Effects of Localized Intraspinal Injections of a Noradrenergic Blocker on Locomotion of High Decerebrate Cats

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Delivet-Mongrain H, Leblond H, Rossignol S. Effects of localized intraspinal injections of a noradrenergic blocker on locomotion of high decerebrate cats. J Neurophysiol 100: 907–921, 2008. First published June 11, 2008; doi:10.1152/jn.90454.2008. Previous studies demonstrated that neuronal networks located in midlumbar segments (L3–L4) are critical for the expression of locomotion in cats following complete spinalization. In the present study the importance of several thoracolumbar segments (T8–L7) for the generation of spontaneous hindlimb locomotion in decerebrate cats was evaluated. Experiments were performed in high decerebrate cats (n = 18) walking spontaneously. Yohimbine, an alpha2-noradrenergic antagonist, was microinjected intraspinally in various thoracolumbar segments. Locomotor performance was evaluated with kinematics and electromyographic (EMG) recordings before and after each injection. When and if spontaneous locomotion (SL) was abolished, skin or perineal stimuli (exteroceptive stimuli) were used to trigger locomotion (exteroceptive-induced locomotion [EL]). Yohimbine injections at L3 or L4 completely inhibited SL and EL. In contrast, injections at T8 did not interfere with SL or EL. Injections at T10, T11, T12, L5, L6, and L7 inhibited SL but EL could still be evoked. Injections at T13, L1, and L2 had similar effects except that the quality of locomotion evoked by exteroceptive stimulation declined. Combined injections at T13, L1, and L2 abolished SL and EL, in contrast to injections restricted to the same individual segments. Simultaneous injections at L5, L6, and L7 also abolished SL but EL could still be induced. These results suggest that noradrenergic mechanisms in L3–L4 segments are involved in the expression of locomotion in decerebrate cats, whereas antagonizing noradrenergic inputs in individual rostral or caudal segments may alter the expression and overall quality of the locomotor pattern without abolishing locomotion.

INTRODUCTION

Following a complete section of the spinal cord several species can recover hindlimb locomotion (Delcomyn 1980; Orlovsky et al. 1999; Rossignol 2000). The reexpression of locomotion depends on the presence of a spinal locomotor central pattern generator (CPG) (Grillner 1981; Rossignol 1996, 2006; Rossignol and Dubuc 1994). This propriospinal network that generates the pattern of hindlimb muscles activity is, under normal conditions, modulated by supraspinal inputs (Armstrong 1986) and peripheral feedback (Rossignol et al. 1988, 2006). The importance of this spinal CPG is highlighted by the fact that adult chronic spinal cats can walk with their hindlimbs on a treadmill some weeks after a complete spinalization at the last thoracic segment (T13) with intense treadmill training (Barbeau and Rossignol 1987; Rossignol et al. 2002, 2004). Hindlimb locomotion can also be evoked in adult cats, immediately after spinalization, with intraperitoneal, intravenous (iv), or intrathecal (i.t.) administration of clonidine, an alpha2-noradrenergic agonist (Barbeau and Rossignol 1994; Barbeau et al. 1987; Chau et al. 1998a,b; Forssberg and Grillner 1973; Rossignol 1996). Furthermore, a locomotor pattern can be evoked after administering 3,4-dihydroxy-L-phenylalanine (l-DOPA), a noradrenergic precursor (Grillner and Zangger 1979) and recorded on peripheral nerves in curarized cats spinalized at T13, clearly showing the existence of a spinal CPG at the lumbar level.

Although the existence of locomotor CPGs for the hindlimbs is well established in the cat, its localization within the thoracolumbar spinal cord is unclear. Experiments on adult cats suggest that L3–L5 segments play a leading role during fictive scratching, although the generation of rhythmic oscillations was distributed throughout the lumbosacral spinal cord (Dellagena et al. 1983). Such potential for segmental rhythmogenesis of a reduced number of lumbar segments was also shown using intravenous l-DOPA in fictive conditions (Grillner and Zangger 1979).

Pharmacological tools were used to study the segmental localization of circuits important for the generation of locomotion in the cat, by restricting the effects of noradrenergic agonists or antagonists to specific spinal segments. For example, intraspinal microinjections of the alpha2-noradrenergic antagonist yohimbine restricted to L3, L4, or L5 segments but not in L6 blocked locomotion induced by an iv injection of clonidine in spinal cats (Marcoux and Rossignol 2000). It was also shown, in the same cats, that lesioning L3 or L4 segments permanently abolished the expression of locomotion by intravenous clonidine. The importance of these midlumbar segments was confirmed later in chronically spinalized (T13) cats. Indeed, after these cats recovered spontaneous hindlimb locomotion, a second complete transection at L2 or rostral L3 did not block the expression of spinal locomotion but a second transection at caudal L3 or L4 completely and irreversibly abolished the capacity to trigger hindlimb locomotion even several weeks after the section (Langlet et al. 2005). Finally, recent work with intraspinal microstimulation (ISMS) also showed that, in spinal cats (T13), locomotion induced by electrical stimulation and clonidine depended on the integrity of the L3–L4 segments (Barthélyem et al. 2007). Indeed, microinjections of yohimbine in L3 or L4 segments or a...
complete second spinal lesion at L3–L4 abolished all locomotor activity evoked by ISMS applied at more caudal segments.

Altogether, these results indicate that the integrity of noradrenergic mechanisms at L3–L4 spinal segments is required for the expression of spinal locomotion in cats with a complete spinal section at T13. These midlumbar segments contain interneurons that are active during brain stem–evoked fictive locomotion (Shchekly et al. 1990) and, as such, should be considered as prehindlimb motoneuron segments since most of the hindlimb muscles are innervated by motoneurons located at L5–S1 segments, thus caudal to these midlumbar segments (Vanderhorst and Holstege 1997). Therefore it may be suggested that these premotoneuronal segments play a major role in hindlimb locomotion of spinal cats by driving motoneurons located in more caudal segments.

However, whether these segments also play such a crucial role in the expression of locomotion in high decerebrate walking cats, in which all forebrain structures are removed but descending pathways originating from brain stem motor areas are intact, is unclear. It is well established that high decerebrate cats (section at a precollicular–premammilar level) can walk spontaneously on a treadmill (Orlovsky 1969; Shik and Orlovsky 1976).

