Comparison of the Effect of Intrathecal Administration of Clonidine and Yohimbine on the Locomotion of Intact and Spinal Cats

NATHALIE GIROUX, TOMÁS A. READER, AND SERGE ROSSIGNOL
Centre de Recherche en Sciences Neurologiques, Département de Physiologie, Faculté de Médecine, Université de Montréal, Montreal, Quebec H3C 3J7, Canada

Received 30 June 2000; accepted in final form 9 February 2001

Giroux, Nathalie, Tomás A. Reader, and Serge Rossignol. Comparison of the effect of intrathecal administration of clonidine and yohimbine on the locomotion of intact and spinal cats. J Neurophysiol 85: 2516–2536, 2001. Several studies have shown that noradrenergic mechanisms are important for locomotion. For instance, L-dihydroxyphenylalanine (L-DOPA) can initiate “fictive” locomotion in immobilized acutely spinalized cats and α2-noradrenergic agonists, such as 2,6-dichloro-N-2-imidazolidinylidenebenzenamine (clonidine), can induce treadmill locomotion soon after spinalization. However, the activation of noradrenergic receptors may not be essential for the basic locomotor mechanism because chronic spinal cats can walk with the hindlimbs on a treadmill in the absence of noradrenergic stimulation because the descending pathways are completely severed. This suggests that locomotion, in intact and spinal conditions, is probably expressed and controlled through different neurotransmitter mechanisms. To test this hypothesis, we compared the effect of the α2 agonist, clonidine, and the antagonist (16a, 17a)-17-hydroxy yohimbin-16-carboxylic acid methyl ester hydrochloride (yohimbine), injected intrathecally at L3–L4 before and after spinalization in the same cats chronically implanted with electrodes to record electromyograms (EMGs). In intact cats, clonidine (50–150 μg/100 μl) modulated the locomotor pattern slightly causing a decrease in duration of the step cycle accompanied with some variation of EMG burst amplitude and duration. In the spinal state, clonidine could trigger robust and sustained hind limb locomotion in the first week after the spinalization at a time when the cats were paraplegic. Later, after the spontaneous recovery of a stable locomotor pattern, clonidine prolonged the cycle duration, increased the amplitude and duration of flexor and extensor bursts, and augmented the foot lag at the onset of swing. In intact cats, yohimbine at high doses (800–1600 μg/100 μl) caused major walking difficulties characterized by asymmetric stepping, stumbling with poor lateral stability, and, at smaller doses (400 μg/100 μl), only had slight effects such as abduction of one of the hindlimbs and the turning of the hindquarters to one side. After spinalization, yohimbine had no effect even at the largest doses. These results indicate that, in the intact state, noradrenergic mechanisms probably play an important role in the control of locomotion since blocking the receptors results in a marked disruption of walking. In the spinal state, although the receptors are still present and functional since they can be activated by clonidine, they are seemingly not critical for the spontaneous expression of spinal locomotion since their blockade by yohimbine does not impair spinal locomotion. It is postulated therefore that the expression of spinal locomotion must depend on the activation of other types of receptors, probably related to excitatory amino acids.

INTRODUCTION

Lundberg and coworkers (Jankowska et al. 1967a,b) have shown that the intravenous (iv) administration of L-dihydroxyphenylalanine (L-DOPA), a precursor of noradrenaline, together with the monoamine oxidase inhibitor nialamide, in acutely spinalized and paralyzed cats profoundly modified spinal circuits. Indeed, long-latency and -duration discharges, often rhythmically organized in antagonist muscle nerves, were evoked by stimulation of sensory nerves. The same drugs were also found to induce a detailed pattern of locomotion in paralyzed cats, indicating the existence in the cat spinal cord of a central pattern generator (Grillner and Zangger 1979). In acutely spinalized adult cats, Forssberg and Grillner (1973) showed that 2,6-dichloro-N-2-imidazolidinylidenbenzenamine (clonidine), an α2-noradrenergic agonist injected iv, could evoke hindlimb locomotion on a treadmill. In chronic spinal cats (T13), clonidine and other α2-noradrenergic agonists such as tizanidine and oxymetazoline injected intraperitoneally or intrathecally have been shown (Barbeau et al. 1987; Chau et al. 1998a) to initiate locomotion within the first week following spinalization. In late spinal cats capable of walking with the hindlimbs without drugs, these α2 agonists exert a potent modulation of the locomotor pattern. For example, clonidine increases the cycle duration and flexor muscle burst duration as well as decrease the extensor activity and consequently the weight support.

The major source of spinal noradrenaline (NA) afferents are cell groups in the brain stem, namely the locus coeruleus and subcoeruleus, the nucleus of Kölliker-Fuse as well as the medial and lateral parabrachial nuclei (Dahlström and Fuxe 1964a,b). After a complete spinal transection, all descending pathways, including NA fibers, are destroyed. Nevertheless adult cats usually recover within a few weeks the ability to walk with the hindlimbs on the treadmill (for a review, see Rossignol 1996; Rossignol et al. 1999). These results indicate that in the adult spinal cat, since the descending monoaminergic systems are absent, the stimulation of noradrenergic receptors is not essential for triggering or organizing the basic locomotor pattern. Therefore locomotion in the spinal state may depend on other transmitter systems still present after the spinal transection. However, this does not prevent the stimulation of these receptors from affecting locomotion in the
spinal state as mentioned in the preceding text nor does it negate that in intact cats, NA receptor stimulation may be essential for the normal modulation of the locomotor pattern.

