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Inhibitory synaptic mechanisms regulating upper airway patency

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Abstract

The breathing cycle of vertebrates comprises three phases (inspiration, postinspiration and expiration) that are apparent in the activities generated in the ponto-medullary respiratory network. A large body of evidence now indicates that in adult mammals generation of this three-phase pattern is based on reciprocal synaptic inhibition between distinct subsets of respiratory neurones. This review summarises our recent experiments focused on the role of glycinergic inhibition in respiratory pattern formation: e.g. in co-ordinating the activity of spinal and cranial motor outputs that drive the ventilatory pump (thoracic and abdominal muscles) and adjust airflow by regulating laryngeal resistance (laryngeal abductors and adductors). We used arterially perfused in situ preparations of neonatal and mature rat and show that specific blockade of glycine receptors within the ponto-medullary network caused a severe disruption of the co-ordination of spinal and cranial motor outputs: postinspiratory neurones lose their characteristic inspiratory inhibition revealing excitatory synaptic drive coincident with inspiratory phrenic nerve activity. The resulting simultaneous discharge of inspiratory and postinspiratory neurones caused co-activation of both glottal abductors and adductors during neural inspiration. The latter resulted in a paradoxical inspiratory adduction of the vocal fold and severe disruption of the eupneic breathing pattern.

The effect of blocking glycine receptors was the same in both mature and newborn rats suggesting that glycinergic inhibition is essential for co-ordinating cranial and spinal motor outputs from birth.

Keywords: Control of breathing; pattern formation; inhibition; Mammals; rat; Neurons; post-inspiratory; Transmitters; glycine

1. Eupnoea is characterised by co-ordinated activity of cranial and spinal motor outputs

The respiratory cycle of higher vertebrates including reptiles, amphibians and mammals comprises three phases: inspiration, postinspiration (expiration stage I) and expiration (stage II). Each of these phases is accompanied by the contraction

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of specific muscle groups of the thorax and abdomen to pump air into and out of the lungs, respectively. The thoracic and abdominal muscles are controlled by motor outflows originating from cervical, thoracic, and lumbar spinal cord (Monteau and Hilaire, 1991; Iscoe, 1998). The discharge of spinal motor nerves occurs during inspiration (phrenic; external intercostals) and stage II expiration (active expiration only; internal intercostals, abdominal). The phrenic nerve can show postinspiratory 'after discharge' but this seems more prevalent in larger mammals such as the cat and less obvious in rodents (e.g. mouse: Paton, 1996a,b; rat: Paton et al., 1999; Schwarzacher et al., 1991). In contrast, postinspiratory activity is pronounced in some cranial motor nerves (e.g. both recurrent and superior laryngeal nerves) and originates from functionally and anatomically different sets of respiratory motoneurones located in the nucleus ambiguus, which are embedded into the ventral respiratory group of the medulla oblongata. These motoneurones innervate muscles of the larynx and pharynx (Barillot et al., 1990; Bieger and Hopkins, 1987) to actively control respiratory airflow by adjusting upper airway resistance during the respiratory cycle (Harding, 1984; Bartlett, 1986). The larynx is the valve of the respiratory system providing a major source of resistance in the upper airway during the early part of expiration. The recurrent laryngeal nerve (RLN) provides primary efferent innervation of the larynx. The RLN is a mixed motor nerve displaying two discharges comprising an incrementing inspiratory discharge followed by a decrementing discharge; the latter is postinspiratory activity (also termed passive expiration; see Fig. 1A). These discharges lead to a phase-locked contraction of two opposing muscle groups in the larynx. During inspiration laryngeal abductors (e.g. cricoarytenoid muscles) are activated to open the vocal cords and decrease laryngeal resistance, while during postinspiration laryngeal adductors (e.g. thyroarytenoid muscles) contract to increase glottal resistance (Fig. 1A). The phase of laryngeal constriction is essential to both brake expiratory airflow, thereby increasing time for pulmonary gas exchange, and maintain functional residual capacity to prevent atelectasis (Bartlett, 1986).