In the present work, we assessed the role of different thoracolumbar segments in the generation of spontaneous hindlimb locomotor movements on the treadmill in high decerebrate cats. To this end, yohimbine was injected intraspinaly to selectively block noradrenergic mechanisms at various thoracolumbar segments (T8–L7). Our results indicate that the L3–L4 segments play a key role in the expression of hindlimb locomotion in the decerebrate cat, as is the case for complete spinal cats, since their inactivation blocks spontaneous locomotion even with exteroceptive stimulation. Other segments (above and below) contribute to a lesser extent to the actual rhythmogenesis since, even though spontaneous locomotion can be blocked by their inactivation, locomotion can still be triggered by exteroceptive stimuli, although the quality of walking is degraded. Preliminary results were published in abstract form (Delivet-Mongrain et al. 2006; Leblond and Rossignol 2003).

METHODS

General protocol

For this study, 18 adult cats of either sex (3.0–7.3 kg) were used to perform acute experiments in which yohimbine, an alpha2-nodrenergic antagonist, was injected intraspinally in individual segments of the spinal cord from T8 to L7 or, in some cases, in multiple segments simultaneously. Electromyographic (EMG) recordings and kinematic analyses were used to quantify various aspects of hindlimb locomotion before and after each injection.

Surgery

All surgical procedures were performed in aseptic conditions and approved by the Comité de Déontologie pour l’Expérimentation Animale from Université de Montréal. Before each surgery, cats were preanesthetized using acepromazine maleate (Atravet, 0.001 mg/kg), glycopyrrolate (0.01 mg/kg), and ketamine (10 mg/kg) administered intramuscularly. Thereafter, 2% isoflurane-95% O2 was initially administered through a mask and then, after a tracheotomy, through an endotracheal tube. End-tidal pCO2 was continuously monitored (Datex Normocap 200) and maintained between 3.5 and 4.5% by adjusting the frequency and volume of a respiratory pump when needed. A feedback-controlled heating pad, wrapped around the thorax, was servo-controlled using a rectal thermometer to maintain body temperature at around 38°C. A cannula was inserted in the urethra and maintained throughout the experiment to drain urine. The left carotid artery was cannulated to monitor blood pressure and the right carotid artery was ligatured just prior to decerebration. The right jugular vein was also cannulated for administration of fluids. A laminectomy was performed at two or three selected thoracic or lumbar vertebrae (T8–L7). The animal was then placed in a spinal-fixation unit mounted over a motor-driven treadmill belt and was stabilized with pairs of lateral pins inserted in the iliac fossae as well as on the vertebral bodies of L2 and L4 (Fig. 1). The vertebral fixation served to restrain movements of the vertebral canal and the spinal cord to ensure the accuracy of intraspinal injections, especially when repeated injections at the same site were needed. The head of the cat was fixed in a stereotaxic frame with ear bars and a mouthpiece. After craniotomy, a careful decerebration was performed using cauteryization of cortical vessels and aspiration of all nervous tissue rostral to the precollicular–premammilar level.

FIG. 1. Experimental setup. After a precollicular–premammilar decerebration, the cat is fixed with a rigid frame over the treadmill with 2 pairs of lateral vertebral pins and one set of pins inserted in the iliac fossae. Reflective markers were placed on the skin over various joints of the left hindlimb to allow the reconstruction of movements according to the kinematics model indicated. The angular orientation was chosen so that the excursion of all joints would be represented by a decrease in angular values in the first part of swing. Electromyogram (EMG) wires were inserted percutaneously. A Hamilton microsyringe was fixed on a stereotaxic manipulator over the spinal cord to inject yohimbine in the targeted spinal segments indicated in blue.
Evaluation of locomotor capabilities

The capacity of cats to walk with the hindlimbs was tested before drug injections. Usually, within the first 150 min after decerebration, cats walked spontaneously on the motor-driven treadmill belt without any drugs and, most of the time, without exteroceptive stimulation (except that provided by the treadmill belt). Such exteroceptive stimuli consisted in touching or pinching the skin areas around the ears (pinna stimulation), neck, thorax, shoulder, back, abdomen, or perineal region. These stimulations were used in two circumstances. First, when the cat did not walk spontaneously, these stimuli were used to trigger locomotion to ensure that locomotor movements could still be triggered. Second, these stimuli were also used when the cat walked spontaneously, to assess their effects on locomotion, as a baseline value, with the aim of comparing locomotor sequences before and after injection with the same stimuli. The locomotion triggered by exteroceptive stimulation was termed “EL”, for exteroceptive locomotion. Only 2 of the 18 cats (cat M and cat P) never walked spontaneously before the injections. However, because the general excitability of the cats generally diminished several hours after decerebration, 23 of the 62 drug injections were performed on cats walking while using exteroceptive stimulation (see Table 1). These trials were performed to confirm previous observations on the effects of yohimbine in the same cat. After each yohimbine injection, sequences of spontaneous locomotion (SL), when present, and exteroceptive-induced locomotion (EL), when present, were then reevaluated.

Evaluation of the locomotor capabilities of the animal were tested throughout the post decerebration period and injections were stopped when some significant deterioration occurred such as a major decrease in cardiac or respiratory rhythms, blood pressure, pCO₂, or responsiveness to exteroceptive stimulations.

Intraspinal microinjection protocol

Before injecting yohimbine, the dura was cut over the spinal cord at the desired segmental levels and a bath of warm paraffin oil was made to prevent desiccation. The spinal cord was first punctured at the desired segmental levels and a bath of warm paraffin oil was separated by about 2 mm paramedially in one of the selected segments (T8–L7). Clonidine (10 mg/ml), an alpha2-noradrenergic agonist, was microinjected intraspinally (2.5 μl/injection, four injections/segment bilaterally) to ensure that the observed effects following yohimbine injections were not due to mechanical damage of the cord. In some experiments, clonidine (10 mg/ml), an alpha2-noradrenergic agonist, was microinjected intraspinally (2.5 μl/injection, four injections/segment bilaterally) to reverse the effects of yohimbine and ensure that the observed effects of yohimbine administration were due to the inactivation of noradrenergic receptors on cellular elements of the selected segment (Marcoux and Rossignol 2000). Figure 2 illustrates the sequence and the time (in minutes postdecerebration) of yohimbine (individual or simultaneously), clonidine, or sham injections in each cat. On average, injections were separated by about 93 ± 52 min.