The aim of the present study was thus to investigate the role of the noradrenergic system on locomotion by injecting intrathecally (i.t.) the $\alpha_2$-noradrenergic agonist clonidine and the $\alpha_2$-noradrenergic antagonist (16α, 17α)-17-hydroxy yohimbine-16-carboxylic acid methyl ester hydrochloride (yohimbine at lumbar segments (L2–L5) in the same cats, before and after a complete spinal cord transection at T13. It is indeed important to know the effects of drugs in the intact and spinal states since the state of the receptors change after spinalization (Giroux et al. 1999b). Furthermore as spinalization elimates presynaptic receptors by removing all descending noradrenergic terminals, receptors activated in the spinal state are located postsynaptically that allows discrimination between pre-versus postsynaptic effects of drugs. Preliminary results have been published in abstract form (Giroux et al. 1996, 1998, 1999a).

METHODS

General protocol

Adult cats ($n = 3$) were trained during a 3- to 4-wk period to walk at different speeds (0.2–0.8 m/s) on a motor-driven treadmill. When the cats had been trained to walk at a constant speed for ~15–20 min, they were implanted with chronic electromyographic (EMG) electrodes in muscles of the hindlimbs (Belanger et al. 1996) as well as with an i.t. cannula. Nerve-cuff electrodes were placed on the superficial peroneal nerve just above the ankle of both sides to test reflexes. After obtaining baseline values for locomotion in the intact state, drug-injection experiments were performed while in the intact state. The cats were then spinalized at T13 under general anesthesia and trained for 3–4 wk to walk on the treadmill. When the cats recovered spinal locomotion, the same drugs were re-injected to allow a comparison of the effects of the same drugs in the intact and the spinal states. Overall these experiments lasted for periods of 6 mo to 2 yr.

Implantations

All surgical procedures were performed in aseptic conditions and approved by the Comité de Déontologie pour l’Expérimentation Animale from the Université de Montréal. The cats were premedicated with acepromazine maleate (Atravet, 0.1 mg/kg), ketamine (10 mg/kg) male from the l’Université de Montréal. The cats were premedicated with acepromazine maleate (Atravet, 0.1 mg/kg), ketamine (10 mg/kg) and glycopyrrolate (0.01 mg/kg) injected subcutaneously with acepromazine maleate (Atravet, 0.1 mg/kg), ketamine (10 mg/kg) and glycopyrrolate (0.01 mg/kg) injected subcutaneously and anesthetized with 1–3% isofluorane. Lactate Ringer solution was infused externally by counting spinous processes. The cannula was flushed with saline solution when appropriate.

Drug injections

The $\alpha_2$-noradrenergic agonist 2,6-dichloro-N-2-imidazolinylidenebenzamine (clonidine) from Sigma and the antagonist (16α, 17α)-17-hydroxy yohimbine-16-carboxylic acid methyl ester hydrochloride (yohimbine) from RBI were used in this study. The drugs were injected as a bolus of 100 μl into the subarachnoid space of the spinal cord through the inlet of the cannula, and a subsequent bolus injection of saline (100 μl) was made to flush the drug outside the cannula, the dead space of the cannula being about 10–20 μl. Yohimbine was dissolved in sterile distilled water and given at a concentration of 5.1–40.9 mM while clonidine was dissolved in saline solution (0.9%) and administered at a dose of 1.5–5.6 μl. These values were based on previous studies with these drugs in chronic spinal cats (Brustein and Rossignol 1999; Chau et al. 1998a).
Recording and analysis procedures

After the implantation and before any drug injections, recordings of locomotion were done in the intact state as the cat walked freely at different speeds (0.2–0.8 m/s) and tilts (15° up or 15° down) on a treadmill belt. The cats were also trained to walk on a horizontal ladder with eight round rungs (3 cm in diameter) spaced by ~20 cm. The later task was recorded only on video tape and studied only in the intact state. These recording served as baseline controls (intact trials).

During each drug-injection trial, similar recording were done before (predrug trial) and at different times after the drug injection (postdrug trial).

The experiments with the spinal cats were made when they had recovered a well-coordinated locomotor pattern of the hindlimbs with full weight support of the hindquarters and plantar foot placement. For spinal locomotion, the forelimbs were placed on a platform situated 2 cm above the treadmill, while the hindlimbs walked on the belt and the tail was held to maintain equilibrium of the hindquarters. A Plexiglas separator was place between the hindlimbs to prevent crossing of the hindlimbs.

The EMG signals were differentially amplified (bandwidth of 100 Hz to 3 kHz) and recorded on a 14-channel tape recorder (Vetter Digital, model 4000A PCM recording adapter) with a frequency response of 1.2 kHz per channel. The EMG recordings were synchronized to the video images of the hindlimbs using a digital SMPTE (Society for Motion Picture and Television Engineers) time code. This time code was recorded simultaneously on the EMG tape, the audio channel of the VHF tape as well as into the video image.

