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Review

Development of synaptic transmission to respiratory motoneurons☆

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ABSTRACT

Respiratory motoneurons provide the exclusive drive to respiratory muscles and therefore are a key relay between brainstem neural circuits that generate respiratory rhythm and respiratory muscles that control moment of gases into and out of the airways and lungs. This review is focused on postnatal development of fast ionotropic synaptic transmission to respiratory motoneurons, with a focus on hypoglossal motoneurons (HMs). Glutamatergic synaptic transmission to HMs involves activation of both non-NMDA and NMDA receptors and during the postnatal period co-activation of these receptors located at the same synapse may occur. Further, the relative role of each receptor type in inspiratory-phase motoneuron depolarization is dependent on the type of preparation used (in vitro versus in vivo; neonatal versus adult). Respiratory motoneurons receive both glycinergic and GABAergic inhibitory synaptic inputs. During inspiration phrenic and HMs receive concurrent excitatory and inhibitory synaptic inputs. During postnatal development in HMs GABAergic and glycinergic synaptic inputs have slow kinetics and are depolarizing and with postnatal development they become faster and hyperpolarizing. Additionally shunting inhibition may play an important role in synaptic processing by respiratory motoneurons.

1. Introduction

This review is focused on the postnatal development of fast ionotropic synaptic transmission to respiratory-related motoneurons, with an emphasis on studies of hypoglossal motoneurons (HMs) during the postnatal period. This review will not cover synaptic transmission involving modulatory neurotransmitters such as serotonin, norepinephrine and neuropeptides, nor the metabotropic receptors such as mGlu- and $GABA_B$-receptors (see an earlier review by Rekling et al., 2000 for information on these subjects).

Before discussing the development of fast ionotropic synaptic transmission to HMs it is important to understand the target muscle of these motoneurons. The tongue muscle is heterogeneous, containing 8 difference muscle groups, 4 are extrinsic and 4 are intrinsic (Sokoloff and Deacon, 1992). The tongue takes part in a number of basic functions including mastication, swallowing, suckling, and vocalization. It is also important in respiration due to its position in the upper airway, thereby having a role in upper airway patency. Regarding the latter function the main protrusor muscle group of the tongue, the genioglossus muscle, exhibits inspiratory-related electrical activity. Thus inspiratory-phase (I-phase) activation of this tongue muscle group promotes stabilization and patency of the upper airway posterior to the tongue. Failure of this activation to adequately occur is thought to be an important contributing factor to obstructive sleep apnea.

2. Morphology of hypoglossal motoneurons with respect to synaptic transmission

Brainstem motoneurons, such as HMs, are found in distinct bilaterally located nuclei within which the motoneurons are somatotopically organized. This is in contrast to spinal motoneurons which are found in longitudinally organized columns in the spinal cord ventral horn. A number of anatomical studies have shown that HM dendrites, while elaborate within the XII nucleus itself, also penetrate into reticular formation around the lateral and ventral sides of the XII nucleus (Altschuler et al., 1994; Nunez-Abades et al., 1994; Tarras-Wahlberg and Rekling, 2009; van Brederode et al., 2011).

Although we do not know the relative distribution of inhibitory versus excitatory synapses onto HMs some indication of this may be gleaned from detailed studies at the ultrastructural level regarding this distribution in adult cat spinal motoneurons (Ornung et al., 1998). In spinal motoneurons it is the dendritic tree and not the somal membrane that dominates the synaptic receptive area by over 9 to 1. In primary first order (stem) dendrites of spinal motoneurons about 70% of synaptic boutons are γ-aminobutyric acid (GABA) and/or glycine containing, while only approximately 20% are glutamate containing. In non-primary den-

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drites approximately 55% are GABA and/or glycine containing, while approximately 40% are glutamate containing. Thus in the dendritic space close to the soma there is a strong inhibitory synaptic influence (Ornung et al., 1998). This may have importance with respect to synaptic integration that is described later in this review.

3. Glutamatergic synaptic transmission

There has been a recent overall review of ionotropic glutamate receptors by Traynelis et al. (2010). Ionotropic glutamate receptors which mediate fast excitatory synaptic transmission have for the most part been separated into three broad classes based on structural, functional and pharmacological characteristics. These three classes are kainate receptors; AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors; and NMDA (N-methyl-D-aspartic acid) receptors. These are assembled from different combinations of four large glutamate receptor subunits (Traynelis et al., 2010). AMPA and kainate receptors have fast activation and deactivation rates as well as strong and rapid desensitization. NMDA receptors have slower gating kinetics and deactivate over a longer time-scale. NMDA receptors also exhibit modest and slow desensitization (Traynelis et al., 2010).

Developmental changes in glutamatergic synaptic transmission to HMs have been demonstrated. It has been shown that tritiated glutamate binding within the rat hypoglossal motor nucleus peaks in intensity at around nine days postnatal and then declines progressively at later postnatal ages. This likely reflects changes in glutamatergic receptor density and/or glutamate affinity (Rao et al., 1995).

Another feature of glutamatergic transmission is the presence at single postsynaptic sites on respiratory motoneurons of both non-NMDA and NMDA receptors. O'Brien et al. (1997) studied the properties of glutamatergic miniature excitatory postsynaptic currents (mEPSCs) recorded in neonatal rat HMs. They observed dual component mEPSCs that were generated by non-NMDA and NMDA receptors (Fig. 1). The separation of these components on the basis of their kinetics (decay times) led to the conclusion that non-NMDA and NMDA receptors are co-localized at individual synapses (O'Brien et al., 1997).

