

Monoaminergic and catecholaminergic activation of the central pattern generator for locomotion following spinal cord injury^{☆●}

Innovative therapeutic approaches

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Abstract: The development of secondary health complications following spinal cord injury has been increasingly recognized by healthcare professionals as a major concern. These problems most specifically affect complete or near-complete spinal cord injury patients (e.g., those with minimal mobility), who are not typically rehabilitated with treadmill training approaches, because motor control and leg movements are largely impaired. However, recent pharmaceutical advances in central pattern generator activation may provide new therapeutic hopes for these spinal cord injury patients. This article provides a comprehensive overview, for the non-specialist, of the most recent advances in this field.

Key Words: central pattern generator; locomotion; spinal cord injury; mice
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INTRODUCTION

There are more than 500 000 spinal cord injury (SCI) patients in North America and Europe, with approximately 25 000 new cases each year. To date, no cure exists, although surgery (e.g., removal of bone fragments and decompression) and administration of methylprednisolone (corticosteroid) can reduce, to some extent, neuronal damage and improve neurological dysfunction. Unfortunately, complications are complex and are not entirely limited to injury and paralysis *per se*. SCI results in an irreversible loss of motor and sensory functions below the site of injury (i.e., paraplegia or tetraplegia). However, the state of chronic immobility progressively leads to severe complications, including cardiovascular and respiratory problems, osteoporosis, muscle atrophy, immune system deficiencies, and life-threatening infections.

Based on encouraging results unexpectedly obtained with actor Christopher Reeve several years ago (*The Guardian*,

September 17, 2002), advanced locomotor therapies have been considered to be a highly promising and novel tool to treat chronic SCI patients. Mr. Reeve exhibited exciting progress, which was attributed to his training. From a state of complete tetraplegia, he regained limited, but significant, motor control, bone density, muscle mass, and strength. Perhaps, most importantly, locomotor training strengthened his immune system, which was substantiated by a reduced need for hospitalization and antibiotic treatment. Unfortunately, Mr. Reeve's therapy was very costly, primarily due to extensive medical assistance (i.e., a minimum of four physiotherapists passively moved his legs on a treadmill). These factors have likely contributed to the limited potential of these training methods for treating the most severely injured patients.

MATERIALS AND METHODS

Data retrieval

Retrieval-related contents: Pierre A. Guertin.

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Time period searched: from 1990 to 2009.

Retrieval words: non-exclusively locomotion, spinal cord injury, central pattern generator (CPG), secondary complications.

Retrieval database: PubMed

(<http://www.ncbi.nlm.nih.gov/PubMed>).

Retrieved articles: 59 articles.

Retrieval methods

Inclusion criteria: articles with key findings.

Exclusion criteria: non-significant articles.

Literature selection: the articles were initially screened by reading the titles and abstracts to exclude ones that were not closely related.

Literature type: peer-reviewed articles.

OVERALL EVALUATION

Pharmacological aids in locomotor training following SCI

In recent years, therapeutic hope for SCI patients has emerged from studies performed in adult paraplegic cats, showing that basic hindlimb stepping is partially restored with regular treadmill training and administration (intrathecally or intraperitoneally) of monoaminergic drugs, such as clonidine, an alpha-2 noradrenergic agonist^[1-3]. In some cases of previously wheelchair-bound, incomplete SCI patients, regular treadmill training, combined with clonidine and other monoaminergic drugs, has resulted in walking episodes with the aid of Canadian crutches^[4-5]. Clonidine is believed to facilitate walking by decreasing spinal reflexes and, hence, spasticity and clonus^[5-10]. Unfortunately, at doses required for locomotor enhancement in incomplete paraplegic patients, clonidine also induces severe side effects (e.g., bradycardia, sedation, and hypotension). Therefore, the development of an effective treatment, which safely and specifically enhances functional recovery in chronic SCI patients, is imperative. It is believed that such potential treatment would ideally comprise non-peptide small molecules, which can cross the blood-brain barrier (BBB), and activate the CPG through selective receptor-mediated actions. This promising avenue could be used to treat SCI-related health complications by proper and specific, upon systemic delivery, triggering episodes of active and self-generated stepping movement on a treadmill. This would afford a large number of patients the opportunity to receive advanced locomotor training and reduce the need for assistance, lower costs, and hopefully allow them to train at home using conventional equipment.