Video images of the side view of the left hind limb during locomotion were captured using a digital camera (Panasonic 5100, shutter speed 1/1000 s) and recorded on a video cassette recorder (Panasonic, AG 7300). Reflective markers were glued to the skin over the bony landmarks (iliac crest, femoral head, knee joint, lateral malleolus, metatarso-phalangeal or MTP joint and the tip of the 4th toe) of the left (ipsilateral) hind limb. Two additional markers placed on the trunk of the animal served for calibration (10 cm) to reduce the parallax error.

The kinematic analyses were carried out using a two-dimensional Peak Performance system (Peak Performance Technologies, Englewood, CO). The video images were digitized and the x-y coordinates of different joint markers were obtained at a frequency of 60 fields/s. These coordinates were used to calculate angular joint movements and could be displayed as continuous angular displacements or stick diagrams of one step cycle.

Reflex testing

ELECTRICAL STIMULATION. The superficial peroneal nerve was stimulated by a single pulse of 250 μs at 0.45 Hz (Grass S88 stimulator, Quincy, MA) through the nerve-cuff electrodes. The stimulation was delivered at rest when the cat was laying down on the treadmill. Selected EMGs were displayed on an oscilloscope (Tektronix 2214) and the threshold of stimulation was set at the current value required to evoke a small short-latency (10 ms) response in the semitendinosus muscle (St) in 50% of the trials.

FAST PAW SHAKE. Fast paw shake was elicited by holding the spinal cat in the air and then dipping the paw into a bowl of warm water. During the fast paw shake, EMG signals and video images were recorded, but only the EMG signals were analyzed.
Histology

At the end of the experimental series, the animals were killed with an overdose of pentobarbital sodium, and the spinal cord were removed and divided into 4- to 6-cm-thick blocks that were rapidly frozen for autoradiographic analysis in other studies. To assure the completeness of the spinal transection, the segment of the encompassing lesion was completely removed and cut in sagittal 10-μm-thick sections that were stained with the Klüver-Barrera method for histological observations.

RESULTS

These results were obtained from three adult cats in both the intact condition and after a complete spinal transection. In each condition, drugs were injected several times on different days and at different concentrations. The schedule and dosages of clonidine and yohimbine injection during control and spinal states are shown in Fig. 2. Cat NG2 was kept 658 days in the intact state and 158 days after the spinal transection while both NG3 and NG5 were kept 263 and 127 days as intact and 114 and 70 days after the spinalization, respectively. The results reported in this study are from selected experiments in different cats in both conditions. Even if some experiments were not analyzed in detail, the videotapes and EMG data were always reviewed to verify the similarities or differences in the effect of drugs. In the spinal condition, except for one experiment with clonidine (Fig. 6), all drugs were administered when the cat had recovered a well-organized locomotor pattern with adequate foot placement and weight support.

Effects of clonidine on locomotion

INTACT CAT. Level walking. In this study, high doses of clonidine correspond to 100–150 μg/100 μl and are well known to have important locomotor effects in spinal cats (Chau et al. 1998a). Since clonidine induced in the intact cats side effects, i.e., nausea and drowsiness, lower doses (50 μg/100 μl) were used in a few trials.

The effects of clonidine administration in the intact condition are illustrated for cat NG2 in Fig. 3, E–H. The locomotion during the intact condition before clonidine injection (Fig. 3, A–D) will serve as a reference for locomotion of the same cat after spinalization as shown in Figs. 6 and 7. The characteristics of normal walking are demonstrated by the stick diagrams of the left hind limb representing one step cycle (Fig. 3A), the angular displacement of the hip, knee, ankle, and MTP joints (Fig. 3B), and the duty cycle of right/left limbs as illustrated by the horizontal bar, the arrow heads indicating foot contact (↓) and foot lift (↑) (Fig. 3D). The raw EMG traces obtained from the intact cat illustrate the complex pattern of activity in flexor and extensor muscles (Fig. 3C), each with more or less its own signature. Thirty-eight minutes after a high dose of clonidine (100 μg in a bolus of 100 μl), there were no dramatic changes in the locomotor pattern when compared with the locomotion before clonidine. However, there was a decrease in the step length (84% of predrug) as shown in the stick figure (Fig. 3E) and a decrease in the angular excursion of all joints (Fig. 3F). The decrease in angular excursion was evident at the end of the swing phase (Fig. 3F) and resulted in a decrease in the forward placement of the paw. The step cycle duration also decreased (to 86% of the predrug value) after clonidine, leading to an overall decrease in flexor and extensor bursts duration (Fig. 3G). Furthermore, there was a reduction in EMG amplitude of the knee extensor vastus lateralis (VL) for both hindlimbs (Fig. 3G) but variable changes could be seen for flexor and ankle extensor muscles. As shown in Fig. 3G, the amplitude of ankle extensor EMGs for gastrocnemius lateralis (GL) and gastrocnemius medialis (GM) increased by 113 and 122%, respectively, compared with the predrug values, while the knee extensor VL decreased to 85%. This increase extensor EMGs could result in the generally more extended posture observed especially at the onset and offset of stance (see stick diagram in Fig. 3E).

