

Anxiolytics may promote locomotor function recovery in spinal cord injury patients

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Abstract: Recent findings in animal models of paraplegia suggest that specific nonbenzodiazepine anxiolytics may temporarily restore locomotor functions after spinal cord injury (SCI). Experiments using *in vitro* models have revealed, indeed, that selective serotonin receptor (5-HTR) ligands such as 5-HTR_{1A} agonists, known as relatively safe anxiolytics, can acutely elicit episodes of rhythmic neuronal activity referred to as fictive locomotion in isolated spinal cord preparations. Along the same line, *in vivo* studies have recently shown that this subclass of anxiolytics can induce, shortly after systemic administration (eg, orally or subcutaneously), some locomotor-like hindlimb movements during 45–60 minutes in completely spinal cord-transected (Tx) rodents. Using ‘knock-out’ mice (eg, 5-HTR₇^{-/-}) and selective antagonists, it has been clearly established that both 5-HTR_{1A} and 5-HTR₇ were critically involved in mediating the pro-locomotor effects induced by 8-OH-DPAT (typically referred to as a 5-HTR_{1A} agonist) in Tx animals. Taken together, these *in vitro* and *in vivo* data strongly support the idea that 5-HTR_{1A} agonists may eventually become constitutive elements of a novel first-in-class combinatorial treatment aimed at periodically inducing short episodes of treadmill stepping in SCI patients.

Keywords: 5-HT agonists, anxiolytics, locomotion, SCI

Introduction

Spinal cord injury (SCI) generally causes permanent disability or loss of movement (paralysis) and sensation below the site of the injury leading either to paraplegia (thoracic level-injury) or tetraplegia (cervical level-injury). It is well established also that many SCI patients will develop anxiety and depression problems as well as other severe health complications such as obesity, type II diabetes, cardiovascular diseases, hormone dysregulation, muscle and bone loss, immune system deficiencies and life-threatening infections (Bauman et al 1999; Bauman and Spungen 2000; Cruse et al 2000). No pharmacological treatment is currently available to treat these so-called secondary health complications. In turn, the benefits of regular exercise training on secondary health complications and the quality of life are increasingly documented especially in incomplete SCI patients (Heath and Fentem 1997; Hicks et al 2003; Ditor et al 2003; Martin Ginis et al 2008; Ditor et al 2005). However, exercise training has remained difficult and is poorly adapted for people with a complete SCI (absolutely no voluntary motor control) who represent 40%–45% of all SCI cases in the United States (National Spinal Cord Injury Statistical Center). Experiments using animal models of SCI have recently offered hope for complete SCI patients. Researchers have recently found in completely spinal cord-transected (Tx) rodents that 5-HTR_{1A} agonists, normally used as anxiolytics, can also partially re-activate lumbar spinal cord networks that control locomotion (ie, typically referred to as central pattern generator or CPG) (Antri et al 2003, 2005; Landry et al 2006). This breakthrough finding may contribute to the development of a first-in-class drug treatment as ‘CPG activator’ that could elicit episodes of exercise training in complete SCI individuals.

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The 5-HT system

Serotonin (5-hydroxytryptamine or 5-HT) is an important endogenous monoamine neurotransmitter found in the central nervous system (ie, CNS: the brain and spinal cord), peripheral systems (eg, gastrointestinal tract), and plants. In the CNS, 5-HT is mainly synthesized by specialized neurons in the brainstem (ie, raphe nucleus and parapyramidal region) that send projections throughout the brain as well as in the spinal cord as far as into the lumbar and sacral segments (Hochman et al 2001). This system has clearly been shown to play a determinant role in functions and behaviors such as in body temperature, anger, mood, sleep, and sexual functions. The release of 5-HT by this system is known to influence the activity of other neurons via 14 genetically, pharmacologically and functionally distinct 5-HT receptors (5-HTR) belonging to seven families termed 5-HTR₁ through 5-HT₇ (Barnes and Sharp 1999; Fink and Gothert 2007).

