Ionotropic 5-HT$_3$ Receptor Agonist-Induced Motor Responses in the Hindlimbs of Paraplegic Mice

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INTRODUCTION

The effects of serotonin (5-hydroxytryptamine or 5-HT) in the peripheral and CNS are mediated by 13 G-binding protein-coupled receptors and one ligand-gated ionic channel (Barnes and Sharp 1999). The latter is a nonselective cationic receptor (Derkach et al. 1989; Maricq et al. 1991) for which two classes have been identified: 5-HT$_3$ and 5-HT$_4$. Central 5-HT$_3$ receptors (5-HT$_3R$) are well known for their role in wakefulness, cognition, and nociception. However, clear evidence of their participation in motor control is still lacking despite specific 5-HT$_3$ expression in hindlimb motor areas of the spinal cord (i.e., lumbar laminae VII-IX). Here, we studied the acute effects of 4-amino-(6-chloro-2-pyridyl)-1-piperidine hydrochloride (SR 57227A), a potent and selective 5-HT$_3$ receptor agonist, on hindlimb movement generation in complete paraplegic mice. The induced movements were assessed in open-field, air-stepping, and treadmill conditions using a combination of qualitative and quantitative methods. The results revealed that SR 57227A (1–4 mg/kg ip) induced hindlimb movements corresponding to scores ranging from 1 to 5 on the motor scales of Basso, Beattie, and Bresnahan and of Antri, Orsal, and Barthe. Additional analyses revealed that one-third of the movements displayed on a treadmill were “locomotor-like” (i.e., bilateral alternation), whereas only nonlocomotor movements were observed in the other testing conditions suggesting a task-dependent contribution of peripheral afferent inputs to these effects. Locomotor-like movements could also be induced in open field and air stepping if SR 57227A was combined with subthreshold doses of 5-carboxytryptamine (5-HT$_1A$ receptor agonist), suggesting synergistic actions of these drugs on central neurons. These results demonstrate that 5-HT$_3R$ activation can induce motor activity and, under some conditions, rhythmic locomotor-like movements in the hindlimbs of paraplegic mice providing evidence of an unsuspected role for this receptor subtype in hindlimb motor control.

METHODS

Animal model

All experimental procedures were conducted in accordance with the Canadian Council for Animal Care guidelines and accepted by the Laval University Animal Care and Use Committee. Twenty-nine (n = 29) adult mice (male CD1, Charles River Canada, St-Constant, Quebec) initially weighing 35–40 g were used for this study. In brief, a complete transection of the spinal cord was performed using micromanipulators to ensure complete elimination of hindlimb movements. Drug-induced effects on hindlimb movements were examined using a variety of assessment methods and testing conditions in adult mice whose low-thoracic spinal cord was transected 1 wk prior to testing.

However, some of the most densely 5-HT$_3$-labeled CNS neurons have been found in the ventral horn and intermediate gray matter zone of the lumbar spinal cord (Morales et al. 1998). This is of particular interest because this area of the spinal cord also corresponds with segments and laminae containing elements of the central pattern generator (CPG), a neuronal network involved in the production of basic commands for hindlimb locomotor movement generation (Kiehn and Butt 2003).

Surprisingly however, 5-HT$_3$s have never been reported to participate directly in spinal-cord-mediated motor functions. In vitro and reduced models, 5-HT$_3$s agonists and antagonists have been shown, in fact, not to affect N-methyl-d-aspartate (NMDA)- or 5-HT-induced fictive locomotion in lampreys (Wikstrom et al. 1995) and neonatal rats (Cazalets et al. 1992). 5-HT$_3$s agonists and antagonists have been shown to decrease in the presence of NMDA-induced depolarizations of frog motoneurons (Legendre et al. 1989) and rat lumbar motoneurons (Roberts et al. 1988). In turn, NMDA-induced depolarizations of frog motoneurons have been shown to decrease in the presence of 5-HT$_3$s agonists (Holohan et al. 1995).

In response to a lack of conclusive evidence from in vitro studies, we decided to examine the effects of 5-HT$_3$s ligands in vivo. More specifically, we examined the possible effects of SR 57227A, a high-affinity 5-HT$_3$, agonist active in vivo upon systemic delivery (Bachy et al. 1993), on spinal-mediated movements in paraplegic mice. Drug-induced effects on hindlimb movements were examined using a variety of assessment methods and testing conditions in adult mice whose low-thoracic spinal cord was transected 1 wk prior to testing.