These overall observations made in the intact cat after clonidine administration were consistently seen in different trials. Table 1 summarizes the effects of both small and high doses of clonidine in different cats. High doses (150 μg/100 μl) of clonidine caused a decreases in the step cycle duration (92% of predrug values) as well as in the duration of the majority of EMGs recorded from muscles. The normalized EMG showed that the amplitude of some EMGs varied after clonidine, but extensor muscles VL always decreased (74% of predrug). With smaller doses (50 μg/100 μl), there was a decrease in amplitude and duration of some flexor and extensor EMGs but neither the step cycle duration nor the step length changed significantly after clonidine.

Despite these changes in EMG amplitude and duration, the general EMG profile for each flexor and extensor muscle was

---

**FIG. 2.** Schedule and doses of 2,6-dichloro-N-2-imidazolidinylid-enebenzenamine (clonidine) and (16α, 17α)-17-hydroxy yohimbine-16-carboxylic acid methyl ester hydrochloride (yohimbine) injections in the control state and after spinalization in cats NG2, NG3, and NG5. Each vertical tick represents 1 trial, and the tick length is the dose expressed in μg/100 μl that is injected as a bolus of 100 μl. The x’s indicate the day of the spinal transection. The time scale is different for each cat for the control period but is the same for the postspinalization period.
not markedly different after clonidine. Figure 4A shows a block diagram representing changes in the timing and amplitude of the EMGs for the intact cat NG2 before and after clonidine. After one bolus injection of 100 μg/100 μl (highest dose), there was some decrease in amplitude and duration in the flexor and in some extensor muscles, but the EMG onset and offset for each individual muscle was relatively similar to predrug values. However, at low treadmill speed (0.2 m/s) in the intact cat, a small increase in the delay between the onset of the knee flexor semitendinosus (St) and hip flexors sartorius (Srt) and Ip (not illustrated), which sometimes seen in intact cat at low treadmill speed, appeared more frequently after clonidine and led to a more segmented swing phase.

The effect of clonidine (highest dose) on the regularity and
maintenance of the walking pattern was also tested in the intact cat NG2. Figure 5A illustrates the consecutive step cycle duration before and after a bolus injection of 100 μg/100 μl of clonidine. Before the drug, the cat could, as expected, walk at different speeds (0.4–0.6 m/s) on the treadmill with more regular walking at high speed as reflected by the reduced fluctuation in the step cycle duration. Note that lower speeds (0.2–0.3 m/s) are not represented in this graph; in the intact situation, speeds lower than 0.4 m/s were too slow for this cat. Thirty minutes after administration of clonidine, the cat exhibited a more stereotyped locomotor pattern and seemed to be less disturbed by the environment. This is shown by a reduced variability in the consecutive step cycle when compared with a control trial at the same speed (Fig. 5A; 0.4 m/s). The improvement in the regularity was observed in all experiments in all three intact cats and was also evident in the EMG traces showing a more defined and homogeneous pattern of activity in flexor and extensor muscles. There was also a marked decrease in the step cycle duration (77% of predrug value), and the cat had some difficulty walking at higher speeds (≥0.5 m/s), probably because of minor secondary effects of central origin, as evidenced by some salivation, nausea, and drowsiness, that could be observed during the first 2 h postinjection.

Walking on slopes and ladder. Intact cats were also tested for their capability to adapt their locomotion to various external conditions, such as walking on uphill and downhill slopes as well as on a ladder; these evaluations were carried out both before and after clonidine administration. In the intact cat, walking on a 15° uphill slope produced a general increase in EMG amplitude, in particular extensor muscles such as GM and GL, leading to an increase in the stance length. After low doses of clonidine, the kinematics, the step cycle duration as well as the amplitude and duration of flexor and extensor bursts were similar when compared with the uphill predrug session. At higher doses (150 μg/100 μl), there were no major changes in the kinematics except for an increase in the step and stance durations (114–119 and 111–127% of predrug) and a decrease in the angular excursion at the knee and ankle joints that may be the result of a more extended position of the hindlimbs. The amplitude of the knee extensor VL decreased while the amplitude of ankle extensors (GL and GM) increased, and this may also have contributed to the more extended posture observed after clonidine.

In 15° downhill slopes, when compared with level walking, there was a decrease in step cycle duration and a general decrease in duration or amplitude of flexor and extensor muscles. Some changes were observed in the mode of discharge of the flexor muscle Srt in which a second burst of activity is now seen during the stance phase. Following clonidine injection, the kinematics and EMG pattern was similar to the downhill control. At high doses (150 μg/100 μl), there were no changes in step length or duration. However, the knee often sagged at the end of the stance, and this was probably due to the decrease in amplitude of the knee extensor VL present after clonidine. The rest of the EMG remained unchanged after clonidine except for a small decrease in the amplitude and duration of the flexor St.