Records and analyses

Locomotor capabilities of the cats were evaluated at a speed of 0.3 m/s with EMG recordings synchronized to video recordings. Reflective markers were placed on the skin of the left hindlimb at the level of the iliac crest, the femoral head, the knee joint, the lateral malleolus, the metatarsophalangeal (MTP) joint, and the tip of the third toe (Fig. 1) for kinematic measurements and movement reconstruction. Walking sequences were captured by a digital camera system.

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**TABLE 1. Effects of yohimbine injections at different spinal cord segments tested for each cat**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Trials</th>
<th>Cat</th>
<th>Preyohimbine</th>
<th>Postyohimbine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SL/Trials</td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>4</td>
<td>G, H</td>
<td>4/4</td>
<td></td>
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<td>2</td>
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<tr>
<td>T12</td>
<td>4</td>
<td>H, L</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
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<td>6</td>
<td>D, E, H, L</td>
<td>3/6</td>
<td>3/6</td>
</tr>
<tr>
<td>L1</td>
<td>4</td>
<td>I, K, L</td>
<td>3/4</td>
<td>3/4</td>
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<tr>
<td>L2</td>
<td>6</td>
<td>J, K</td>
<td>3/6</td>
<td>6/6</td>
</tr>
<tr>
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<td>8</td>
<td>A, B, C, D, E</td>
<td>3/8</td>
<td></td>
</tr>
<tr>
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<td>4</td>
<td>G, N</td>
<td>4/4</td>
<td></td>
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<td>4</td>
<td>G, N</td>
<td>3/4</td>
<td>1/4</td>
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<td>G, N</td>
<td>3/3</td>
<td>2/3</td>
</tr>
<tr>
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<td>P, Q, R</td>
<td>3/6</td>
<td>1/6</td>
</tr>
<tr>
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<td>4</td>
<td>S, T</td>
<td>3/4</td>
<td>1/4</td>
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</tbody>
</table>

For each segment tested (Segment) the number of injections performed (Trials) on each cat (Cat) and the number of trials in which spontaneous locomotion (SL) was observed before injection (SL/Trials) are indicated. Spontaneous locomotion was not always observed before each trial. In those cases, the effect of yohimbine injections was evaluated on the exteroceptive-induced locomotion (EL). Four different effects were observed: no effect (No Effect); spontaneous locomotion was blocked but exteroceptive stimulation could reinstate a similar pattern (Block Only SL); spontaneous locomotion is blocked and exteroceptive-induced locomotion (EL) showed deficits (Block SL Deficit EL); locomotion is completely abolished (Block SL and EL). For each category of effects, the number of trials where it was observed and the number of trials where recovery of this particular effect was observed are indicated (Recovery).
(NTSC Panasonic WV-CL920) with a shutter speed of 1/1,000 s at 30 frames/s and recorded on DVD (Sony RDR-GX315). Frames were then deinterlaced to obtain 60 fields/s and a temporal resolution of 16.6 ms between fields. Custom-made software was used to detect the reflective markers. The X and Y coordinates served to reconstruct hindlimb locomotor movements and calculate the time course of angular excursion of the various joints (hip, knee, ankle, and MTP). These measurements were synchronized with visually detected step cycle events such as paw contact (left contact, right contact) or paw lift (left lift, right lift). Three flexor muscles were acutely implanted (semitendinosus [St], sartorius anterior [Srt], and tibialis anterior [TA]) and two extensor muscles (vastus lateralis [VL] and gastrocnemius lateralis [GL]) of both hindlimbs. The muscles were implanted with enamel-insulated copper wires (32AWG) and the signals were amplified by AC-coupled amplifiers (Neuralynx lynx-8) (bandwidth of 300 Hz to 10 kHz) digitized (data acquisition board: National Instruments PCI-6071E) at 1 kHz using custom-made acquisition software and recorded on-line on a computer. EMG activity was synchronized to the recorded video by a digital Society for Motion Picture and Television Engineers time-code generator/reader.

A complete step cycle consists of stance and swing phases. The stance phase starts when the foot strikes the treadmill belt and terminates when the foot starts its forward movement. The swing phase begins at this point and terminates as soon as the foot contacts the treadmill belt. Activity was considered to be locomotor only when there was an alternation between flexor and extensor muscles of the same hindlimb and an out-of-phase alternation between homologous muscles of both hindlimbs for a minimum of five consecutive step cycles.

Many parameters were measured to evaluate the locomotor characteristics of a given walking sequence. Step cycle duration was defined as the time elapsed between successive contacts of the same foot and step length as the distance traveled between these two events. The values of interlimb coupling correspond to the phase value of the onset of the foot contact relative to the onset of the foot contact of the other limb. The peak-to-peak angle amplitude for each joint was obtained by subtracting the mean values of the minimum and maximum joint angles of the excursion for each step cycle in a given walking sequence. Custom-made interactive software was used to determine the onset and offset of EMG bursts in each of the ten implanted muscles and to measure burst duration, amplitude, and phase. To represent the mean EMG activity of different episodes of locomotion, the EMG signals of each step cycle, synchronized on the contact of the left foot, were extracted and normalized to 1,024 bins and then averaged. Differences between these different measurements were compared using a multiple-way ANOVA (Tukey’s post hoc test) and were considered to be significant if the probability of an α-type error was ≤0.05.

RESULTS

The following describes the effects of microinjecting yohimbine in different segments of the spinal cord on the locomotion of decerebrate cats and groups the results according to different segmental sections: L3–L4, T8–L2, and L5–L7. Table 1 summarizes these effects and indicates the number of times yohimbine was injected (trials) in each segment as well as the number of experimental cats, which are identified by a capital letter.