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of the spinal cord lesion, and histological examination in some cases of coronal or midsagittal spinal cord sections stained with luxol fast blue/cresyl violet solutions that stain for myelinated axons and nissl substance, respectively. Only data from animals with complete spinal cord transection were used for further analyses. Animals were left in their cage with food and water ad libitum for 7 days to allow sufficient rest and recovery from surgery before testing.

Experimental protocol

All tests were performed on day-7 postsurgery to allow sufficient recovery postspinalization and distinguish drug-induced effects on hindlimb movements from those that may spontaneously occur after the first week postspinalization (Guertin 2005). Prior to testing, the left hindlimb was entirely shaved, and pen marks were drawn on the skin over the iliac crest, femoral head, knee joint, lateral malleolus, and second external digit (i.e., longest) of the left hindlimb. A pen mark was also placed on the homonymous digit of the right hindpaw for bilaterally displaced placement (displacement shown in Figure 2A) (also Quantitative assessment methods). Drug administration was performed intraperitoneally with either 1–4 mg/kg SR 57227A hydrochloride (Tocris, Ellisville, MO) or 1–4 mg/kg 5-carboxamidotryptamine (5-CT, Sigma, St-Louis, MO) or both. Some animals were also pretreated (i.e., 15 min prior to agonist administration) with a highly selective 5-HT₃ antagonist, ondansetron hydrochloride (1–2 mg/kg, GlaxoSmithKline, Montreal, Quebec, Canada) in an attempt to prevent, and selectively block 5-HT₃-mediated effects to verify 5-HT₃ agonist specificity. Immediately before (control) and 30 min after agonist(s) administration (test), mice were filmed (see Video camera recording) during 4 min in each of three conditions: air stepping, open field, and treadmill.

Testing conditions

In the open-field condition, mice were examined inside a closed circular 60 × 60-cm arena entirely made of transparent plexiglass walls to facilitate video camera monitoring (Guertin 2005). In the air-stepping condition, mice were completely suspended (i.e., no foot contact with the ground) with a harness placed around the upper body (torso) and waist (e.g., see Fig. 2A) (see also Guertin 2004a,b). In the treadmill condition, we used a custom-made adjustable-speed motor-driven treadmill running at a constant speed of 8–10 cm/s (Guertin 2004b, Landry and Guertin 2004).

Quantitative assessment methods

Semi-quantitative method. This method is also used routinely in our laboratory (Guertin 2004b, 2005; Landry and Guertin 2004). In addition to being more objective, it allows straightforward quantification (i.e., except for amplitudes) of movements. It thus constitutes a useful method for easily distinguishing and quantifying locomotor-like movements (LMs) from nonlocomotor movements (NLMs). One LM was defined as an entire step-like cycle (in the hindlimbs only) consisting of an extension phase or stance followed by a flexion phase or swing occurring in both hindlimbs consecutively (i.e., bilaterally alternated or “out-of-phase”). "Extension" began with foot contact onset (i.e., dorsal or plantar foot) until lift off or end of foot contact with the ground or treadmill belt. "Flexion" began with foot contact ending (lift off) until next foot contact or extension onset. In the case of "air-stepping" or when the foot never quite cleared off the ground or was constantly rubbing against the treadmill belt, then extension was more generally defined as when the hindlimb was in a relatively extended position and flexion when it was not extended and generally flexed (e.g., see Fig. 2B). One NLM was defined as one nonbilaterally coordinated movement (i.e., not followed by a flexion-extension on the other side) including typically unilateral movements, jerks, brief sequences of fast-paw shaking (typically lasting 1–2 s/episode and counted as 1 NLM), twitches, and kicks. “Amplitude” was evaluated as either slight or extensive (i.e., less than half or more than half the normal range of joint motion) as in the BBB and AOB methods except that they were given the value “1” or “2,” respectively for statistical purposes. “Incidence” corresponds with the number of mice (of all mice tested in a group) in which drug-induced effects were observed. As mentioned in the preceding text, all these characteristics were assessed during a 4-min bout of activity which was video recorded immediately prior to and 30 min after drug administration.