CPG for locomotion

To investigate this innovative avenue, since 2001, the main aim of the research program in our laboratory has been to elucidate some of the fundamental mechanisms underlying drug-induced CPG activity following SCI. The CPG is a network of neurons located in the lumbar area of the spinal cord, which is capable of producing basic

commands for stepping, even when isolated from supraspinal and sensory inputs^[11]. Early evidence of a CPG emerged one century ago from the pioneering work of Sherrington^[12] and Brown^[13]. In the 1970s, low-thoracic spinalized rabbits and cats were used to show that endogenously released 5-hydroxytryptamine (5-HT), induced by 5-hydroxytryptophan, can generate spinal locomotor-like rhythms^[14] in acute spinal cord-transected animals or increase extensor muscle activity in regularly treadmill-trained spinal cord-transected animals^[15-17]. A clear demonstration of the CPG was provided in 1979 by Grillner, who induced, through the use of L3, 4-dihydroxyphenylalanine (L-DOPA), locomotor-like neural activity in the motor nerves of completely deafferented, curarized, and spinalized cats^[11].

In rats, the CPG was located, *via* activity-dependent labeling (e.g., c-fos), mainly in the rostral segments of the lumbar spinal cord^[18]. Comparable results were observed in mice, where CPG activity was determined to originate from the lumbar segments^[19], with critical elements in L_{1,2}^[20]. In humans, evidence of a CPG was demonstrated by "automatic" (involuntary) stepping, which was triggered by epidural electric stimulation of the L₂ segment in SCI patients^[21-22]. Sensory inputs (*i.e.*, muscle proprioception, vision, *etc.*) have been found to provide feedback information to the CPG to re-enforce muscle contraction and adapt stepping to external disturbances^[23-24].

Evidence of drug-induced CPG activation in *in vitro* preparations

In the 1980s and 1990s, *in vitro* isolated spinal cord preparations were extensively used to study CPG activation at system and cellular levels. Initially discovered in lampreys, bath application of N-methyl-D-aspartate (NMDA) was found to induce rhythmic activity (recorded from ventral roots) that shared locomotor characteristics and, therefore, was termed "fictive locomotion". This provided evidence that even a perfectly isolated CPG can be activated with drugs^[25-26]. Subsequently, neonatal mammalian preparations were used to study drug-induced CPG-mediated hindlimb fictive locomotion *in vitro*. These studies revealed that bath-applied ligand combinations, such as NMDA, 5-HT, and dopamine (DA), can induce high-quality and robust fictive locomotor activity in isolated spinal cord preparations from mice^[20, 27-30] and rats^[18, 31-36]. Specific lesions in mice demonstrated that locomotor activity was mainly generated by networks located in the rostral lumbar spinal cord^[20]. Although, these studies revealed that several drug families are necessary for enhanced CPG activation, most of the compounds used *in vitro* were synthetic neurotransmitters (e.g., 5-HT and DA). Synthetic neurotransmitters are not good candidates for drug treatments, because of poor selectivity (e.g., full activation of all receptor subtypes) and inability to cross the BBB. These reasons have led to more research aimed at studying the potential role of specific receptor subtypes.