L3–L4 segments

Injections at L3 were performed eight times on five different cats (A, B, C, D, and E; see Table 1). In all cases, spontaneous locomotion (SL) and exteroceptive-induced locomotion (EL) were completely blocked 5 min after yohimbine injections. The EL was reinstated within an average of 75 ± 20 min (60–130 min) and, when recovered, SL was observed within an average of 101 ± 16 min (85–115 min) after the injection. Figure 3 illustrates such an injection at L3 in cat C. This cat walked spontaneously on the treadmill 110 min after decerebration. Before injecting yohimbine the needle was inserted in four sites bilaterally at rostral and caudal L3 to ensure that mechanically puncturing the spinal cord did not prevent locomotion.
Yohimbine at L3

Locomotion was unaffected as illustrated in the stick diagrams (Fig. 3A), the averaged angular excursions (Fig. 3B), and the rectified averaged EMGs (Fig. 3C) of selected muscles of both hindlimbs during consecutive step cycles (n = 22). Yohimbine was then injected at L3 in the same four above-mentioned sites. Approximately 5 min after injecting yohimbine, SL and EL were abolished (Fig. 3, D–F) and even strong exteroceptive stimulation could not induce locomotion. About 30 min postinjection, some faint rhythmical movements (e.g., erratic alternating bursts between flexor and extensor) were observed with strong skin stimulation but there was no SL (not shown). At 70 min postinjection EL was observed but became more robust at 80 min (Fig. 3, G–I). As seen in the stick figures (Fig. 3G), joint angular excursions (Fig. 3H), and EMG bursts (Fig. 3I), the locomotor pattern was similar to pre-yohimbine even though there were still slight differences such as reduced excursion of the MTP joint. A full recovery of SL was observed 115 min postinjection (Fig. 3, J–L) with a different but consistent EMG pattern. In all trials locomotion recovered except for two trials (last trial for cats A and B; see Fig. 2) where no recovery was observed after >140 min. Note that cat B had previously recovered a proper SL after a first trial.

The effect of yohimbine on locomotion was also tested at L4 for a total of four trials in two different cats (cats C and G; see Table 1). Similarly to L3, yohimbine injections completely blocked locomotion in all cases. EL was reinstated in both cats after 35 and 50 min in cats G and C, respectively. A full SL recovery was observed 85 min postinjection in cat G. To ascertain the effects of yohimbine, a second series of injections were performed in cat C after the recovery of EL (e.g., before the recovery of SL). Again yohimbine blocked locomotion but, in this case, a recovery of locomotion was not observed because of the general deterioration of the preparation late in the experiment.

FIG. 3. Effects of yohimbine (4 × 2.5 μl solution 8 mg/ml) at L3 in cat C at 5, 80, and 115 min. In the top row (A, D, G, J), the stick diagrams illustrate the swing and stance phases separately for one representative step cycle in each condition. In the middle row (B, E, H, K), the averaged joint angular displacement (mean ± SD over n cycles) of the left hip, knee, ankle, and metatarsophalangeal (MTP) joints synchronized to contact of the left foot and repeated twice for the sake of clarity. In the bottom row (C, F, I, L) are represented the rectified, normalized, and averaged EMGs of the hindlimb muscles also synchronized on contact of the left foot for the same number of cycles. A–C: spontaneous locomotion (SL) at 0.3 m/s before drug injection (22 step cycles). D–F: locomotion is abolished 5 min after yohimbine injection. G–I: recovery of exteroceptive-induced locomotion (EL) 80 min postyohimbine (17 step cycles). J–L: recovery of spontaneous locomotion 115 min postyohimbine (26 step cycles). L, left; R, right; St, semitendinosus; Srt, sartorius; VL, vastus lateralis; GL, gastrocnemius lateralis.

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In one cat, yohimbine was injected at L4 on the right side only (four injections of 2.5 μl of yohimbine, 8 mg/ml). Five minutes after the injection, SL was observed exclusively in the left hindlimb (e.g., contralateral to the injections) but perineal stimulation induced bilateral walking. During this EL if the right hindlimb was mechanically fixed in a vertical position the left hindlimb could still walk; however, if the left leg was restrained, the right leg could not make any locomotor movement.

Thus injecting yohimbine at L3–L4 completely abolished locomotion (SL and EL) for a period of 30–60 min. Thereafter, strong exteroceptive stimulation could trigger locomotion and SL eventually returned.

Segments T8–L2

**INDIVIDUAL T8–T12 SEGMENTS.** Effects of intraspinal injections of yohimbine on locomotion were studied in segments rostral to L3–L4: T8 (n = 4 trials in two cats), T10 (n = 2 in two cats), T11 (n = 3 in one cat), T12 (n = 4 in two cats), and T13 (n = 6 in four cats), L1 (n = 4 in three cats), and L2 (n = 6 in two cats).

Injections at T8 were tested four times on two cats walking spontaneously (cats G and H; see Table 1). In all cases injections at T8 had no effect on SL, as illustrated for cat H in Fig. 4. Indeed, the stick figures of representative step cycles of SL before (Fig. 4A) and 5 min after (Fig. 4D) yohimbine are similar, with good foot placement in front of or under the hip.

The angular excursions before (Fig. 4B) and after yohimbine (Fig. 4E) were also similar. The small SD reflects the stability of walking during these consecutive step cycles. Selected EMG bursts before (Fig. 4C) and after yohimbine (Fig. 4F) were also similar. The SL remained unaffected over time following injections at T8 (not shown).

Although there were differences between the effects produced by injections at individual segments from T8 to T12 (as shown in Table 1 for three cats), they were similar enough to justify the regrouping of the data obtained for these segments. First of all, yohimbine at T10, T11, and T12 blocked only SL but not EL. Indeed, exteroceptive stimulation could reinstate a locomotor pattern similar to that generated spontaneously before the injection. Cat M never walked spontaneously, contrary to cats H and L. In each case, SL, when present, was blocked by yohimbine injections but exteroceptive stimuli remained effective. The SL recovered 10 min postyohimbine at T10 and 18 min postyohimbine at T12 in cat H and 14, 170, and 45 min postyohimbine at T12 for the three trials on cat L. Locomotion of cat H before yohimbine injection at T10 is illustrated in Fig. 5, A–C. SL was completely blocked from 5 to 10 min postinjection. However, during this time interval, EL similar to that obtained before injections could be induced (Fig. 5, D–F), but with slight differences, such as the angular excursions of the left knee joint, which ranged from 119.7 ± 1.4 to 151.2 ± 3.4° preinjection compared with 123.3 ± 2.1 to 135.9 ± 2.2° postinjection (P < 0.05) (Fig. 5, B–E). However, yohimbine injections at T10 did not affect the locomotor capabilities of the cat during EL in terms of consecutive step cycles (>20 in both case), step cycle length (preyohimbine: 32.75 ± 2.23 cm; postyohimbine: 31.16 ± 1.80 cm; P = 0.059) of the left leg, and the timing between right and left foot contact (preyohimbine: 0.48 ± 0.036; postyohimbine: 0.474 ± 0.026; P = 0.238). The SL recovered 10 min postinjections.