Kinematic analysis. This entirely quantitative method required anatomical markers (e.g., iliac crest, knee, etc., see Experimental protocol) which, once captured by the second video camera system (see Video camera recording), were digitized frame-by-frame (Freehand 9, Macromedia, CA) and used for the creation of stick diagram representations of the induced movements (e.g., Fig. 2A). Bouts of activity that were recorded immediately prior to and 30 min after drug administration were chosen for recording with a second video camera system (see Video camera recording). When one marker (e.g., knee) was not visible at some point during the cycle, its position for that video frame was estimated with calculations using the corresponding segmental length and joint angle. As this approach is time consuming, detailed kinematic analyses were performed only for representative bouts of activity or for bouts of interest. Stick diagram representations of the induced hindlimb movements (e.g., Fig. 2A) were created by placing, side by side, all sticks obtained from a consecutive bout of activity (i.e., 8–10 s). All sticks were aligned horizontally using the iliac crest pen mark as a reference point. Some of the sticks were subsequently colored in red to distinguish more easily the flexion phase (or part of it) from the extension phase. Mirror images (i.e., left is right) were used for the making of figures because natural progression is generally expected to be seen from left to right rather than from right to left. Joint angular excursion that was expressed in degrees was calculated as the minimum and maximum angle values reached for each cycle during flexion and extension respectively. These were

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averaged for a number of analyzed cycles considering flexion and extension phases separately (see Fig. 2D). Foot displacement expressed in centimeters (cm) represented the step length or amplitude measured using the second digit (i.e., longest) as a reference (e.g., Fig. 4G).

**Video camera recording**

Hindlimb movements were filmed using two separate digital video camera systems. The first, used for its capacity to store a large amount of data, was a digital video camera (Sony DCR110) fixed on a tripod and positioned at a 45° angle above (open-field arena) or behind (motor-driven treadmill and air-stepping conditions) to observe most hindlimb movement characteristics including bilateral alternation. Data were stored directly on a Sony miniDV tape for both subsequent display on a standard 19-in TV and analysis by two individuals using qualitative and semi-quantitative assessment methods (see preceding text). The second video camera system was used specifically for the kinematic assessment and entirely quantitative two-dimensional (2-D) analysis of hindlimb movements in the air-stepping and treadmill conditions (i.e., not in open field because trajectories explored by mice in that condition are 3-D). This second system (3Com, HomeConnect, 30–50 frames/s, shutter speed: 1/200) was placed on the left end side of animals with a 90° angle to capture movements of the respective hindlimb in 2-D. This allowed subsequent measurements of kinematic parameters such as joint angular excursion, foot displacement (step length), and bilateral coordination. This system was computer driven and also used for on-line monitoring, digital data storage and subsequent analysis (i.e., frame-by-frame digitization of markers, stick diagram representation and quantitative measurements). All graphs were made with Excel 2000 (Microsoft, WA). Data were reported as means ± SE, and statistical analyses (SPSS BI) were conducted using paired Student's t-test (control vs. test) and one-way ANOVA (conditions). P values <0.05 were considered statistically significant.

**RESULTS**

**Effects induced by SR 57227A**

We found that intraperitoneal administration of SR 57227A induces some movements in the hindlimbs of mice with complete spinal cord transection. These were flexions and extensions that were accompanied occasionally with bilaterally coordinated alternations (i.e., defined as LMs). This is reported in Fig. 1A, where a single dose of SR 57227A ranging between 1 and 4 mg/kg ip was shown to induce hindlimb movements in the previously flaccid and immobile hindlimbs of complete paraplegic mice. Examined in air-stepping, open-field, and
treadmill conditions, these movements corresponded with average BBB scores of 1.0 ± 0.1, 0.9 ± 0.1, and 0.9 ± 0.1, respectively (Fig. 1A) whereas, as expected, near-null scores were found prior to drug injection (control). Higher scores were found with the AOB scale (Fig. 1B) used generally to assess more specifically hindlimb motor and locomotor movements in rodents with complete spinal cord transection (i.e., with no re-connection or regeneration across the lesion). Average AOB scores of 1.4 ± 0.2, 1.8 ± 0.3, and 2.3 ± 0.4 were obtained, respectively, in the air-stepping, open-field, and treadmill conditions (Fig. 1B). The AOB scale was discriminating enough to detect differences among conditions, as significantly higher scores were found on the treadmill than in open field and air stepping. AOB scores as high as 5 were even found occasionally on the treadmill, whereas scores lower than 3 were found generally in the other two conditions.

