al., 2000; Guyenet and Wang, 2001; Lai et al., 2001; Phillips et al., 2001; Alheid et al., 2002; Wenninger et al., 2004; Springell et al., 2005; Seyedabadi et al., 2006) and functional phenotype of cardiorespiratory neurons (Smith et al., 1991; Johnson et al., 1994; Pilowsky and Goodchild, 2002; Verner et al., 2004), but the precise physiological role played by the multiplicity of colocalised transmitters is less clear.

Respiratory neurons are commonly defined as having an activity pattern that is clearly in time with the discharge in the phrenic nerve. This pattern can be one of many types including expiratory activation, inspiratory activation, post-inspiratory activation, as well as subtler variations and combinations of these patterns. Unfortunately, many other neurons that lack a consistent and obvious respiratory pattern may still be important in respiration. In fact, depending on the extent of the chemoreceptor input it is theoretically possible for almost any muscle, and its antecedent neural inputs, to have some degree of respiratory modulation. Indeed chemoreceptor neurons themselves often do not have an obvious respiratory modulation, yet they clearly have an influence on the firing pattern of both respiratory and cardiovascular neurons. Therefore, for the sake of sanity we shall restrict our discussion here to those cardiovascular neurons whose activity is changed by the activation of baroreceptor inputs, and respiratory neurons will be considered to be those whose activity relates prominently to phrenic discharge during quiet breathing and in conditions of

* Tel.: +61 2 9850 4015; fax: +61 2 9850 4010.
E-mail address: paul.pilowsky@mq.edu.au.
URL: http://www.pilowsky.org.

1569-9048/$ – see front matter.
doi:10.1016/j.resp.2008.07.016

normoxic (or even hyperoxic, when peripheral chemoreceptors are effectively silent) normocapnia.

Perhaps the most important example of a neuronal subtype that is neither obviously cardiovascular nor respiratory, but which is crucial to the operation of both systems, is the central chemoreceptor. For the most part, fast neurotransmission between neurons in the chemosensory system and the respiratory rhythm and pattern generating systems is due to the release of GABA, glycine and glutamate, acting on ionotropic receptors. Although these neurotransmitters are crucial for rapid inhibition and excitation, most neurons have other neurochemicals that are co-stored and in some circumstances co-released. These additional neurochemicals are mostly peptides but can also include gases such as nitric oxide, as well as purines such as ATP and cannabinoids. Perhaps the best known non-amino acidergic neurotransmitters are the amines including: serotonin, acetylcholine, dopamine, noradrenaline, octopamine, histamine and adrenaline. To add to the complexity, each of the different neurotransmitters is commonly able to act on many different receptors which in turn may be coupled to a multiplicity of second messenger systems. What is the physiological significance of so much variation? By itself, this is a thorny question, but the issue of the expression of neurotransmitter phenotypes in individual cardiovascular and respiratory neurons is a harder problem to address. Although I cannot hope to answer all of these questions here, I will attempt some speculation that may (or may not) be useful for future work.

2. Fast neurotransmitters

Clearly, fast neurotransmission is essential for the proper maintenance of respiration in a timely way. Glycine is a key neurotransmitter that is now thought to be the inhibitory neurotransmitter released by Bötzinger neurons (Schreihofer et al., 1999; Ezure et al., 2003; Tanaka et al., 2003) and the expression of its receptors increases with age (Wong-Riley and Liu, 2005).

On the other hand, the excitatory neurons in the pre-Bötzinger complex are believed to be reliant on the excitatory neurotransmitter glutamate to elaborate a normal synchronised activity once a population discharge threshold is achieved in cat (Solomon, 2004). The discharge threshold is reached by an interaction of intracellular pathways and membrane currents that are discussed elsewhere (Paton et al., 2006; Koizumi and Smith, 2008). Glutamatergic neurons rely on the action of vesicular transporters to accumulate glutamate from the cytoplasm into small clear vesicles in preparation for regulated release by neuronal varicosities. Glutamate itself is made in several ways, one mechanism being by conversion of glutamine to glutamate through the action of the mitochondrial enzyme phosphate-activated glutaminase (Kaneko et al., 1989). Morphological studies reveal large numbers of terminals that are immunoreactive for vesicular glutamate transporter type 2, with few type 1 immunoreactive varicosities (Liu et al., 2003). Mice that lack the vesicular glutamate transporter 2 are not able to breathe and die immediately after birth (Wallen-Mackenzie et al., 2006). Unfortunately, since glutamatergic neurotransmission is essential for virtually all rapid neurotransmission in the central nervous system, knockout experiments of this type do lack subtlety and can be difficult to interpret.

3. Metabotropic receptors ((slow) neurotransmitters)

Here I will discuss some aspects of the role of aminergic and cholinergic (muscarinic) neurotransmitters in the generation of respiratory frequency, and the elaboration of the frequency and amplitude components of the respiratory-related discharge in motor ouputs. When acting on metabotropic receptors, as opposed to ionotropic receptors, these neurotransmitters, and many others like them, act over a much longer time-course ranging from minutes to hours.

Many neurotransmitters, including amines, acetylcholine and neuropeptides exert effects by acting upon G-protein coupled and other non-ionotropic receptors to exert 'slow' but quite dramatic effects on breathing: some of these are discussed below.

3.1. Acetylcholine

Cholinergic mechanisms are involved in sleep–wake cycles and the nature of the receptors involved has been studied extensively (Kubin and Fenik, 2004). Broadly there are two types of cholinergic receptor: nicotinic and muscarinic receptors. Muscarine, along with muscimol, the potent GABA-A agonist, can be derived for so-called 'recreational use' from the fly agaric mushroom (Fig. 1). There are five muscarinic receptors M1, M3 and M5 that are excitatory through coupling to $G_{q/11}$ and formation of phospholipase C. The M2 and M4 receptors are inhibitory through coupling to Gi/o and a decrease in the activity of adenylate cyclase. Despite being a receptor family that is of considerable importance and one which has widespread action in the central nervous system, there are currently a paucity of pharmacological agents that will adequately discriminate between the five receptors. Recently, knockout mice that lacked either the M1 and M3 or the M2 and M4 receptors were developed in order to gain insights into the role of these receptors (Boudinot et al., 2008). The principal finding of this study was that an absence of the excitatory M1 and M3 receptors results in an increase in tidal volume while removal of the M2 and M3 receptors augmented breathing frequency and ventilation (Boudinot et al., 2008). This may suggest a more important role for M2 and M3 receptors in the generation of respiratory rhythm, but such conclusions await the development of more selective pharmacological agents (Peretto et al., 2007).

Fig. 1. A fly agaric mushroom (*Amanita muscaria*) growing in the mountains to the east of Sydney, Australia. The colour and pattern of white flecks is unmistakable, and the mushrooms emerge from the ground with this appearance. They are best known as a source of the hallucinogenic alkaloids, muscarine and muscimol.