When cats were evaluated in their ability to walk on a ladder, they were all able to walk without any difficulty over the round rungs during the predrug trial. After clonidine, the cats were still capable of walking on the ladder, albeit with some difficulty. Although they could correctly place the foot on the rungs of the ladder, at the hindpaw contact, the paw

### Table 1. Timing and amplitude changes after clonidine in intact cats

<table>
<thead>
<tr>
<th></th>
<th>Predrug</th>
<th></th>
<th>Clonidine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration</td>
<td>Normalized amplitude</td>
<td>Duration</td>
<td>Normalized amplitude</td>
</tr>
<tr>
<td>LSt 131 (50 μg, 25 min, 0.3 m/s)</td>
<td>229 ± 24 (11)</td>
<td>100 ± 9</td>
<td>140 ± 32** 61% (12)</td>
<td>123 ± 13**</td>
</tr>
<tr>
<td>LSt 229</td>
<td>396 ± 57 (11)</td>
<td>100 ± 4</td>
<td>244 ± 24* 62% (12)</td>
<td>108 ± 13</td>
</tr>
<tr>
<td>LVL 713</td>
<td>713 ± 145 (11)</td>
<td>100 ± 19</td>
<td>839 ± 70* 117% (12)</td>
<td>74 ± 6**</td>
</tr>
<tr>
<td>LGM 745</td>
<td>745 ± 146 (11)</td>
<td>100 ± 19</td>
<td>727 ± 160 97% (12)</td>
<td>93 ± 14</td>
</tr>
<tr>
<td>Step cycle 1251</td>
<td>1251 ± 181 (11)</td>
<td>—</td>
<td>1154 ± 71** 92% (12)</td>
<td>—</td>
</tr>
<tr>
<td>LSt 614</td>
<td>614 ± 45 (7)</td>
<td>—</td>
<td>560 ± 26* 91% (10)</td>
<td>—</td>
</tr>
<tr>
<td>LSt 283</td>
<td>283 ± 32 (11)</td>
<td>100 ± 10</td>
<td>287 ± 41 101% (30)</td>
<td>114 ± 12**</td>
</tr>
<tr>
<td>LVL 536</td>
<td>536 ± 49 (11)</td>
<td>100 ± 31</td>
<td>535 ± 67 100% (30)</td>
<td>106 ± 21</td>
</tr>
<tr>
<td>LGM 595</td>
<td>595 ± 90 (11)</td>
<td>100 ± 13</td>
<td>506 ± 100* 87% (30)</td>
<td>94 ± 17</td>
</tr>
<tr>
<td>Step cycle 920</td>
<td>920 ± 74 (11)</td>
<td>—</td>
<td>920 ± 68 100% (30)</td>
<td>—</td>
</tr>
<tr>
<td>Step length, cm 492</td>
<td>492 ± 28 (7)</td>
<td>—</td>
<td>558 ± 59 113% (7)</td>
<td>—</td>
</tr>
<tr>
<td>LSt 131</td>
<td>131 ± 44 (17)</td>
<td>100 ± 32</td>
<td>81 ± 15** 62% (10)</td>
<td>60 ± 32**</td>
</tr>
<tr>
<td>LSt 314</td>
<td>314 ± 22 (17)</td>
<td>100 ± 13</td>
<td>256 ± 40* 82% (10)</td>
<td>88 ± 14*</td>
</tr>
<tr>
<td>LVL 520</td>
<td>520 ± 74 (17)</td>
<td>100 ± 13</td>
<td>504 ± 52 97% (9)</td>
<td>79 ± 11**</td>
</tr>
<tr>
<td>LGM 612</td>
<td>612 ± 79 (15)</td>
<td>100 ± 25</td>
<td>537 ± 50 89% (9)</td>
<td>55 ± 22**</td>
</tr>
<tr>
<td>Step cycle 947</td>
<td>947 ± 76 (17)</td>
<td>—</td>
<td>923 ± 85 97% (10)</td>
<td>—</td>
</tr>
<tr>
<td>Step length, cm 473</td>
<td>473 ± 29 (8)</td>
<td>—</td>
<td>485 ± 17 103% (8)</td>
<td>—</td>
</tr>
</tbody>
</table>

Step length, cycle, and burst duration are expressed in means ± SD. The duration and length values after 2,6-dichloro-N-2-imidazolidinylidenebenzenamine (clonidine) injection are also expressed as a percentage of the predrug values. The normalized and averaged amplitude of the electromyographic (EMG) bursts are given as a percentage of the predrug values ± the coefficient of variation (CV). The number of average step cycles is enclosed in parentheses. Student’s t-tests were performed to compare the pre- and postdrug values, * P ≤ 0.05 and ** P ≤ 0.01. For each cat, the data in parentheses indicate first, the dose in 100 μl; second, the time after the injection; and third, the treadmill speed. LSt, left semitendinosus; LSrt, left sartorius; LVL, left vastus lateralis; LGM, left gastrocnemius medialis.
Often slipped off; thus they had to constantly readjust the foot placement before the weight transfer, otherwise they fell off. Generally, after clonidine administration, cats took more time to perform this task, and this could be due to changes in reflex sensitivity (see following text).