Results obtained with the semi-quantitative method show that the movements induced by SR 57227A were mainly NLM scores. This is reported in Fig. 1C, where 2.0 ± 0.7 NLM/min were found on average to be induced by SR 57227A in the air-stepping condition. Significantly increasing values that reached 3.4 ± 1.1 and 5.1 ± 1.3 NLM/min (as high as 13 NLM/min) were found in open-field and treadmill conditions, respectively (Fig. 1C). In contrast, only rare LM were induced by SR 57227A in air-stepping and open-field conditions (0.1 ± 0.1 and 0.3 ± 0.2 LM/min), although values as high as 8 LM/min (mean: 2.4 ± 0.8 LM/min) were found occasionally on the treadmill (Fig. 1D). Regarding incidences, LM were found in <57% of the mice tested, whereas NLM were found in >85% of them (Fig. 1F). Regarding amplitudes, values assessed for NLM and LM were reported near the low range portion of the scale (i.e., 1.2–0.9) in all three testing conditions (Fig. 1E). Note that none of the mice tested with SR 57227A displayed weight-bearing hindlimb movements (e.g., Fig. 2B).

Some of these effects are illustrated in Fig. 2A using video frames and stick diagram representations. Hindlimb represented-stick diagrams (left) were created for a series of consecutive video frames belonging to a 10-s bout of activity. In the air-stepping condition, injection of SR 57227A (2 mg/kg) in this mouse induced rhythmic hindlimb movements characterized by small amplitude flexions and extensions (flexions in red, Fig. 2A, right). In Fig. 2B, stick diagrams accompanied by their corresponding video frames (displaying rate of 0.1 Hz) show one particular cycle executed by that same mouse on a motor-driven treadmill running at 8 cm/s. The hindlimb is seen moving progressively from an extended position (a) to a flexed position (d) and, again, to a relatively extended position (h). Using the second digit of the foot as a reference mark (see METHODS), distances measured between the most extended and the most flexed positions revealed a foot displacement or step length of 3.8 cm for this particular cycle (see also, superimposed sticks in Fig. 2C, or the average value for four consecutive cycles in Fig. 2D, gray bar foot). Measured for the entire 10-s bout of activity including four consecutive cycles, joint angular excursions at the hip, knee, and ankle levels reveal that movements involved mainly rotations at the knee and ankle levels. Indeed, no significant difference in angle values among the flexion and extension phases was found at the hip level (Fig. 2D).

Although not studied systematically, we noticed that SR 57227A did not generally induce hypothermia, hypermetria, fast-paw shaking, or tremor in the hindlimbs in contrast to what
has been reported with similar doses of 5-HT_{2A/2C} agonists (Guertin 2004a,b; Landry and Guertin 2004).

**Effects induced by SR 57227A + 5-CT**

Tests were also performed with SR 57227A combined with low doses of 5-CT, a 5-HT_{1A/7} receptor agonist that was shown recently to participate in the production of central locomotor-like rhythms in in vitro isolated mouse spinal cord preparations (Madriaga et al. 2004; see also evidence from Liu and Jordan 2005). This was done to examine whether greater locomotor effects could be induced possibly through synergistic actions of multiple drugs on spinal cord neurons. The results revealed that SR 57227A + 5-CT produced hindlimb movements corresponding with scores lower than 2 on the BBB scale (n = 10). This is shown in Fig. 3A, where similar BBB scores were reported in air-stepping, open-field, and treadmill conditions. As expected, higher scores were obtained with the AOB rating scale in all three conditions (Fig. 3B). The combined treatment generated relatively high numbers of NLM compared with LM. For instance, in the air-stepping condition, 6.7 ± 2.4 NLM/min and 1.0 ± 0.5 LM/min were found on average (Fig. 3, C vs. D).

![Figure 3A](image_url)

![Figure 3B](image_url)

![Figure 3C](image_url)

![Figure 3D](image_url)

![Figure 3E](image_url)

![Figure 3F](image_url)

Also, it is worth noting that LMs were found to be induced by SR 57227A + 5-CT in all three testing conditions in contrast with results obtained with SR 57227A alone (Fig. 3, D vs. D). Regarding incidences and amplitudes (Fig. 3F), movements induced by SR 57227A + 5-CT were found in >87% of the cases for NLM (open field) and in >50% for LM (air stepping) whereas average amplitude values ranged between 1.4 and 0.9 (Fig. 3E).