A role for serotonin receptor subtypes (5-HTRs) in CPG activation following SCI

Recently, *in vivo* evidence primarily from our laboratory has suggested that CPG activation can be specifically mediated by subsets of spinal cord receptors. For instance, in the rat spinal cord, 5-HTR_{2A} and 5-HTR₇ are expressed in motor control and CPG-associated areas, such as the ventral horn and intermediate zone (e.g., 5-HTR_{2A} and 5-HTR₇). However, other subtypes (e.g., 5-HTR_{2C}) have been observed throughout the grey matter, which does not preclude a specific role in locomotor rhythmogenesis^[37-40]. Furthermore, our team of researchers observed high 5-HT_{2A} expression in the upper lumbar spinal cord segments of mice, where critical CPG elements have been previously reported in mice^[20]. Supporting these observations, we showed that the 5-HTR_{2A/2C} agonist quipazine, intraperitoneally injected alone or in combination with other monoaminergic drugs, can acutely trigger “automatic” stepping-like movement in the hindlimbs of paraplegic mice^[41-42]. In contrast, we also showed that 5-HTR_{2B/2C} (m-CPP) and 5-HTR_{1B/2B/2C} (TFMPP) agonists did not induce stepping-like, only non-locomotor hindlimb movements (*i.e.*, kicks, fast-paw shakes, toe spanning, *etc.*)^[42], suggesting that 5-HTR_{2B/2C} is associated with non-locomotor effects and 5-HTR_{2A} with locomotor effects. Behavioral and kinematic analyses revealed quipazine-induced locomotor-like movements in spinal cord transected Tx mice that were non-pre-treated or pre-treated with selective 5-HTR_{2B} and/or 5-HTR_{2C} antagonists^[43]. In contrast, in animals pre-treated with selective 5-HTR_{2A} antagonists, such movements were not induced by quipazine. Altogether, these results provided strong evidence that 5-HTR_{2A} is specifically associated with spinal locomotor network activation and locomotor-like movement generation induced by quipazine in spinal cord transected animals. Treatment with quipazine was found to induce greater locomotor recovery than in combination with DOI (5-HT₂ agonist with high 5-HT_{2C} affinity)^[44-46] or with clonidine (an alpha-2 noradrenergic agonist)^[47].

In paraplegic mice, our laboratory demonstrated that a single dose (1 mg/kg, intraperitoneal) of 8-OH-DPAT, a potent and selective 5-HTR_{1A/7} agonist, but not SR57227 (highly selective 5-HTR₃ agonist), acutely induces hindlimb movement that shared some characteristics with locomotion^[48-49]. These effects were significantly reduced in mice pre-treated with the selective 5-HTR_{1A} antagonists (WAY100135 and WAY100635) or 5-HTR₇ antagonists (SB269970). Moreover, the effects were completely abolished in mice pre-treated with both types of antagonists, or in 5-HTR₇^{-/-} KO mice pre-treated with 5-HTR_{1A} antagonists^[48]. Overall, these studies have shown that 5-HTR_{1A/2A/7} agonists (but not 5-HTR_{2B/2C/3}) activate the CPG following SCI.

A specific role for dopamine receptors in spinal stepping generation following SCI

Compelling evidence also suggests that the spinal DA

system is of particular interest in SCI research.

Traditionally, the dopaminergic system has been studied, primarily for its role in Parkinson's disease. In fact, the dopaminergic system in other areas of the central nervous system, such as in the spinal cord, has received considerably less attention (*i.e.*, 98% of all PubMed articles on central nervous system-dopaminergic research are brain-related, whereas only 2% are spinal cord-related). Yet, the existence of a widespread and well-developed dopaminergic receptor system in the spinal cord has been clearly established^[50]. Most spinal dopamine is released by descending axons originating from the A11 region of the hippocampus, which projects down the cord *via* the dorsal funiculus. All five receptor subtypes (D1–D5) have been identified in the spinal cord (D1-like: D1 and D5; D2-like: D2, D3, and D4), and extensive dopaminergic projections have been detected at all segmental and laminar levels^[50-52]. Recent data has also shown that transcripts for each subtype are found in the murine spinal cord^[53-54].