The role of glutamate-receptor mediated respiratory-cycle related excitation of respiratory motoneurons has been studied extensively. The excitatory synaptic input to respiratory motoneurons is a consequence of the synaptic release of glutamate from glutamatergic synaptic terminals apposed to these motoneurons. Receptor expression studies have revealed that the three classes of the glutamate receptor are present in HMs (Rekling et al., 2000). Functionally both non-NMDA and NMDA gated channels have been demonstrated on HMs (Berger et al., 1998; Funk et al., 1993; O'Brien et al., 1997; Rekling, 1992), and this is shown in Fig. 1.

The relative contribution of non-NMDA and NMDA receptor activation to the respiratory-related drive to respiratory motoneurons is controversial and depends on the preparation used. For example, Funk et al. (1993), using the in vitro rhythmic rat medullary slice preparation, found that local blockade of NMDA-receptors in the hypoglossal nucleus did not alter the inspiratory burst amplitude observed in HMs, but blockade of non-NMDA-receptors caused a dose-dependent reduction in inspiratory burst amplitude. Thus even though HMs possess both non-NMDA and NMDA receptors they concluded that it was activation of primarily non-NMDA receptors that mediate transmission of inspiratory drive to HMs (Funk et al., 1993). Subsequently this group showed in an in vitro preparation from mutant neonatal mice lacking the important NMDA-receptor gene (they were unresponsive to application of exogenous NMDA), that respiratory-related activity recorded from either HMs or phrenic motoneurons was virtually identical with activity in control animals (Funk et al., 1997). Thus, these in vitro neonatal studies indicate that it is the non-NMDA receptor mediated I-phase excitatory synaptic input to these motoneurons that mediates the inspiratory-related drive. These in vitro neonatal results leave open the question of whether later in life there is a role for NMDA-receptors in the respiratory drive to motoneurons. Additionally, the in vitro nature of these experiments may influence the results.

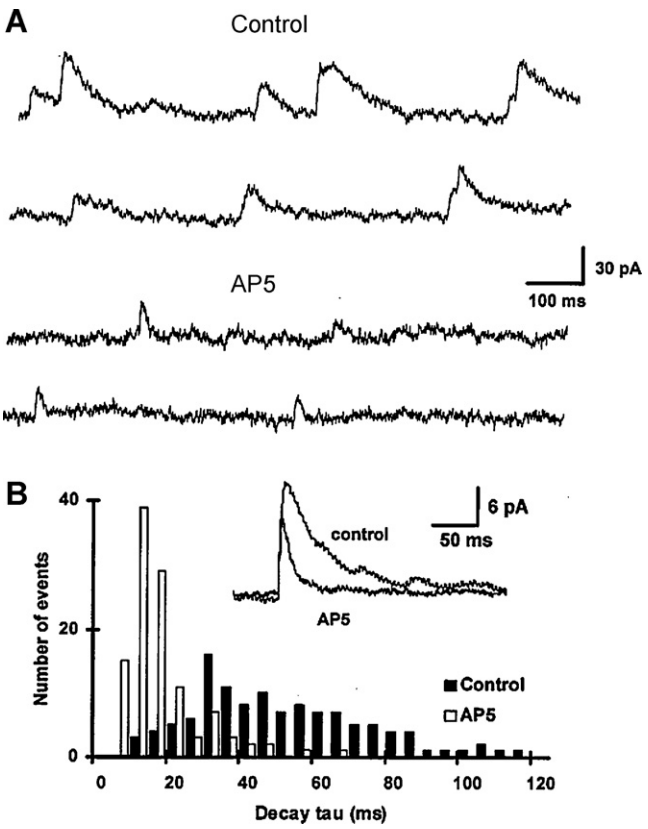

Fig. 1. NMDA and non-NMDA glutamatergic receptors are co-localized in the postsynaptic membrane of hypoglossal motoneurons. This is shown by the presence of spontaneous miniature excitatory postsynaptic currents (mEPSCs) that have dual temporal components. (A) When recorded in the presence of bicuculline to block $GABA_A$-receptors, strychnine to block glycine receptors and TTX to block action potential generated synaptic events, the majority of mEPSCs show slow decay kinetics (Control – top two traces). With the addition of AP5 to block NMDA receptors the slow decaying component is abolished leaving only a fast decaying component due that is due to activation of non-NMDA receptors (AP5 – lower two traces). (B) Distribution of mEPSC decay time constants before and after application of AP5. Inset: Average of mEPSCs before and after addition of AP5. Data show that the AP5 sensitive component of the mEPSC has a significantly longer decay time constant. Data from HMs voltage clamped at +50 mV to remove the voltage-dependent Mg block of the NMDA receptor.
(adapted from O'Brien et al., 1997).

Indeed a role for NMDA-receptors in mediating respiratory drive to respiratory motoneurons has been demonstrated in in vivo experiments involving mature animals. For example, it has been shown that in the adult rat both non-NMDA and NMDA receptors are involved in the inspiratory-drive that is seen in phrenic motoneurons (Chitravanshi and Sapru, 1996). Recently, it has been shown in the anesthetized adult rat, using microdialysis perfusion of non-NMDA and NMDA receptor antagonists into the hypoglossal nucleus, that activation of both non-NMDA and NMDA glutamate receptor types are involved in the excitatory synaptic I-phase drive to HMs (Steenland et al., 2006, 2008). Significant reductions in respiratory-related genioglossus muscle activity with blockade of non-NMDA receptors by the antagonist CNQX (Fig. 2A), and separately the blockade of NMDA receptors by the antagonist D-