**Spinal Cat.** *Level walking: initiation in early spinal cat.* The ability of clonidine to trigger locomotion in complete spinal cats has already been well documented (Barbeau et al. 1987; Chau et al. 1998a), but the comparison of the effects of clonidine in the same cat before and at various times after spinalization has not been made. In Fig. 6, three days after the spinalization, the same cat as shown in Fig. 3 in the intact state is now paraplegic. A strong perineal stimulation could sometimes produce episodes of faint stepping on the treadmill (Fig. 6A), but this locomotor activity was not well organized as shown by the duty cycle of the left/right limbs and EMGs traces (Fig. 6, D and C) and was so small that the hip, knee, ankle, and MTP joints barely moved (Fig. 6B). The paws were always dragged on the treadmill belt, and no plantar foot placement nor weight support were seen before clonidine injection. As illustrated in Fig. 6, E–H, one bolus injection of clonidine (100 µg/100 µl) triggered an adequate locomotor pattern with good bilateral placement of the feet on the plantar surface and weight support of the hindquarters, requiring only

---

**FIG. 4.** Block diagram of the activity of the principal hindlimb muscles for the cat (NG2) pre- and postclonidine injection (100 µg/100 µl). A: in intact locomotion at 0.4 m/s. B: 121 days after spinalization. For each muscle, the bar length represents the average onset and offset of burst ± SD in a normalized step cycle. The width of the bars indicates the amplitude of the EMG bursts and their percentage change in comparison to predrug values are presented below (significant changes at the level of *P < 0.05, **P < 0.01). The duty graphs are shown at the bottom of the graph.
light perineal stimulation. When compared with predrug conditions, there was a marked increase in the step length as shown in the stick diagram (Fig. 6E) as well as by the increase in the total angular excursion at the hip, knee, ankle, and MTP joints (Fig. 6F). This spinal locomotion was stable, adaptable to speed, and sustained, i.e., the animal could walk for a long period of time (10–15 min), at different treadmill speeds (0.1–1 m/s), and with a regular locomotor pattern.

Clonidine-induced locomotion in the early spinal cat that resembled the intact locomotion illustrated in Fig. 3, E–H, after drug injection, and this locomotor pattern could last for several hours (5–6 h). There were, however, some differences. For example, the paw dragged at the beginning of swing phase as indicated in Fig. 6E by the horizontal line below the stick diagram of the swing phase, and this was generally followed by an increase in the elevation of the foot at the end of swing (Fig. 6E).

The EMG activities before clonidine were not organized (Fig. 6C); however, there was some rhythmic activity in extensor muscles (GL and GM), while only tonic activity was seen in flexor muscles (St and Srt). After clonidine injection, a rhythmic alternation of flexor and extensor muscles appeared during treadmill locomotion (Fig. 6G). There was an increase in the duration of the flexors St and Srt when compared with the intact values before the spinalization (132 and 192% of the intact values, respectively; data not shown), and this could have contributed to the marked increase in swing duration.

Level walking: modulation of locomotion in late spinal cat. In the late state, when cats had recovered the ability to walk on the treadmill without any pharmacological treatment, clonidine was injected, and its effects on locomotion are illustrated in Fig. 7. Before clonidine injection in a spinal cat, 125 days after the spinalization (NG2, same cat as Figs. 3 and 6), the locomotion was well established with placement of the foot and full weight support (Fig. 7, A–D). The same dose (100 μg/100 μl) that produced minor effects in the intact and triggered the locomotion in the early spinal now induced important changes in the locomotor pattern in the late spinal animal. Thirty minutes after clonidine, there was a marked increase in the step length (137% of predrug) and in the joint angular excursions particularly at the knee joint, leading to an increase in the amplitude of the swing phase (Fig. 7, E and F). A more pronounced feet drag during the initial swing was also observed after clonidine (Fig. 7E), followed by an increase in amplitude of the swing. There was a marked increase in the amplitude (140 and 160% of the predrug values) and in the duration (190 and 104%) of the knee and the hip flexors, St and Srt, respectively. This could contribute to the increase in the swing duration. The duration and amplitude of ankle extensor muscles, GL and GM, also increased after clonidine (114 and 105%, respectively, for the amplitude and 133 and 120% for the duration) contributing to an increase in the duration of stance.

The general EMG profile for each individual muscle undergoes some modifications after clonidine. As shown in the block diagrams (Fig. 4B), the EMG timing in the late spinal cat NG2 before any drug injection, was relatively similar to the intact pattern. When compared with the intact animal before any drug injection (Fig. 4A), there was a decrease in the delay of the onset of St with the onset of the swing phase or paw lift, but the general EMG timing for each extensor muscle was not markedly different. Also, following spinalization, the amplitude of flexors (St and Srt) increased while the amplitude of extensors (GL and GM) decreased (Fig. 4, A and B). One bolus injection of clonidine (100 μg/100 μl) in the late spinal cat caused no major change in the timing of the EMG except for a marked decrease in the delay between the knee flexor St and the hip flexor Srt (Fig. 4B). Also, there was a pronounced increase in the general amplitude (173–253% of predrug) of both flexors and extensors when compared with the predrug values before any drug injection. These changes in the burst duration and amplitude, as well as in the timing of EMGs observed after clonidine, were constantly seen in all the spinal cats after clonidine. Furthermore, in some spinal cats, the EMG bursts became fragmented (clonic) after spinalization (see for instance RGL raw EMG in Fig. 7C) and, after clonidine, discharged in a much smoother desynchronized burst (see RGL in Fig. 7G).