Thus although yohimbine at T10, T11, or T12 blocked SL for a minimum of 10 min a proper EL could be induced during that time.

**INDIVIDUAL T13–L2 SEGMENTS.** Yohimbine injections at T13, L1, or L2 also abolished SL but, contrary to what was observed at T10, T11, or T12, EL was significantly altered or exceptionally blocked. Microinjection of yohimbine at T13 was tested six times on four cats (cats D, E, H, and L) that all walked spontaneously except for cat D that needed exteroceptive stimulation. Cat E will be discussed separately. In cats H and L yohimbine injection blocked SL in all cases for 40 and 55 min, respectively. EL was altered in cats D, H, and L and blocked in one trial in cat D. Figure 6 illustrates this effect in cat H, the same cat illustrated in Figs. 4 and 5 for the effect of yohimbine injection at T8 and T10 (see Fig. 2). The SL observed (Fig. 6, A–C) was completely blocked 5 min postinjections at T13. Exteroceptive stimulation could produce some alternating rhythmical movements in the hindlimbs 5 min postyohimbine (Fig. 6, D–F). These faint movements with no foot placement can be seen in the small amplitude of the stick diagram of the stance and swing phases of a typical step cycle (Fig. 6D) and in the angular excursions of the joints (Fig. 6E). Nevertheless, some rhythmical movements are apparent in the averaged EMGs, especially between bursts in GL and TA in both hindlimbs (Fig. 6F). These small movements were also

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**FIG. 4.** Effects of yohimbine injected intraspinally at T8 in cat H. Same display as Fig. 3. A–C: SL before any drug injection (20 step cycles). D–F: SL 5 min after yohimbine (17 step cycles).
observed in cat D after yohimbine injection. However, the deficits observed in cat L after the injections were different. The EL was robust and sustained, although the cycle period deficits observed in cat L after the injections were different. However, the cycle period deficits observed in cat D after yohimbine injection. However, the cycle period deficits observed in cat L after the injections were different.

Figure 7 illustrates some of these deficits observed during locomotion from 5 to 75 min postyohimbine injections at L1 in cat L. For instance, the timing between stance of the right and left hindlimbs was irregular, as shown by the duty cycles (Fig. 7C). During those consecutive steps, excursions at the hip and knee were also variable (Fig. 7D). In addition, the feet rarely made contact in front of the hip (Fig. 7G). Other EMG (amplitude and duration) and kinematic (percentage of the swing in the step cycle) parameters of locomotion were measured and varied in often different manners after yohimbine injection, so no significant trends were found. Overall, the effects of yohimbine restricted to T13, L1, or L2 segments blocked SL and strongly altered EL.

In summary, yohimbine injection at segments T8–L2 had different effects on the locomotion of decerebrate cats. There was no effect when injected at T8. However, injections at T10 to T12 blocked SL but EL continued relatively unaltered. Finally, when injected in single segments from T13 to L2, yohimbine blocked SL and strongly altered EL.

SIMULTANEOUS INJECTIONS AT T13/L1/L2. Injections were done in succession (<5 min from each other) in segments T13, L1, and L2 to determine whether simultaneously inactivating these adjacent segments had a cumulative effect. Locomotion was assessed immediately after injections at each segment before targeting the next one. Successive injections at T13, L1, and L2 segments, in different orders, were tested six times on three different cats (cats P, Q, and R) with two trials per cat (see Table 1). Locomotion was completely abolished in all trials except in one case (cat R) where locomotion could still be evoked with very strong exteroceptive stimulation, albeit with important deficit. The recovery of a proper locomotion as seen before the injection was observed 80.0 ± 17.3 min (70–100 min) postinjections except for two trials (last trials of cats P and R) in which there was a deterioration of the preparation.

Figure 8 illustrates the effect of yohimbine injections in close succession at T13, L1, and L2 in cat Q that walked with exteroceptive stimulation before yohimbine injections (Fig. 8, A and B). Five minutes after the injections, locomotion ceased and rhythmic activity in all muscles was completely abolished (Fig. 8, C and D) despite exteroceptive stimulation. The EL recovered 60 min after the final injections and SL, which was not observed before yohimbine injections, was expressed 70 min after the end of injections (Fig. 8, E and F). Fifty minutes later, a second series of injections with the order L2, L1, and T13 was performed (not shown). Both SL and EL were immediately abolished but no recovery was observed. To test whether this was due to an inability to generate locomotion, clonidine was injected at T13, L1, and L2 200 min after the end of the final series of yohimbine injections. EL recovered 10 min after clonidine injections and the walking pattern was
more robust for the right hindlimb. However, at this point, the vital signs of the animal declined.

In summary, injecting yohimbine in close succession at T13, L1, and L2 completely abolished locomotion, contrary to individual targeting of these same segments. It is important to recall that injections of physiological saline solution at T13, L1, and L2 were simultaneously performed on a spontaneously walking cat (cat R) and no effect on either SL or EL was observed.

**Segments L5–L7**

Intraspinal injections of yohimbine were also tested in segments caudal to L3–L4: L5 (n = 4 trials in two cats), L6 (n = 4 in two cats), and L7 (n = 3 in two cats), as shown in Table 1.

After yohimbine injections at L5, L6, or L7 performed on cats G and N, SL was generally blocked but EL remains. For cat G, yohimbine injection at L5, L6, or L7 blocked systematically SL for a minimum of 15 min. During this time, EL was always observed but sometimes with some differences. Note that cat G was the same cat used to test yohimbine injection at T8 where locomotion was unaffected and yohimbine injection at L4 where locomotion was completely abolished (see Fig. 2). For cat N, after yohimbine injection at L5, L6, or L7, SL and EL were always observed. Although the locomotion was robust and sustained for prolonged periods, foot drag and reductions of the step cycle length and duration were observed for the left hindlimb. This alteration of the left hindlimb pattern cannot be conclusively attributed to yohimbine administration because no full recovery was observed. Thus yohimbine injections at L5, L6, or L7 abolished SL but did not block EL.