We propose that the pattern of normal breathing, so called eupnoea, should be characterised by periodic and precisely-regulated phase-locked discharges in cranial and spinal motor outflows during the respiratory cycle. This co-ordinated activity of various pulmonary pump, laryngeal and accessory muscles, such as the inspiratory abduction and postinspiratory adduction of the vocal cords, is essential for efficient breathing (Dutschmann et al., 2000a). The eupneic respiratory motor pattern is influenced by a number of sensory afferent inputs, particularly from pulmonary stretch receptors, and is ultimately controlled by the discharge of pre-motoneurones of the respiratory central pattern generator (CPG) located in the ponto-medullary brainstem. However, in contrast to respiratory rhythm generation the role of neuronal mechanisms (including glycinergic inhibition) in co-ordinating spinal and cranial motor outputs are only rarely investigated.

To study these synaptic mechanisms we employed the intra-arterially perfused working heart-brainstem preparation (WHBP; Paton, 1996a,b). This in situ preparation allows a spectrum of analyses (from intracellular recording to measures of glottal resistance) in a single preparation and exerts an eupneic-like respiratory pattern as determined from in vivo experiments. Importantly, the preparation permits application of toxic drugs systemically (e.g. strychnine) without the complications of changes in arterial pressure that could alter the oxygenation and pH of the brainstem thereby confounding interpretation of data. Finally, it allows a direct comparison of data from neonatal and mature rat from the same preparation.

2. Glycinergic inhibition is essential for the co-ordination of cranial and spinal respiratory motor outputs in both the mature and neonatal respiratory network of rat

The formation of the respiratory pattern is dependent on reciprocal synaptic interaction between different subsets of respiratory neurones as well as on their intrinsic biophysical properties (for review see Richter and Spyer, 2001). Fast,

chloride-mediated synaptic inhibition, via glycine and $GABA_A$ receptors, is the foundation of many models explaining central respiratory pattern formation (Bianchi et al., 1995; Bonham, 1995; Richter, 1996; Ogilvie et al., 1992; Rybak et al., 1997; Smith et al., 2000; Richter and Spyer, 2001). However, there is still a lack of experimental data to support for these models. Nevertheless, recent studies in juvenile rats and mice in situ (Büsselberg et al., 2001; Dutschmann and Paton, 2002b) indicate the importance of glycinergic inhibition for respiratory pattern formation. Block of glycinergic inhibition by systemic application of strychnine (0.01–0.2 μM in mature rat, 0.5–1 μM in neonatal rat) caused dramatic changes in the firing of postinspiratory neurones and RLN dis-