Effects induced by this drug combination are also illustrated with video frames and stick diagrams. This is shown in Fig. 4, where a mouse that had received SR 57227A (2 mg/kg) and 5-CT (2 mg/kg) displayed flexion-extension movements on a treadmill running at 8 cm/s. Illustration of one particular cycle revealed its hindlimb moving progressively from extension (a) to flexion (c) and, again, to extension (Fig. 4A, g and h). Superimposed sticks for the entire cycle illustrate the extent of foot displacement (2 cm) and the progressive changes that occurred at the hip, knee, and ankle (Fig. 4B). Plotted for four consecutive cycles, joint angular values between flexion and extension are shown to significantly change at all three joints which led to an average foot displacement of 2.3 cm (Fig. 4C).
Figure 4 shows also the same mouse displaying both NLM and LM in a 10-s bout of air-stepping activity. The stick diagrams illustrate four consecutive flexions (in red); two of small amplitude followed by two of rather larger amplitude (Fig. 4E). Superimposed sticks for the third and fourth flexions show angular excursion changes at all three joints as well as the extent of foot displacement (Fig. 4F). Foot displacement data plotted simultaneously for both feet (see E, left) reveal that the ipsilateral foot (black circles) moved upward four consecutive times (red arrows, Fig. 4G). During that time, the contralateral foot (blue circles) moved upward with an out-of-phase relationship (i.e., bilaterally alternating movements or LMs) with the ipsilateral foot for all cycles but the third one. Indeed, the first two contralateral flexions (1st and 2nd blue arrows, Fig. 4G) were out-of-phase with the first two ipsilateral flexions (corresponding red arrows). For the third cycle, the contralateral flexion was in-phase with the ipsilateral one because both feet moved upward simultaneously (3rd blue and black arrows, Fig. 4G). While for the fourth cycle, a return to an out-of-phase relationship was found because the ipsilateral flexion (4th red arrow) occurred simultaneously with a contralateral extension rapidly followed with a flexion (4th blue arrow, Fig. 4G).

We tested, also, 5-CT administered individually. With doses of 1–4 mg/kg, 5-CT induced nearly no hindlimb movement in paraplegic mice. Scores ranging from 0 to 0.4 were reported with the BBB and AOB scales in the air-stepping and open-field conditions. Although some NLM were found to be displayed on the treadmill (i.e., effects in 2/5 mice), there was absolutely no case of 5-CT-induced LM during any testing condition.

A clear lack of effect was also found in five (n = 5) additional mice pretreated with a highly selective 5-HTR3 antagonist called ondansetron (1–2 mg/kg ip) 15 min prior to SR 57227A administration. A few NLMs were found occasionally especially on the treadmill. However, these values were not significantly different compared with control values prior to ondansetron administration.

**DISCUSSION**

The results show that a single intraperitoneal injection of 1–4 mg/kg SR 57227A, a highly selective 5-HTR3 agonist, can
acutely induce movements in the hindlimbs of adult paraplegic mice. Some of the movements induced by SR 57227A on a running treadmill were LM because rhythmic and bilaterally alternating actions were observed. LMs were also found in the air-stepping and open-field conditions if SR 57227A were combined with subthreshold doses of 5-CT, a high-affinity 5-HT₁₄/₅ agonist.

Activation of spinal neurons

The results show clearly that highly potent and selective 5-HT₃ agonists such as SR 57227A can acutely trigger motor responses in the hindlimbs of paraplegic mice (Figs. 1 and 2). Because these mice had their spinal cord completely transected at the thoracic level, this study becomes the first to report that 5-HT₃ can be involved in spinal cord-mediated motor control.

Results obtained mostly from in vitro isolated spinal cord preparations have reported, indeed, that 5-HT₃ ligands have no detectable effect on motoneurons (see INTRODUCTION). On the other hand, tests performed in in vivo models have reported that 5-HT₃ ligands typically modulate some motor behaviors. For instance, 5-HT₃ agonists were found to increase wakefulness and associated locomo-tor activity in rats (Gillies et al. 1996). 5-HT₃ antagonists such as ondansetron were reported to inhibit morphine-induced grooming, locomotor activity, and sniffing (Pei et al. 1993) as well as to decrease EtOH-induced hyperlocomotor activity in mice (Kostowski et al. 1995). Those effects have been attributed to central actions on supraspinal structures given the specific distribution of 5-HT₃ expression in various areas of the brain well known to be associated with these behaviors (Tecott et al. 1993). However, in light of the present results using spinal-cord-transected animals, we provide clear evidence that some of the effects described in those earlier studies could have been mediated, at least partially, by direct actions of 5-HT₃ ligands on spinal cord neurons.