Results from our laboratory and other studies in awake and freely moving decerebrate rats and spinal mice have shown that L-DOPA acutely induces real spinal stepping-like movements^[55-56], which are blocked in dopamine receptor antagonist-pre-treated animals^[57]. This provides *in vivo* evidence of a role for spinal dopaminergic receptors in stepping generation following SCI. We recently conducted an extensive study using a variety of dopamine receptor agonists and antagonists^[57]. Administration of D1/D5 receptor agonists (0.5–2.5 mg/kg, intraperitoneal) was found to acutely elicit rhythmic locomotor-like movements and non-locomotor movements in untrained and non-sensory stimulated Tx mice. Comparable effects were observed in mice lacking the D5 receptor (D5KO), whereas D1/D5 receptor antagonist-pre-treated animals (wild-type or D5KO) failed to display D1/D5 agonist-induced locomotor-like movements^[57]. In contrast, administration of broad spectrum or selective D2, D3, or D4 agonists consistently failed to elicit significant hindlimb movements^[57]. These results provide clear evidence that the spinal D1 receptor may be critical for spinal-mediated reflexes and rhythmic behaviors, such as locomotion.

Serotonergic and catecholaminergic drug combinations

Although these studies have identified several CPG-activating compounds, none of these molecules have been shown to generate over-ground stepping movements, *per se*, in untrained, non-assisted, and non-sensory stimulated Tx animals. In other words, none of these molecules, separately administered, induced real stepping movements (*i.e.*, weight bearing and plantar foot placement). Only movements resembling crawling were induced, which suggested that only partial CPG-activating effects were generated by 8-OH-DPAT, quipazine, L-DOPA, or SKF-81293 in these experimental conditions. Given these findings, we also studied the effects induced by combining drugs. In brief, NMDA and

quipazine (5-HT_{2A/2C} agonist) interacted synergistically to produce “air-stepping” movements in completely suspended Tx mice^[41]. L-DOPA (NA/DA precursor) and quipazine synergistically elicited locomotor-like movements in treadmill and air-stepping conditions^[55-56]. Clear weight-bearing and plantar foot placement were recently observed following administration of SKF-81297 (D₁/D₅ agonist) and 8-OH-DPAT (5-HT_{1A/7}), suggesting synergistic CPG-activating actions in untrained, non-assisted, and non-sensory stimulated chronic Tx mice^[58]. These data strongly suggest that at least some drug combinations efficiently activate CPG neurons in low-thoracic SCI subjects. In fact, this hypothesis was strongly supported by *in vitro* data showing that bath-applied cocktails, including dopamine, serotonin, and glutamate receptor agonists, induced robust and stable CPG-mediated fictive locomotor rhythms (in hindlimb motor nerves) in *in vitro* isolated spinal cord preparations^[56]. In turn, recent clinical data provided preliminary evidence of safety (*i.e.*, no atypical side effects were found) in a monoplegic patient, who received a combination of L-DOPA (200, 400, and 600 mg) and buspirone (5, 10, and 15 mg) tablets^[59]. Encouraging efficacy signs were also reported with this combination, with increased blood flow in his paralyzed leg. Clinical trials in motor-complete SCI patients are expected to start in 2010 with different doses of L-DOPA and buspirone to further investigate safety, as well as efficacy (*i.e.*, rhythmic leg movements), in ASIA A and ASIA B SCI patients.

CONCLUSION

This review article primarily reported results from a mouse model of paraplegia, showing that specific ligand families can be used to partially activate (compounds used individually) or fully activate (specific drug combinations) the CPG *in vivo*. These findings may pave the way to the development of novel treatments designed to efficiently treat life-threatening complications associated with poor physical activity in chronic SCI individuals.

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What is already known on this topic: Some families of drugs activate the CPG *in vitro*. However, no studies have clearly shown that pharmaceutical therapies acutely activate the CPG *in vivo* in non-stimulated, and untrained completely paraplegic animals.

What this study adds: Recent studies performed primarily in my laboratory revealed that some ligand families trigger acute CPG activity and corresponding locomotor movements in untrained and non-stimulated completely paraplegic animals.

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