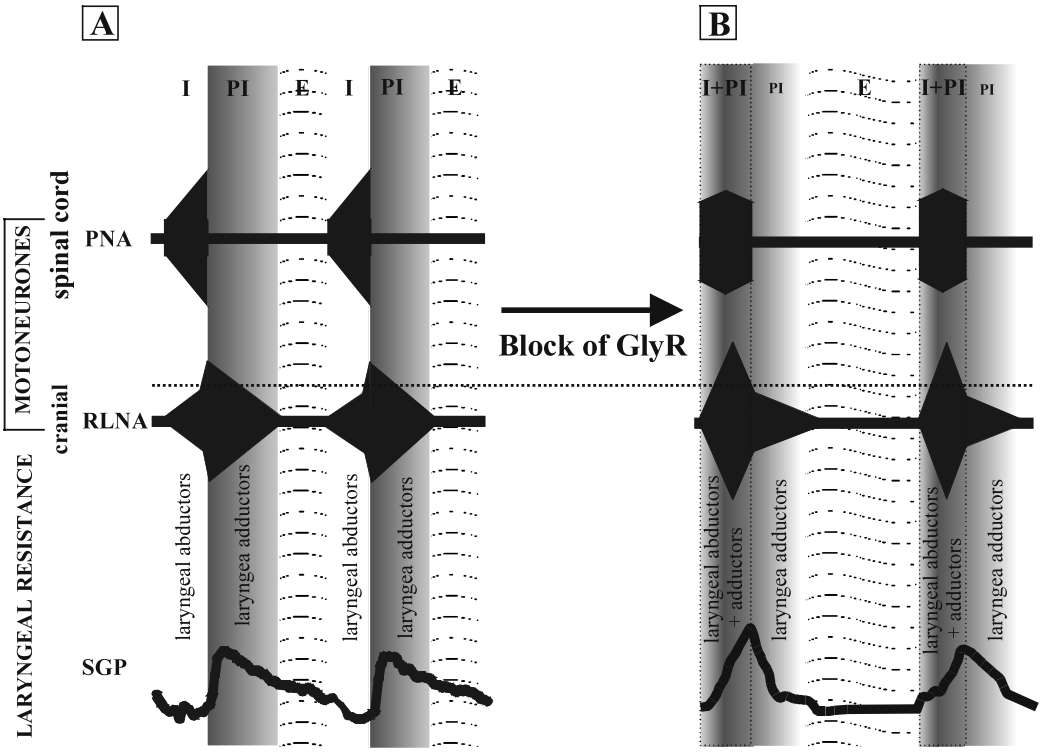

Fig. 1. A schematic summary of the effects of blocking glycine receptors (systemic application of strychnine (0.01–0.2 μM in mature rat, 0.5–1 μM in neonatal rat)) on the pattern of respiratory motor activity. (A) Illustration of the eupneic-like pattern as recorded from the phrenic and RLN under in situ conditions: the ponto-medullary respiratory network generates three neuronal phases. The phrenic nerve activity (PNA) displays a ramp-like discharge pattern. Please note: the incrementing discharge of PNA is usually only expressed consistently in preparations obtained from mature rats, while in neonatal preparations the PNA pattern is difficult to analyse because of its short duration. In contrast, the recurrent laryngeal nerve activity (RLNA) consists of two burst discharges including an incrementing inspiratory followed by a decrementing postinspiratory discharge in preparations from both neonatal as well as mature rat. These discharges drive two opposing muscle groups within the larynx: the laryngeal abductors during inspiration, to decrease laryngeal resistance and the laryngeal adductors that contract during postinspiration to increase laryngeal resistance. We measured changes in pressure recorded below the larynx (i.e. subglottal pressure; SGP) during constant airflow perfusion of the larynx in the expiratory direction. Thus, an increase in SGP indicates adduction whereas a fall reports abduction. The dynamic changes in laryngeal resistance over the respiratory cycle are illustrated by the waveform of the SGP signal. (B) Spinal motor outputs are moderately affected by systemic strychnine application in mature rats as evidenced by a loss of the ramp-like PNA. In contrast, cranial motor output (RLNA) shows a massive increase in the amplitude of inspiratory related discharge (see doted line) while postinspiratory discharge is reduced. Functionally this leads to a simultaneous co-activation of laryngeal abductors and adductors during inspiration causing a paradoxical inspiratory laryngeal adduction (see SGP trace). I, inspiration; PI, postinspiration; E, expiration; PNA, phrenic nerve activity; RLNA, recurrent laryngeal nerve activity; SGP, subglottal pressure.

charge. The characteristic inspiratory hyperpolarisation of postinspiratory neurones was converted into a depolarisation such that these neurones now fired simultaneously with the inspiratory burst recorded from the phrenic nerve (Büsselberg et al., 2001; Dutschmann and Paton, 2002b). The same effect was observed in preparations of neonatal rats (Dutschmann et al., 2000b; Dutschmann and Paton, 2001 see Fig. 1B). The precise mechanism underlying the depolarisation of postinspiratory neurones during inspiration following the block of glycine receptors remains unclear. Either excitatory synaptic drive is revealed during the inspiratory phase, which is overwhelmed by a predominating glycinergic inhibition in the intact network, or blockade of glycine receptors induces such an input to these neurones.

More detailed investigations demonstrated that the absence of glycinergic transmission and resultant simultaneous activation of inspiratory and postinspiratory neurones during the inspiratory burst of the phrenic nerve caused a change in the discharge pattern of the RLN. There was a reduction of postinspiratory activity (amplitude and duration) accompanied by an increase in the amplitude of the discharge during inspiration (Fig. 1B). We suggest that the latter reflects the summed activity of simultaneously firing laryngeal abductor and adductor motoneurones. Based on this interpretation we predicted a major disturbance in the regulation of the upper airway and larynx. Indeed, from airway resistance measurements made across the larynx the reduction in resistance during inspiration and the increase in postinspiration, which is typical of eupnoea, underwent a massive transformation in the presence of strychnine (see Fig. 1A). With glycine receptors antagonised there was a paradoxical inspiratory adduction (see Fig. 1B; Dutschmann et al., 2000a; Dutschmann and Paton, 2002b).