Direct actions of 5-HT₃ agonists in the spinal cord may include a postsynaptically mediated depolarization of target spinal neurons. Indeed, in the CNS, 5-HT₃ are known as high Ca²⁺-permeable ionophores that mediate rapid excitatory responses (in mouse hippocampus, Yakel and Jackson 1988; rat amygdala neurons, Sugita et al. 1992; for a review see Aghajanian et al. 1990). In rat striatal neurons (Ronde and Nichols 1998), their activation has been shown to lead to a significant rise in cytosolic and nuclear calcium. Such rise of Ca²⁺ has been reported also to trigger Ca²⁺-induced-Ca²⁺ release mechanisms associated with L-type channel-mediated Ca²⁺ entry in neuroblastoma × glioma cell lines (Ronde and Nichols 1997).

Yet a postsynaptic action mechanism does not exclude the possibility of presynaptic actions. For instance, 5-HT₃-induced excitation has been found in the striatum to produce dopamine release (Blandina et al. 1989). Because dopamine is known to participate in activation of the CPG in in vitro-isolated mouse spinal cord preparations (Jiang et al. 1999), 5-HT₃-induced release of dopamine intraspinally could possibly constitute a presynaptic mechanism participating in CPG activation [i.e., dopamine-containing spinal neurons reported in some species by Acerbo et al. (2003); Commissiong and Neff (1979); Scholand et al. (1996)]. Although 5-HT₁ agonists such as SR 57227A are considered highly selective for activation of 5-HT₁ (Bachy et al. 1993), the possibility of additional actions at the glycine binding sites by decreasing glycine inhibitory effects (Maksay 1998) or at the nicotinic acetylcholine receptor and the GABAₐ receptor (Reeves and Lummis 2002) cannot be excluded. However, arguing against this possibility, Maksay (1998) has also shown that central SR 57227A-induced effects are blocked by ondansetron, a highly selective 5-HT₃ antagonist with little effect, if any, on glycine-ergic binding sites.

Task- and ligand-dependent synergistic activation of CPG neurons

The results show also that SR 57227A generally induced a mixture of NLM and LM in the hindlimbs of paraplegic mice. Our decision to use several testing conditions has turned out being particularly useful because it allowed the detection of SR 57227A-induced task-dependent effects. Indeed, NLMs were found in all three conditions of testing, whereas LMs were found mostly when mice were examined on a running treadmill or if SR57227A administration was combined with 5-CT.

As mentioned in the preceding text, LMs induced by SR 57227A alone were found mainly on a running treadmill (see Fig. 1, C and D). This provides evidence that peripheral afferent inputs associated with the treadmill condition may have contributed to the production of LM. Given that the air-stepping and open-field tests are less likely to provide high levels of hindlimb afferent inputs compared with the treadmill test, we postulate that afferent inputs associated with the treadmill condition synergistically contributed to SR 57227A-induced LM. This is based on the fact that the treadmill test was the only condition where the entire front part of both hindlimbs including the dorsal paws and front legs of these paraplegic mice had to rub continuously against a moving belt (i.e., rubber treadmill belt running at 8–10 cm/s) before and, most often, even after drug administration. This may have led to some activation of cutaneous receptors and, perhaps, of proprioreceptors (e.g., muscle spindle “stretch” receptors) of the hindlimbs. Such level of treadmill-associated hindlimb afferent feedback activity was most likely not reached in the other conditions. In fact, in the air-stepping condition, the hindlimbs were completely suspended and pendent with no contact with the ground, whereas, in the open-field condition, some activation of cutaneous and stretch receptors was possible although most probably to a lesser extent (i.e., intensity, duration and amount) compared with the running treadmill condition. The idea that higher levels of hindlimb afferent inputs were found on the treadmill is supported also by results showing that some weak hindlimb movements were displayed sometimes even before drug administration only during the treadmill test (Fig. 1, A–C, control–treadmill).

