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BRAIN RESEARCH REVIEWS

Review

Plasticity of spinal cord locomotor networks and contribution of cation–chloride cotransporters

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ABSTRACT

Locomotor burst activity in the mature intact spinal cord alternates between the left and right sides of a segment through reciprocal inhibition. By contrast, all motor bursts are in phase in the fetus. The alternating pattern disappears after neonatal spinal cord transection which suppresses supraspinal influences upon the locomotor networks. These data reveal the plasticity of spinal cord locomotor networks. This review describes recent evidence suggesting that regulation of cation–chloride cotransporter expression and activity may underlie this plasticity. GABA and glycine are classically called "inhibitory" amino acids, despite the fact that their action can rapidly switch from inhibition to excitation and vice versa. This post-synaptic action depends on the intracellular concentration of chloride ions ($[Cl^-]_i$) which is regulated by a protein in the plasma membrane: the K^+-Cl^- cotransporter (KCC2) extruding both K^+ and Cl^- ions. No or a reduced KCC2 expression leads to a depolarizing (excitatory) action of GABA and glycine. This latter situation is observed early during development and in several pathological conditions, such as epilepsy, neuronal injury and chronic pain.

Contents

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1. Introduction

Locomotion results from dynamic interactions between a central program, produced by spinal locomotor networks (central pattern generators, CPGs), and feedback mechanisms. CPGs can function independently of central and peripheral inputs. Locomotor burst activity alternates between the left and right sides of a segment through reciprocal inhibition. Glycine and GABA are the major inhibitory transmitters in the adult mammalian spinal cord. Blocking $GABA_A$ and glycine receptors in the spinal cord disrupts the left–right alternating locomotor pattern and increases tonic activity (e.g. Hinckley et al., 2005; Pflieger et al., 2000), confirming that an optimal excitatory and inhibitory balance is the basis for proper CPGs function.

GABA and glycine are classically called "inhibitory" amino acids, despite the fact that their action can rapidly switch from inhibition to excitation and vice versa. This post-synaptic action depends on the intracellular concentration of chloride ions ($[Cl^-]_i$) which is regulated by a neuron-specific protein in the plasma membrane: the K^+-Cl^- cotransporter (KCC2) extruding both K^+ and Cl^- ions. No or few KCC2 leads to a depolarizing (excitatory) action of GABA and glycine. This latter situation is observed early during development and in several pathological conditions, such as epilepsy, neuronal injury and chronic pain. Cortical neurons lacking KCC2 expression fail to exhibit a developmental decrease in $[Cl^-]_i$ (Zhu et al., 2005). KCC2 knockout mice exhibit severe motor deficits and an inability to breathe, which results in perinatal death (Hübner et al., 2001). This review describes recent evidence suggesting that regulation of cation–chloride cotransporter expression and activity may serve as a mechanism for locomotor network plasticity.

2. The presence of higher centers may be critical for the expression of a left–right alternating pattern in neonates

Pharmacological activation of the CPGs during the first few days following birth in rats evokes a fictive locomotor pattern characterized by alternations between the motor bursts on the two sides of the spinal cord (Fig. 1A, right) as well as between flexor and extensor bursts on the same side. Similar experiments performed a few days prior to birth reveals a motor pattern with all bursts in phase (Iizuka et al., 1997; Fig. 1A, left). These major changes occur in a time window during which the first axons descending from the brain stem start to reach the lumbar enlargement. For instance, serotonergic projections arising from the raphe nuclei are among the earliest axons to reach the upper lumbar segments in the rat (Bregman, 1987; Lakke, 1997; Rajaofetra et al., 1989; Vinay et al., 2000). We hypothesized that these pathways may contribute to some extent to the changes observed in locomotor network operation. We investigated the effects of suppressing inputs from supraspinal structures on the CPGs, shortly after the switch from synchrony to alternation has occurred (Norreel et al., 2003). The spinal cord was transected on the day of birth and the locomotor abilities of the animals were examined during the first postnatal week, both *in vivo* and *in vitro*. Airstepping was the protocol chosen because it represents "the natural capacity of the pattern-generating circuits to regulate stepping" more accurately than treadmill stepping (Bradley and Smith, 1988b). The overall pattern of airstepping switched in cord-transected animals from left–right alternation on postnatal day (P) 1–3 to synchrony at P6–7 (Fig. 1B1–2 left and right, respectively). However, the phase relationships were much more widely distributed in rats of the latter group than in young animals, suggesting that the overall synchrony is obtained by default and results more from the disorganization of the alternating pattern than from the emergence of a new – synchronous – pattern. Activation of CPGs, *in vitro*, by application of excitatory amino acid agonists, induced an irregular motor pattern. At P4–7, the mean correlation coefficient computed from left and right ventral root activities was positive in spinal cord-transected animals (Fig. 1C, black trace) and negative in shams, indicating that the disorganized pattern observed *in vivo* is unlikely to be due to sensory feedback from the moving limbs. Consistent with these results, synchronous airstepping has been observed during the first two postnatal weeks in kittens spinalized at birth (Bradley and Smith, 1988a).

Alternation was restored at P6–7 after the activation of $5\text{-}HT_2$ receptors *in vivo* or addition of 5-HT *in vitro* (Fig. 1C). These results suggest that no structural change within the network underlies the disorganization of the pattern after spinalization. Instead, descending pathways may critically modulate left–right coordinating pathways within the spinal locomotor network. The onset of this modulation during development may be an important step in the maturation of the locomotor pattern. Removing this modulation during a critical period following network assembly may lead to a dedifferentiation or a disorganization of the pattern.

3. Inhibitory and excitatory actions of GABA/glycine

Alternation of muscle activities between the two hindlimbs relies on mutual inhibition of the networks on the two sides of the cord (Grillner et al., 1991), involving both crossed reciprocal inhibitory interneurons and a disynaptic pathway with crossed excitatory interneurons (Kjaerulff and Kiehn, 1997). The developmental transition from synchrony to alternation observed in the pharmacologically evoked motor pattern may be due to the maturation of chloride homeostasis.

In mature neurons, $[Cl^-]_i$ is low; as a result, the activation of $GABA_A$- and glycine-receptor-gated Cl^- channels results in an influx of Cl^- (Fig. 2, top right). Therefore, the inhibitory action of glycine and GABA consists in both shunting incoming excitatory currents and moving the membrane potential away from the action potential threshold. This "classical" hyperpolarizing inhibition is not observed in immature spinal neurons (Gao and Ziskind-Conhaim, 1995; Takahashi, 1984; Wu et al., 1992; Ziskind-Conhaim, 1998); activation of glycine and $GABA_A$ receptors leads to a depolarization of the membrane which can be functionally excitatory if the equilibrium potential of Cl^- (E_{Cl}) is above the action potential threshold (Fig. 2, top left). For instance, a brief application of glycine onto the *in vitro*