This response was observed in the respiratory network of both neonatal and mature rats (Dutschmann and Paton, 2001). These data indicate that glycinergic inhibition within the respiratory network is essential for separating inspiration from postinspiration required for the co-ordination of cranial and spinal motor outflows and therefore for the generation of a eupneic motor pattern from the day of birth.

3. Glycinergic inhibition is essential for reflex control of the upper airways

Postinspiratory neurones are heterogeneous, comprising propriobulbar and motor neurones. They subserve numerous functions including inspiratory off-switching (e.g. Richter and Spyer, 2001) and driving laryngeal adductor muscles (Bianchi et al., 1995). Not surprisingly, post-inspiratory neurones also play major roles in respiratory reflexes arising from receptors in the tracheobronchial tree and upper airways (Widdicombe, 1986; Sant'Ambrogio et al., 1995). Reflexes include pulmonary vagal stretch receptor control of inspiratory duration (Hering-Breuer reflex; Hayashi et al., 1996), coughing (Widdicombe, 1986; Shiba et al., 1999; Gestreau et al., 2000), sneezing (Grélot et al., 1992; Shiba et al., 1999) as well as protective apnoeas (Paton, 1997; Dutschmann and Paton, 2002a). We tested the consequence of a loss of glycinergic inhibition on the diving response as evoked by electrical stimulation of the ethmoidal nerve – a branch of the trigeminal nerve that innervates the nasal mucosa (Dutschmann and Herbert, 1998; McCulloch et al., 1999). The diving response is a potent protective reflex that includes a powerful glottal adduction and central apnoea (Fig. 2A). Both the latter would be essential for preventing entry of water into the lungs. However, after blockade of glycine receptors the reflex glottal constriction was reduced and interrupted intermittently by transient dilations of the glottis (Fig. 2B). This was interpreted as a simultaneous reflex activation of abductors and adductors caused by a synchronisation of inspiratory and postinspiratory phases (see Fig. 1; Dutschmann and Paton, 2002a). Moreover, during the diving response the expiratory apnoea was converted into a stimulus-locked tonic excitation of phrenic nerve discharge. We speculate that the loss of glycine receptor function disrupts the protective glottal adduction, which, together with the paradoxical activation of inspiratory phrenic activity, will lead to aspiration