Consequently, the occurrence of SR 57227A-induced LM particularly on a treadmill may be due to 5-HT₃ excitations and to intense hindlimb afferent inputs acting both synergistically on the spinal locomotor network. Indeed, the CPG is well known to be modulated, excited, and reset by various sources of hindlimb afferent inputs. This has been shown in decerebrate and paralyzed cats where stimulation of Ia- and Ib-proprioreceptor afferents or dorsal cutaneous afferents of the paw can activate and even reset the CPG during fictive locomotion (Guertin et al. 1995; Perreault et al. 1995). Our results also imply that 5-HT₃ agonists, used alone or in testing conditions where only weak afferent inputs are present (i.e., air stepping...
or open field), are not powerful enough to fully activate the CPG (i.e., production of LM—bilaterally coordinated and alternating movements), which would explain why only NLMs and not LMs were found in the air-stepping and open-field conditions.

The idea that CPG neurons can be activated by 5-HTR$_3$ agonists is further supported by data showing that combining SR 57227A with 5-CT, a 5-HTR$_{1A}$ agonist recently shown to partially activate the CPG in vitro (Madriaga et al. 2004), can further activate the CPG given that some LMs ended up being induced even in the air-stepping and open-field conditions. Combining several drugs for enhanced CPG activation is performed routinely in in vitro studies (in mice, Jiang et al. 1999) but is just beginning to receive attention in in vivo paraplegic models (Antri et al. 2005; Guertin 2004a,b; Landry and Guertin 2004). This said, it is worth noticing that our results are in clear contrast with results reported in similar animal preparations by Leblond et al. (2003). They have reported higher recovery levels characterized by steady left-right coordination and weight bearing stepping in complete paraplegic mice that have not even received pro-CPG activating drugs. Reasons for this are unclear. However, their results may have been affected by powerful additional stimuli such as tail pinching and daily testing sessions over several days/weeks (i.e., training experience-induced plasticity changes of subcortical neuronal circuits), which can induce and facilitate CPG-mediated activity (Strauss and Lev-Tov 2003) and functional recovery (de Leon et al. 1998). Supporting this, recent studies from our laboratory show that in absence of tail pinching, drug treatment and regular treadmill training sessions, complete paraplegic mice examined ≤1 mo posttranssection do not display spontaneously recovered hindlimb movements with steady left-right coordination or weight-bearing capabilities in open-field (Guertin 2005) or motor-driven treadmill conditions (unpublished data).

These observations may also explain why in vitro studies with 5-HTR$_3$ ligands have failed to produce any detectable spinal-cord-mediated motor or locomotor effects. Indeed, we provide evidence indicating that 5-HTR$_3$ agonists constitute only weak activators of the CPG unless combined with relatively intense hindlimb afferent inputs (e.g., running treadmill) and/or other subtypes of receptor agonists capable of CPG excitation (e.g., 5-HTR$_{1A}$ agonist). Therefore in absence of these synergistic factors, such as in most types of in vitro studies, one could argue that the sets of conditions that exist in most in vitro testing conditions are not ideal to detect spinal 5-HTR$_3$-mediated actions on motor and locomotor neurons.

Taken together, the results of this study suggest strongly that 5-HTR$_3$ agonist-induced effects were the result of excitatory actions on CPG neurons and hindlimb motoneurons. This is supported also anatomically given that some of the most densely 5-HTR$_3$-labeled CNS neurons have been found in the intermediate gray matter and ventral horn areas of the lumbar spinal cord (Morales et al. 1998) where both CPG neurons and hindlimb motoneurons are located, respectively (Kiehn and Butt 2003).

**Concluding remarks**

5-HTR$_3$ ligands are already used clinically as safe and potent drugs to reduce nausea and vomiting in patients undergoing cancer chemotherapy (Gridelli 2003). Only a few side effects such as moderate changes of body temperature, blood pressure, blood glucose, and heart rate have been reported (Carvalho et al. 2002; Mazzola-Pomietto et al. 1995; Sevoz-Couche et al. 2002; Wilson et al. 1990). However, given that increased 5-HTR$_3$ receptor density has been associated with allodynia in spinal rats, SR 57227A could also possibly exacerbate allodynia after SCI (Oatway et al. 2004). Nonetheless, the present results suggest that 5-HTR$_3$ agonists may constitute relatively safe drugs to moderately facilitate hindlimb movement generation and locomotor function recovery in SCI subjects.

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