**SIMULTANEOUS INJECTIONS AT L5/L6/L7.** Three series of injections at L5, L6, and L7 were also performed in close succession four times in cats S and once in cat T (see Fig. 2). In all cases SL was blocked but EL was always observed, even when the quantity of yohimbine was doubled (i.e., 8 × 2.5 μl, 10 mg/ml injected bilaterally per segments) for the last trial in cat S. Figure 9 illustrates SL of cat S 5 min after injecting yohimbine at L5, L6, and L7 (Fig. 9A) and EL without locomotor deficits 5 min after injecting yohimbine at L5, L6, and L7 (Fig. 9B). SL recovered 40 to 100 min and 17 min postinjections for cats S and T, respectively.
Thus contrary to what was observed after simultaneously targeting T13, L1, and L2, yohimbine injections at L5, L6, and L7 in close succession did not abolish locomotion.

DISCUSSION

The aim of the present study was to assess the importance of noradrenergic mechanisms of spinal thoracolumbar segments in the generation and modulation of hindlimb treadmill locomotion in high decerebrate cats. For this purpose we injected yohimbine, an alpha2-noradrenergic receptor antagonist, directly into different spinal segments and assessed the locomotor ability of the cats. Injecting yohimbine in individual L3 or L4 segments completely abolished the ability of decerebrate cats to generate spontaneous locomotion (SL) and even exteroceptive stimuli could not induce locomotion (EL), suggesting that noradrenergic mechanisms in these segments are important for locomotor rhythogenesis of decerebrate cats. Inactivating the noradrenergic drive of other individual segments above or below L3–L4 produced variable effects. For instance, injections at T8 had no effect on SL or EL, whereas injections at T0–T12 and L5–L7 blocked SL but exteroceptive stimuli could induce a proper EL. Injections in individual T13–L2 segments blocked SL but EL could always be evoked, albeit with some deficits. Simultaneous injections from T13 to L2 also abolished SL and EL but combined injections at L5–L7 abolished SL but not EL. Overall our results indicate the importance of the alpha2-noradrenergic receptors at L3–L4 segments for the expression of locomotion in the decerebrate cat, whereas noradrenergic inputs to other individual segments contribute to a variable extent to expression of the locomotor pattern.

Methodological considerations

Because this work is based on the effect of intraspinal yohimbine injection in different segments of the spinal cord, it is important to justify why we think the results are solely attributable to the drug administration and not to unspecified

FIG. 7. Examples of locomotor deficits observed following intraspinally injected yohimbine at L1 in cat L. A and B: SL before drug injection (9 step cycles). C and D: EL 5 min after yohimbine (8 step cycles) (same results were observed from 5 to 75 min). E and F: recovery of SL 90 min after yohimbine (8 step cycles). A, C, and E: duty cycle at 0.3 m/s. Black bars represent the stance phase of each step cycle. B, D, and F: raw joint angular excursion of the hip, the knee, and the ankle of the left hindlimb for the same step cycles as A, C, and E. G: bar diagram of the position of the toe relative to the hip (vertical line at 0; see inset) at the foot contact and at toe off, in the 3 situations.

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effects of the injections themselves. First, inserting the needle or injecting physiological saline solution intraspinally did not affect the locomotor abilities of the cats on the treadmill. Second, injecting yohimbine at different individual segments in the same cat did not modulate the locomotor expression in the same manner. For example, in cat H yohimbine injection at T13 dramatically altered the locomotor pattern, whereas it had no effect at T8. Third, when injections perturbed or abolished locomotion, the cat could spontaneously recover in most cases a locomotor pattern similar to that obtained before the injections. Fourth, when it was tried, the effect of yohimbine could be reversed by the intraspinal injection at the same site of clonidine, a specific agonist of the alpha2-noradrenergic receptors.

Intraspinal administration of the drug was made to minimize diffusion of the drug into adjacent segments, as would be the case with a bath application. When assessed with a Fast Green dye, such intraspinal injections diffused about 1 mm from the injection site (Marcoux and Rossignol 2000). In cat G, in which bilateral injections of yohimbine at L4 completely abolished locomotion, a subsequent unilateral injection of yohimbine at this same segment (L4) affected only the ipsilateral hindlimb locomotion, indicating that the drug did not diffuse significantly to the contralateral side. In addition, targeting the L5 segment did not block the expression of locomotion, suggesting that the drug did not diffuse to the L4 segment, which would have blocked locomotion.

Consequently, if yohimbine altered or blocked hindlimb locomotion when applied to a restricted segment of the spinal cord, this segment must therefore contain noradrenergic elements important for the generation and/or modulation of the hindlimb locomotor pattern in decerebrate cats. On the other hand, the lack of effect of yohimbine injections in a specific segment does not necessarily mean that this segment is not involved in the control of hindlimb locomotion. Its contribution could implicate other neurotransmitter systems such as serotoninergic or glutamatergic.

In the present study, when SL was reduced or abolished, EL was used to determine whether the spinal network could still generate hindlimb locomotion. For instance, after injecting yohimbine at T10 (Fig. 5) SL was blocked, but EL could be evoked. Although yohimbine injection decreased the excitability of the spinal network and thus SL, the locomotor circuitry could still be activated with exteroceptive stimulation. This differs from injections at L3 or L4 in which case it was impossible to generate hindlimb locomotion even with strong exteroceptive stimulation.

Noradrenergic mechanism

To understand the organization and the mechanism of the central pattern generator (CPG) of locomotion many studies explored different neurotransmitter systems implicated in its modulation in different species (Grillner 1981; Rossignol 1996, 2006; Rossignol and Dubuc 1994). The noradrenergic system is known to influence somatosensory, autonomic, and motor functions. The main source of noradrenaline (NA) to the spinal cord is from cell groups located in the locus ceruleus and subceruleus, the medial and parabrachial nuclei, and the Kolliker–Fuse nucleus (Dahlstrom and Fuxe 1964). Within the lumbar spinal cord, alpha2-noradrenergic receptors are distributed at all levels, with the highest concentration in lamina II and around the central canal (lamina X), and a moderate density in lamina III and IX (Giroux et al. 1999). In intact cat, the segmental distribution of alpha2-noradrenergic receptors is unclear for the thoracic segments but was studied in lumbosacral spinal segments (L1–S3) and their density was higher in S2 and S3 segments (Rossignol et al. 2002). However, preliminary unpublished data in spinal cats indicate that 30 days following spinalization, alpha2-noradrenergic receptors are up-regulated in segments L3–L5 (lamina II, III, V, and X) and down-regulated from L6 or L7 to S3 segments (lamina II, III, V, VI, VII, and X), suggesting the importance of these receptors in midlumbar segments in the expression of spinal locomotion (Chau et al. 2000). In intact rats, alpha2-noradrenergic receptors were correlated with the distribution of NA terminals and were found at all rostrocaudal levels of the spinal cord throughout the whole gray matter with a preferential location in the superficial dorsal horn (Roudet et al. 1994).