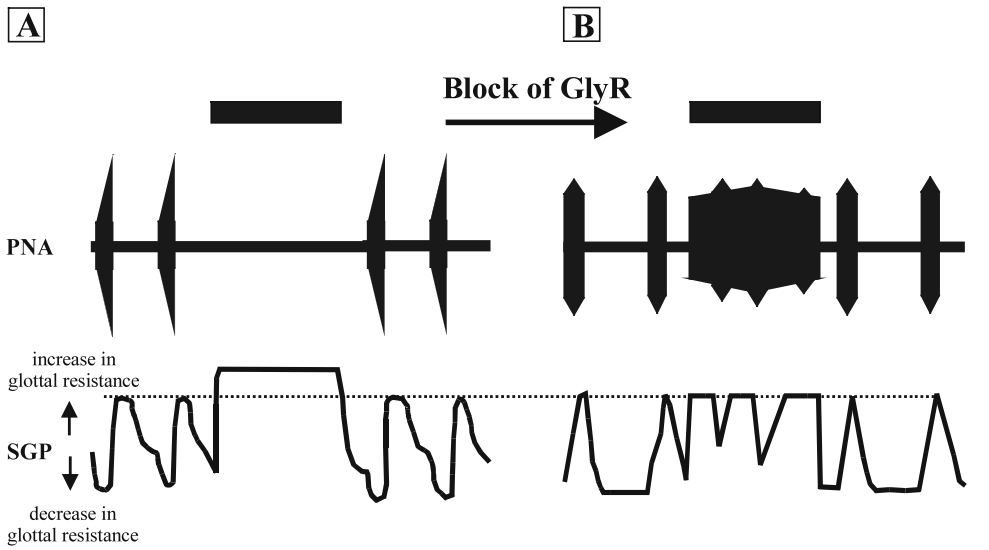

Fig. 2. A schematic summary of the effect of glycine receptor blockade on the diving response. The diving response was evoked by electrical stimulation of the trigeminal ethmoidal nerve (EN5) and includes respiratory reflex responses designed to protect the upper airways and lungs from invasion of potentially harmful substances. (A) Electrical stimulation of the EN5 (black bar top trace) evokes a profound glottal constriction, as indicated by a stimulus-locked increase in sub-glottal pressure (SGP) and apnoea (cessation of phrenic nerve activity, PNA). (B) After systemic administration of strychnine (0.05–0.2 μM) application the EN5-induced reflex glottal constriction (increase in SGP) was reduced compared to control level (see dotted line). Note that the reflexly evoked (adduction) is also interrupted intermittently. The apnoea was abolished and PNA showed tonic activation throughout the EN5 stimulation period. We propose that the absence of the post-inspiratory phase after strychnine affects upper airway control and inspiratory off switching (see text for details).

of water into lungs. Further, we predict that any reflex that commands activation of post-inspiratory neurones (for inspiratory off-switching or reflex glottal adduction – see above for reflexes) will be dramatically affected by an absence of glycinergic inhibition.

4. Remarks on the role of glycinergic inhibition in respiratory rhythm generation

The function of glycinergic inhibition for respiratory rhythm generation remains controversial. In neonates it was shown that block of glycine receptors was largely ineffective in perturbing the respiratory-like rhythm (Paton and Richter, 1995; Ramirez et al., 1996, 1997; Shao and Feldman, 1997; Brockhaus and Ballanyi, 1998). Thus, it was concluded that the activity of glycine receptors was not required for respiratory rhythm generation (Rekling and Feldman, 1998; Ballanyi et al., 1999). In contrast, in more intact preparations (in vivo or in situ) of mature animals, block of glycine receptors lead to a pronounced disturbance (Hayashi and Lipski, 1992; Paton and Richter, 1995) or cessation of rhythmic activity (Pierrefiche et al., 1998). The results of these studies favoured an essential role for inhibitory interactions for respiratory rhythm generation. The discrepancy in studies performed in neonatal and mature animals was the basis for the hypothesis of an increasing functional importance of glycinergic inhibition for central pattern formation and rhythm generation during the postnatal maturation (Paton et al., 1994; Duffin et al., 1995; Paton and Richter, 1995; Smith et al., 2000; Richter and Spyer, 2001).

The effects of blocking glycine receptors on respiratory rhythm described in our recent experiments are difficult to interpret: we can state that there was more of a disturbance to the respiratory rhythm in matures compared to neonates follow-

ing glycine receptor blockade. This supports the above-mentioned hypothesis. However, there was a slight decrease in frequency of PNA bursting in neonates, while an obvious change in the discharge pattern was not detectable. We suggest the latter reflect difficulties attributed to the short duration of the PNA inspiratory burst. It is noteworthy that in neonates PNA burst duration and frequency is similar during eupnoea and gasping (St. John, 1998). Interestingly, glycinergic blockade does not affect gasping (St. John, W.M and Paton, J.F.R.—unpublished data). Thus, strychnine applied to the neonate may alter the operational state of the network from eupnoea (i.e. glycine receptor-dependent) to gasping (glycine receptor-independent) without a detectable change in either pattern or frequency of PNA. Our present level of experimental analysis does not allow us to comment on whether this proposal is correct. We propose that future experiments need to more fully determine the role of glycine receptors in the mature and neonatal pontine-medullary respiratory network for rhythm generation.

It should be stressed that the most important aspect of the respiratory rhythm is that it provides adequate ventilation. Discrimination between mechanisms of rhythm versus pattern formation could be overly interpreted. As we have demonstrated here, the wrong pattern is as likely to cause problems with breathing as disruption to frequency.

5. Conclusions

Glycinergic inhibition is essential for the co-ordination of cranial and spinal motor outputs to produce the normal or eupneic pattern of breathing. This is characterised by precisely controlled phase-locked contractions of laryngeal, thoracic and abdominal respiratory muscles from the day of birth. Block of glycine receptors disrupts the eupneic pattern by causing laryngeal adduction during the inspiratory phase that would presumably impede inspiratory airflow thereby reducing pulmonary ventilation and oxygen uptake. Since these effects could be observed in neonatal as well as mature rats, we conclude that the integrity of glycinergic inhibition in the respiratory network is of fundamental importance from the day of birth.

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