The 5-HT_{1A} receptor subtype

As for all 5-HTR subtypes (except 5-HTR₃), the 5-HT_{1A} is a metabotropic receptor that activates cascades of intracellular events (Barnes and Sharp 1999). The receptor is localized both pre- and postsynaptically (Miquel et al 1992; Radja et al 1992) and is normally negatively coupled (ie, at least the presynaptic one) to adenylyl cyclase via G_i-protein activation (Albert et al 1996) and K⁺ channel opening at least in the brain (Clarke et al 1996). In the spinal cord, 5-HT_{1A} labeling using [3H] 8-OH-DPAT (5-HT_{1A} agonist) has revealed expression in laminae I-IV (dorsal horn) and X (intermediate zone) in cats (Giroux et al 1999). *In situ* hybridization has also revealed 5-HT_{1A} expression in all gray matter laminae with stronger labelling levels in the dorsal horn and intermediate zone in mice (Landry et al 2006). In both cats and mice, increased 5-HT_{1A} receptor expression has been detected in lumbar spinal cord segments shortly after a low-thoracic Tx (Giroux et al 1999; Landry et al 2006). There is compelling evidence associating the effects mediated by 5-HT_{1A} in the brain to antidepressant and anxiolytic actions (Gordon and Hen 2004). However, its specific role in the spinal cord has remained unclear, although recent data have provided evidence of a role in sexual functions (Giuliano and Clement 2006) and pain (Mico et al 2006). Experiments in turtle isolated spinal cord preparations have revealed 5-HT_{1A} on the cellular membrane of hindlimb motoneurons whose activation leads to increased neuronal excitability via inhibition of a leak current (Perrier et al 2003). This finding strongly suggests that pre- and postsynaptic 5-HT_{1A}-mediated mechanisms are different (eg, presynaptic inhibition in the

brain versus postsynaptic excitation in the spinal cord). However, spinal 5-HT_{1A} were not believed until recently to be specifically associated with locomotor functions. Although, preliminary data from *in vitro* preparations have reported early evidence of some role for 5-HT_{1A} agonists in locomotion (Cazalets et al 1992; Hochman et al 2001), a clear demonstration of their effects on spinal locomotor networks has been provided (Antri et al 2003) and attributed only recently to 5-HT_{1A} and 5-HT₇ receptor-mediated actions (Landry et al 2006) which are described in detail in the following sections.

Spinal locomotor networks

The existence of a CPG and its role in locomotor pattern and rhythm generation has been shown in all classes of vertebrate species. In the 1970s, Grillner was the first to demonstrate its existence in mammalian species, when reporting that locomotor activity in hindlimb motor nerves could be acutely induced following L-DOPA (noradrenaline/dopamine precursor) administration (iv) in deafferented and low-thoracic Tx cats (Grillner and Zangger 1979). These experiments have clearly established that a network (or several) of sublesional neurons (caudal to the 12th thoracic segment) referred to as CPG neurons displayed the capacity of generating the basic signals for walking even in complete absence of supraspinal inputs and phasic inputs from peripheral nerves (see also preliminary evidence from Graham-Brown 1911). Nonetheless, a significant role for peripheral afferent inputs to CPG activation and/or modulation has also been shown since greater effects were found in nondeafferented animals placed on a motorized treadmill (Grillner and Zangger 1979). A clear demonstration of its existence in humans has remained difficult since only data from an entirely deafferented and complete SCI patients would satisfy all criteria. Nonetheless, in the 1990s, Calancie and colleagues have reported evidence suggesting the existence of a CPG in a chronic tetraplegic patient (Calancie et al 1994). Although not completely SCI, that patient was shown, once lying down on a table, to spontaneously display locomotor-like movements in the lower limbs. More convincing data were reported a few years later by Dimitrijevic and colleagues (1998) who showed locomotor-like movements induced by epidural stimulation of the spinal cord (ie, lumbar segments) in complete paraplegic subjects. Those results have strongly suggested the existence of critical CPG elements in the lumbar or thoraco-lumbar area of the spinal cord in humans. Although a contribution from peripheral inputs (eg, proprioceptors, skin receptors, etc.) was possible, this

series of experiments has clearly shown that spinal stepping is possible even in complete absence of inputs from the brain. During the same period, automatic air-stepping (in completely suspended subjects) has been successfully induced in healthy persons following tonic muscle vibration which may also partially support the idea of a CPG (activated by afferent inputs) in humans (Gurfinkel et al 1998). Prior to those studies, preliminary evidence of a CPG was found in a complete SCI patient who developed myoclonus and leg movements resembling locomotion (eg, low frequency, rhythmic and bilaterally alternating; Bussel et al 1988. See Illis 1995; Nicol et al 1995 for additional evidence).