Consecutive step cycle duration taken before and after clonidine injection showed that clonidine improved the regularity of walking in the late spinal cat (Fig. 5B). Before the drug, spinal cats have regular and sustained hind limb walking at different treadmill speeds (0.2–1.0 m/s; in Fig. 5B, only speeds 0.4–0.7 m/s are shown). After clonidine injection (100 μg/100 μl), the walking became even more regular as indicated by a much reduced variability in the consecutive step cycle. Clonidine also induced an increase (107–116% of the predrug value) in the step cycle duration (seen at all speeds in
This was in contrast to the intact state, where the cycle duration decreased following clonidine (Fig. 5A).

Similar finding as those observed with clonidine in intact versus spinal cats were seen after administration of the neurotransmitter NA itself (data not shown). In fact, in two cats (NG2 and NG3), the administration of NA (100–200 μg/100 μl) had no major effects on the locomotor pattern (in 5/5 trials), whereas in the same cat after the spinalization, the same doses of the physiological agonist induced an important modulation of the locomotion in three out of four cats; indeed, NA caused an increase in the stance and the swing duration as well as in the angular excursions of all joints. There was also an important increase in the amplitude and duration of flexors and extensor EMG bursts.

**Effects of clonidine on cutaneous reflex excitability**

After clonidine administration (50–150 μg/100 μl), the amplitude of the reflex response evoked by electrical stimulation of the superficial peroneal nerve decreased in both intact (10/12 injections) and spinal cats (4/5 injections); these effects are illustrated for cat NG2 in Fig. 8, A and B. In the intact cat (Fig. 8A), despite a much stronger stimulating current of 600 μA (3 times predrug threshold), there was still a marked decrease in amplitude of the short latency response in the St muscle. Similarly, in a spinal cat at 158 days (Fig. 8B), the same stimulation used before clonidine abolished the response in Srt and tibialis anterior (TA) muscles and markedly decreased the response in the St muscle.

The time course of the effect on the reflex excitability was analyzed in two intact cats (NG2 and NG3) and is shown in Fig. 9, A and B. This time course was evaluated by measuring the stimulation threshold at rest at different time periods following clonidine administration. The threshold of the stimulation was defined by observing a just detectable response in the St muscle at rest. After clonidine, the threshold increased and this persisted for 30–60 min before returning to control values by 120–180 min. During the 30- to 60-min postinjection, the cat was drowsy and unwilling to walk at high treadmill speeds. The return of reflex excitability coincides with the return of the ability to cope with high treadmill speeds. The return of reflex excitability coincides with the return of the ability to cope with high treadmill speeds as observed during the predrug session and with the disappearance of side effects; this time course of clonidine effects seems to be shorter in duration for intact (2–3 h) than for late spinal cats (6 h) (Chau et al. 1998a).

As previously shown in other studies (Barbeau et al. 1987; Chau et al. 1998a), clonidine abolished the fast paw shake
response in 4/4 experiments. Figure 8C shows an example of the fast paw shake response before and after clonidine in cat NG5. In a 41-day postspinalization cat, 30 min after clonidine, the fast paw shake response was completely abolished by a small dose of clonidine (50 μg/100 μl).

In summary, clonidine slightly modulated the locomotor pattern in intact cats. High doses (100–150 μg/100 μl) only caused a decrease in duration of the step cycle and, in some muscles, slight variations in burst amplitude and duration as well as difficulty to follow treadmill speeds higher than 0.4 m/s. Lower doses caused only some minor variations in muscle amplitude and duration. Also, after clonidine all cats were able to walk either on 15° slopes (uphill or downhill) or on a horizontal ladder. On the other hand, in the same cats but in the spinal state, clonidine triggered robust and sustained hind limb locomotion in the first week after the spinalization at a time when cats were paralyzed. Later when the cats had recovered a stable spontaneous locomotor pattern, clonidine prolonged the cycle duration, increased the amplitude and duration of flexor and extensor muscles, and augmented the foot drag at the onset of swing. Furthermore, in both intact and spinal conditions, the excitability of the cutaneous reflexes decreased significantly after clonidine administration.

Effects of yohimbine on locomotion

INTACT CAT. Level walking. The overall effects of yohimbine administration in intact cats NG3 and NG5 are illustrated in Figs. 10 and 11, respectively. Four minutes after a bolus injection of yohimbine (800 μg/100 μl), the cat had walking deficits as shown by the tracing of the hind limb position taken from the video recording (Fig. 10B). There was a clear asymmetrical posture of the hindquarters and an asymmetry of stepping between the two hindlimbs leading to the turning of the hindquarters to one side. There was an abduction of the left hindpaw particularly pronounced at the end of stance and an adduction of the right hindpaw. The side of the turning was different for each cat and was not obviously related to the side of the cannula’s tip as determined at the autopsy. Cats NG3 and NG5 turned on the right side (abduction of the left hindpaw), while NG2 turned on the left side (abduction of the right hindpaw). However, in some experiments, the effect could start on one side and continue on the other side.
One example of the effect of a high dose of yohimbine (1600 mg/100 ml) at the peak period is illustrated in Fig. 11 for cat NG5. Before yohimbine administration, normal walking at 0.4 m/s was characterized by the regularity in the consecutive step cycle and angular displacement of all joints (Fig. 11, A and B). There was also an alternating activity of the swing and stance phase between left and right hindlimbs (Fig. 11C) and rhythmic activity in flexor and extensor muscles as shown in the raw EMG data (Fig. 11D). Ten minutes after yohimbine (1600 µg/100 µl), the cat developed the walking difficulties mentioned in the preceding text and had very poor lateral stability of the hindlimbs, leading to falling on one side. At that time, the cat could hardly step at a treadmill speed of 0.4 m/s and sometimes exhibited a decrease in its step cycle duration (Table 2). As illustrated in Fig. 11, E–H, the stick diagram, the consecutive joints angular excursion traces, the EMG and the duty cycles were extremely irregular and disorganized.