The noradrenergic system was found to activate locomotor activity in cats with complete spinal lesion, as mentioned earlier in the INTRODUCTION and summarized elsewhere (Rossignol et al. 1996). In the present study we showed that the noradrenergic system is important for the expression of locomotion in decerebrate cat, even though other neurotransmitter systems are also most likely involved. On the other hand, it was shown that depletion of brain stem and spinal cord noradrenaline (maximal depletions of NA ≈14% of control in lumbar cord) by administrating 6-hydroxydopamine and alpha-methyltyrosine did not prevent coordinated locomotion evoked by stimulation of the mesencephalic locomotor region (MLR) in mesencephalic cats (Steeves et al. 1980). This could mean that NA release in the lumbar spinal cord may still have been sufficient or that another neurotransmitter system could trigger locomotion in the absence of noradrenergic inputs, with an effective stimulation. Our present results in decerebrate cats are in concordance with the role that the noradrenergic system may play even in locomotion of intact cats since i.t. injections of yohimbine at midlumbar segments caused major walking deficits such as asymmetric stepping, stumbling, and poor lateral stability during voluntary quadrupedal treadmill walking (Giroux et al. 2001). Similar results were found in intact rats, where i.t. administration of yohimbine in segments T12 to L5 produced dose-dependent impairments of hindlimb locomotion, which varied from a transient trunk instability (50 μg/20 μl) to transient hindlimb paralysis (200 μg/20 μl) (Majczynski et al. 2006).

In the present study, yohimbine could have interacted with sensory and/or supraspinal inputs by acting on interneurons

FIG. 9. Effects of yohimbine intraspinally injected in close succession at L5/L6/L7 in cat S. Same display as Fig. 8. A and B: SL before drug injection (16 step cycles). C and D: EL 5 min after yohimbine (12 step cycles).
implicated in the integration of inputs necessary for the expression of locomotion. Activity of both Ia and Ib inhibitory interneurons is depressed by iontophoretic administration of clonidine in midlumbar segments (L3–L5) in adult anesthetized cats (Hammar and Jankowska 2003). Furthermore, the activation of commissural interneurons that coordinate left–right muscle activity by group II afferents is depressed by NA (Hammar et al. 2004). It has also been suggested that the activation of these same commissural interneurons by reticulospinal fibers was depressed by clonidine (Hammar et al. 2007). In addition, it has been shown that clonidine decreases the excitability of motoneurons (Bedard et al. 1987). However, our work demonstrates that injecting yohimbine at L5, L6, and L7 segments individually or simultaneously did not prevent locomotion.

Localization

Based on in vitro preparations of the neonatal rat spinal cord, two different models of rostrocaudal distribution of the locomotor network have been proposed. The first model suggests that the locomotor-generating network is distributed over the caudal thoracic segments and the entire lumbar cord with a higher excitability of the most rostral segments (Cowley and Schmidt 1997; Kjaerulff and Kiehn 1996). The second model suggests a more restricted localization of the locomotor circuitry in rostral lumbar segments (L1–L2), which would project to and drive flexor and extensor motoneurons located in more caudal segments (Cazalets et al. 1995). In the present work we have evidence for the crucial role played by restricted segments L3–L4 but also for a more distributed input from other segments feeding into these L3–L4 segments.

In the cat, the evidence suggesting the importance of L3 and L4 segments for the generation of spinal locomotion is as follows: 1) i.t. injections of clonidine in chronic spinal cats is very effective in evoking locomotion, even when the site of the cannula is at L3–L4 (Chau et al. 1998a,b); 2) intraspinal clonidine and yohimbine at L3–L4 in spinal cats can trigger or abolish locomotion (Marcoux and Rossignol 2000); 3) lesioning L3–L4 in acute or chronic spinal cats (Langlet et al. 2005; Marcoux and Rossignol 2000) abolishes the ability of spinal cats to walk; 4) locomotion induced by intraspinal microstimulation is abolished by intraspinal yohimbine injections at L3–L4 or by lesioning these segments (Barthélem et al. 2007).

The present study further proposes that these premotoneuronal L3–L4 spinal segments are important as well for the expression of locomotion in decerebrate cats. However, some experiments in decerebrate cats suggested that the rhythm generator capacity is distributed along the lumbosacral spinal cord, although with L3–L5 as the leading segments. Decerebrate curarized cats were used to show that fictive scratch reflex could still be evoked after the gray matter of L3–L4 segments was destroyed (Delagina et al. 1983). Nevertheless, these midlumbar segments contain interneurons receiving inputs from group II afferents (Edgley and Jankowska 1987a) and having direct excitatory or inhibitory action on hindlimb motoneurons (Edgley and Jankowska 1987b) that must be involved in locomotion of the cat (Edgley et al. 1988) since they are active during MLR-evoked fictive locomotion in decerebrate cats (Shefchyk et al. 1990). Also, interneurons receiving inputs from reticulo- and/or vestibulospinal neurons that influence the activity of the contralateral hindlimb muscles were found in L3–L5 segments (Krutki et al. 2003).

The key role of premotoneuronal segments in the expression of the locomotion is also observed in rats. As is the case for L3–L4 in cats, the L1–L2 segments in rats, which correspond to spinal regions above the main hindlimbs motoneuronal pools, are largely responsible for generating the rhythmic alternating pattern of locomotion, which has given rise to a localized rhythm-generating network model. Indeed, recordings of lumbar ventral roots of the isolated rat neonatal spinal cord have shown that bath application of a mixture of serotonin (5-HT) and N-methyl-d-aspartate (NMDA) restricted to L1–L2 induced rhythmic locomotor-like activity in all lumbar segments (L1–L5) (Cazalets et al. 1995). Moreover, sectioning the spinal cord between segments L2 and L3 suppressed this rhythmic activity recorded in caudal segments but not at L2 (Bertrand and Cazalets 2003). In addition, it was shown that injuries in the upper lumbar segments (L2) had more severe consequences on locomotor output of the hindlimbs than injuries targeting thoracic segments (Garcia-Alias et al. 2006; Magnuson et al. 2005). These effects can be attributed to cellular elements within the spinal cord and not to white matter tracts because a loss of midthoracic gray matter (by intraspinal administration of the excitotoxin kainic acid) had no effect on locomotion, whereas a loss of gray matter in the rostral lumbar segments provoked major locomotor deficits (Magnuson et al. 2009). These studies confirm that, as is the case for decerebrate and spinal cats, interneurons at premotoneuronal level of the spinal cord are critical for locomotion in rats.