Anxiolytic effects induced by 5-HTR_{1A} agonists

Typically used for short-term relief of anxiety, antidepressants and anxiolytics are generally divided into two groups, benzodiazepines and nonbenzodiazepines. Medications of the latter group are increasingly prescribed since compelling evidence suggests that they lack the sedation- and dependence-related side effects associated with benzodiazepines. 5-HTR_{1A} agonist-induced anxiolytic/antidepressive effects are believed to be mainly mediated by a progressive desensitization of presynaptic autoreceptors in the raphe nucleus leading to a reduction of autoreceptor-mediated neuronal inhibition (De Vry et al 1992). Unfortunately, as with most secondary health complications experienced by paralyzed individuals, depression and anxiety, although frequently reported after SCI, remain largely untreated (Smith et al 2007).

Activation of spinal locomotor networks by 8-OH-DPAT

Although a role of 5-HT in spinal motor control was proposed several years ago (a facilitating role, see Jacobs and Fornal 1993), a determinant role in the control of locomotion *per se* has been demonstrated more recently (Schmidt and Jordan 2000; Jordan et al 2008). 5-HT was found indeed to trigger bouts of fictive locomotor rhythms (ie, bilaterally alternating and rhythmic ventral root-monitored activity) in *in vitro* isolated spinal cord preparations (mice, Jiang et al 1999; Nishimaru et al 2000; rats, Cazalets et al 1992). *In vivo*, transplantation of embryonic 5-HT cells or intrathecal administration of 5-HT was also reported to promote the recovery of locomotor functions in SCI rats (Feraboli-Lohnherr et al 1997, 1999; Ribotta et al 2000). Further supporting a determinant role of 5-HT in spinal locomotor activity, increased 5-HT release was measured in the lumbar

cord using implanted microdialysis probes in freely moving animals during locomotion (Gerin et al 1995).

As mentioned earlier, recent data from this laboratory and others have provided convincing evidence of a specific role for 5-HTR_{1A} in spinal locomotor rhythmogenesis (eg, Landry et al 2006). *In vitro* data from Hochman and colleagues (2001) have shown that bath application of 5-carboxamidotryptamine (5-CT, a 5-HTR_{1A/5A/7} agonist) combined with low doses of *N*-methyl-*D*-aspartate (NMDA: agonist at the corresponding glutamatergic receptor) elicited fictive locomotor rhythms in murine isolated spinal cord preparations. Other *in vitro* experiments have revealed that 5-HTR_{1A} antagonists could block 5-HT-evoked fictive locomotion in neonatal rats (Cazalets et al 1992) and eliminate the afterhyperpolarization-enhancing effect of 5-HT during fictive locomotion in lampreys (Wikstrom et al 1995). Altogether, results from *in vitro* isolated spinal cord preparations tend to suggest that spinal 5-HTR_{1A} activation can induce and modulate CPG-mediated rhythms in the mammalian spinal cord.

The above hypothesis has been confirmed recently in *in vivo* models of SCI. For instance, chronic administration of 8-OH-DPAT (5-HTR_{1A} agonist), alone or in combination with quipazine (5-HTR_{2A/2C} agonist), was shown to significantly improve the recovery of locomotor functions in low-thoracic Tx rats regularly trained on a treadmill (Antri et al 2003, 2005). We have recently found in low-thoracic Tx mice that 8-OH-DPAT can also acutely elicit short episodes of locomotor-like movements. Indeed, a single dose of 8-OH-DPAT administered subcutaneously or intraperitoneally in untrained and otherwise non-stimulated Tx mice was found to induce bilaterally alternating hindlimb movements (Landry et al 2006). This finding has provided clear evidence that 5-HTR_{1A} agonist administration can, in itself (without tail pinching, body weight-support assistance or other stimulation) activate CPG neurons and, hence, generate some hindlimb locomotor-like movements in complete paraplegic animals. This said, only partial CPG-activating effects were found in those experiments since the movements acutely induced by 8-OH-DPAT in Tx mice remained incomplete (ie, small amplitude rhythmic movements with no weight-bearing and plantar foot placement capabilities). However, as reported in a number of *in vitro* studies, full CPG-activating effects may be obtained by combining several different types of ligands (eg, Jiang et al 1999; Whelan et al 2000) which, in turn, suggests that combinatorial drug treatments would further enhance CPG activation and spinal stepping generation. This idea is supported also by evidence from *in vivo* models showing that greater (ie, larger amplitude or frequency)

hindlimb movements can be induced by combining several compounds (McEwen et al 1997; Guertin, 2004a, 2004b; Antri et al 2005; Guertin and Steuer 2005).

5-HTR_{1A} and 5-HTR₇-mediated effects induced by 8-OH-DPAT

We have recently established that 8-OH-DPAT-induced locomotor-like movements were mediated by both the 5-HTR_{1A} and 5-HTR₇. Paraplegic mice pretreated with selective 5-HTR_{1A} or 5-HTR₇ antagonists were found indeed to display similar reductions of 8-OH-DPAT-induced movements (Landry et al 2006). A complete blockade of 8-OH-DPAT-induced movements was obtained in wild-type mice pretreated with both antagonists or in 5-HTR₇^{-/-} knock-outs pretreated with 5-HTR_{1A} antagonists (Landry et al 2006) which, altogether, strongly supports the existence of a role for both receptor subtypes in spinal locomotor network activation. This conclusion is in line with data from other laboratories that have also demonstrated a role of both 5-HTR_{1A} and 5-HTR₇ in 8-OH-DPAT-induced control of body temperature and circadian rhythms (Hedlund et al 2004; Sprouse et al 2004). A role of 5-HTR₇ in CPG-mediated activity is also supported by *in vitro* data showing that 5-HTR₇ antagonists can block electrically-evoked or 5-HT/NMDA-evoked fictive locomotion (Madriaga et al 2004; Liu and Jordan 2005; Pearlstein et al 2005).

Clinical relevance

As mentioned earlier, many SCI patients will develop severe health complications such as obesity, type II diabetes, cardiovascular diseases, hormone dysregulation, muscle and bone loss, immune system deficiencies, and life-threatening infections (Bauman et al 1999; Bauman and Spungen 2000; Cruse et al 2000). While activity-based training (eg, manually-assisted training) may contribute to reduce secondary complications in incomplete SCI subjects (Heath and Fentem 1997; Hicks et al 2003; Ditor et al 2003, 2005; Martin Ginis et al 2008), it has generally failed to produce significant effects in complete SCI patients (Harkema 2008). Given the findings reported here, CPG-activating drugs may eventually become the 'gold-standard' to enhance the outcome of activity-based training in complete SCI patients. This said, additional experiments and clinical trials clearly need to be performed in order to clarify a number of issues. For instance, it is unclear whether assisting-devices (eg, harness, robotic system, etc.) or functional electrical stimulation will be required in humans for the maintain of trunk stability and equilibrium (although not essential in mouse quadrupedal

locomotion). It remains unclear also whether supraspinal (in incomplete SCI patients) or propriospinal input will contribute or interfere with drug-induced CPG activation in humans. Nonetheless, the exciting results obtained in paraplegic mice suggest that CPG-activating drugs such as 5-HTR_{1A/7} agonists, may very well become effective treatments to trigger CPG activity and, hence, spinal stepping after SCI. It may thus be postulated that regular drug-induced training will partially reverse or prevent the development of secondary health complications since many of these problems are indeed related with chronic immobility after SCI (eg, cardiovascular problems, immune deficiencies, osteoporosis, muscle atrophy, etc.).

Concluding remarks

As mentioned earlier, 5-HTR_{1A} agonists are better known as nonbenzodiazepine anxiolytics that can induce a short-term relief of anxiety with less side effects on sedation, dependence and cognitive impairment than benzodiazepines. Data presented in this mini-review suggest the existence of a novel therapeutic application for 5-HTR_{1A} agonists. In addition to treating anxiety and depression after SCI (brain-mediated presynaptic autoreceptor inhibition), they could eventually be part of a combinatorial drug treatment to reactivate locomotor circuits (spinal cord-mediated post-synaptic excitation/depolarization) in complete paraplegic or tetraplegic patients. If future clinical trials are to reveal safety and efficacy for that novel indication, such treatment may eventually contribute to treat anxiety problems as well as immobility-related secondary health complications in chronic SCI patients.

Disclosure

The author reports no conflicts of interest.

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