However, our results suggest that these midlumbar segments should be considered as the endpoints of a chain of more rostral segments that, although individually less important for locomotion, exert powerful effects on locomotion when simultaneously inactivated. This is compatible with the work of others in the rat that suggested that the rhythm-generating network is distributed throughout the caudal thoracic and lumbar region of the spinal cord with a rostrocaudal excitability gradient (for a review see Kiehn 2006). In the isolated neonatal rat spinal cord preparation regular rhythmic activity induced by application of a 5-HT/NMDA mixture persists in both rostral and caudal isolated lumbar parts of the spinal cord after transecting the cord at mid-L3 (Kremer and Lev-Tov 1997), which suggests that the rhythmogenic elements are not restricted to L1–L2 in rats. In addition, in the same preparation, left/right alternation was preserved after a mid sagittal lesion between T12 and L2 segments (Kjaerulff and Kiehn 1996). More recently, it was shown that, when isolated, thoracic, lumbar, and sacral regions could generate right and left alternating motor bursts induced by the 5-HT/NMDA mixture (Falgairolle and Cazalets 2007). Specialization of different parts of the spinal cord could result for the distribution of a different neurotransmitter system used to induce rhythmic activity in rats. For example, in the rat, 5-HT alone produced locomotor-like activity when applied to the caudal thoracic or rostral lumbar, but not in the caudal lumbar region of the cord (Cowley and Schmidt 1997), whereas a mixture of 5-HT/NMDA or acetylcholine/acetylcholine esterase inhibitor induced locomotor-like activity when applied in both rostral and caudal parts of the lumbar cord (Cowley and Schmidt 1997; Gabbay et al. 2002; Kjaerulff and Kiehn 1996).
The present work also illustrates the relative contribution of other segments (rostral or caudal to L3–L4) in the expression of locomotion in decerebrate cats. Indeed, simultaneously inactivating T13, L1, and L2 segments completely blocked locomotion. However, they are not as important as L3–L4 segments for the generation of the locomotor pattern since individually targeting one of those segments (T13, L1, or L2) produced important locomotor deficits but did not abolish locomotion. The role of these segments rostral to L3–L4 could be to integrate descending supraspinal inputs from the brain stem before relaying this information to a more caudal locomotor network (Shik 1983). Such an organization was described at the cervical level. A propriospinal system at segments C3–C4 of the cat spinal cord, projecting to motoneurons located at C6–T1 segments, mediates cortico-, rubro-, reticulo-, and tectospinal inputs (for review see Alstermark et al. 2007). In addition, the isolated cervical region can generate the locomotor pattern of the forelimbs as shown in cats (Zangger 1981) and in rats (Ballion et al. 2001). This spinal interneuronal organization could also exist for the hindlimbs. Experiments done by Shik on the initiation of locomotion in mesencephalic cat suggest a relation between reticulospinal and propriospinal systems at the thoracic level of the spinal cord (Shik 1997). This lesion study indicates that some propriospinal cells at the thoracic level could be implicated as a relay between reticulospinal descending inputs and the locomotor network in the lumbar enlargement. More recently, using in vitro neonatal rat spinal cord preparations, it was shown that rhythmic lumbar root discharges (recorded at L2 and L5 level), evoked by electrical stimulation of the brain stem, was abolished following the suppression of synaptic activity in the cervicothoracic region (C1–T8) (Zaporozhets et al. 2006). Thus injecting yohimbine at low-thoracic or high-rostral levels in decerebrate cats could have altered the modulatory role of such an integrating propriospinal network located rostral to the CPG.

In light of our results, we propose a model (Fig. 10) of a segmental organization of the locomotor network for the hindlimbs of the cat as viewed from the point of view of one neurotransmitter system: noradrenaline. Although this model emphasizes the critical role played by noradrenergic mechanisms in these midlumbar spinal segments L3–L4, it also recognizes the importance of combined inputs from more rostral segments in rhythmogenesis. An essential feature of this model is that segments rostral to L3–L4 have multisegmental connections, so that inactivation of one segment does not prevent the action of more rostral segments on L3–L4 segments. A strictly serial activation scheme is not compatible with our results because with such an arrangement, inactivation say of L2 individually would have the same effect as simultaneously inactivating T13, L1, and L2, which is not the case. Furthermore, as mentioned, descending supraspinal inputs that project directly—and hypothetically via T13, L1, L2—to segments L3–L4 must be required for the full expression of locomotion in the decerebrate cat. Afferent inputs projecting to midlumbar segments may also be of critical importance since midlumbar interneurons receiving peripheral inputs from group II afferents were shown to discharge during MLR-activated fictive locomotion (Shefchyk et al. 1990). Finally, the work of Barthélémy (Barthélémy et al. 2007) shows that ISMS is probably acting mainly through the activation of sensory inputs and also that inputs ascend from lower segments through ventral/ventrolateral pathways (Barthélémy et al. 2007; Riddell and Hadian 2000).

Therefore in the scheme proposed, the midlumbar L3–L4 segments would occupy a focal point for the generation of locomotion in the spinal cat and in the decerebrate cat with regard to noradrenergic mechanisms. Several inputs would converge at L3–L4 and the integrity of these segments is
essential for the expression of the full hindlimb locomotor pattern. This of course does not mean that other segments do not play a role in the final expression of hindlimb locomotion, but their inactivation does not have the profound effect it has when midlumbar spinal segments are blocked by yohimbine. Although this scheme best represents our results on noradrenergic mechanisms, it is possible that the distribution of the relative segmental importance might differ for other neurotransmitter systems in line with the distribution of their various subreceptors. For example, since i.t. administration of antagonizing NMDA or non-NMDA receptors in lumbar segments blocked hindlimb treadmill and fictive locomotion induced by MLR stimulation (Douglas et al. 1993), it will be interesting to intraspinally inject various other neurotransmitter blockers in preparations similar to those used in this study.

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REFERENCES


Magnuson DS, Trinder TC, Zhang YP, Burke D, Morassutti DJ, Shields CB. Comparing deficits following excitotoxonic and contusion...