The time course of the effects of yohimbine, evaluated by the maximal speed that the animal could follow on the treadmill, is shown for three intact cats in Fig. 12. The effects started rapidly (2–5 min); indeed, within 2 min postinjection, some cats already presented some sign of stepping asymmetry and abduction of one leg. These effects gradually increased to attain a peak at 10 min postinjection; by this time, the cats had major difficulties in maintaining balance, which led to them to fall on one side. In some cats (see cat NG2), these effects limited them to a few steps at a time at very low treadmill speeds (0.2 m/s) and in other cases to the inability to walk on the treadmill (see cats NG3 and NG5 at high doses in Fig. 12).

![Figure 8](http://jn.physiology.org/)

**FIG. 8.** Responses to electrical stimulation of the superficial peroneal nerve at rest and fast paw shake before and after clonidine (100 µg/100 µl). A: averaged responses of 14 and 15 stimuli in the intact cat and the same cat before and 1 h 10 min postclonidine injection. The currents delivered before clonidine and after were 200 and 600 µA, respectively. B: averaged responses of 13 and 15 stimuli at a current of 200 µA in a spinal cat (158 days) and 1 h 15 min postclonidine, respectively. C: fast paw shake responses in a spinal cat (41 days) before and 30 min after clonidine 50 µg/100 µl. TA, tibialis anterior.

![Figure 9](http://jn.physiology.org/)

**FIG. 9.** Time course of the effects of clonidine in 2 intact cats. The changes in the threshold of cutaneous reflex responses of left St muscle at rest as a function of time after clonidine injection were measured to evaluate the effect of the drug. A: intact cat NG2. At 150–180 min after clonidine (high doses of 100–150 µg/100 µl), the reflex responses return to predrug values. B: intact cat NG3. Small doses of clonidine (50 µg/100 µl) lasted 120–150 min. No changes in the reflex responses were seen at 25 µg/100 µl.
FIG. 10. Effect of yohimbine on locomotion in an intact cat. Figurines redrawn from the video recordings, show the hindlimb position at middle stance, offset of swing, middle swing, and onset of swing, respectively from left to right. A: intact cat NG3 before clonidine. B: 10 min after yohimbine injection (800 μg/100 μl).

B and C). The effects gradually dissipated, and normal locomotion returned after 20–30 min. These effects of yohimbine appeared to be dose dependent. During the peak period, high doses (800–1,600 mg/100 g) caused major walking difficulties, including stumbling and poor lateral stability, while low doses (400 mg/100 g) had but slight effects, such as abduction of one of the hindlimbs and turning of the hindquarters to one side. However, after yohimbine, the cats never manifested secondary central effects as those observed after clonidine, and even during the peak period, the cats were always playful and alert.

The stepping irregularity induced by yohimbine is also shown in Fig. 13, A and B, for the intact cat NG2. Before the injection, the spinal cat had a regular and stable walk at different treadmill speeds (0.3–0.7 m/s) as shown by the smaller step by step fluctuation in cycle duration (Fig. 13A) and interlimb coupling between the right and left lift (Fig. 13B). After 6–10 min following administration of a bolus of yohimbine (1,600 mg/100 g), there was a pronounced increase in the variability in the consecutive step cycle duration and in interlimb coupling (Fig. 13, A and B). There was also a general decrease in the step cycle duration (77% of predrug). Even at this high dose (1,600 mg/100 g), cat NG2 was less affected by yohimbine and remained capable of following a treadmill speed of 0.6 m/s. The effects of yohimbine on the irregularity of the stepping were consistent in all experiments and in all intact cats. Table 2 summarizes the effect of yohimbine (400–1,600 mg/100 g) on the step cycle duration and interlimb coupling in some experiments with intact cats. About 8–10 min postinjection, the mean step cycle was the same, or even shorter (43–77% to predrug values), but the coefficient of variation (CV) dramatically increased in comparison to predrug; although the mean interlimb coupling did not change significantly after yohimbine, there was a major increase in the CV.

Walking on slopes and ladder. During the peak of effects of yohimbine, i.e., ~10 min, the cats exhibited major walking difficulties on the horizontal treadmill. Outside this peak period, the cats could walk without too many problems at the horizontal level but showed major difficulties if the demand was increased, such as walking on slopes or ladder. Figure 14, A–C, shows an example from cat NG3. At 6 min after yohimbine administration, even if the presence of abduction and asymmetry was observed between the two hindlimbs, the cat could easily walk on the horizontal treadmill at a speed of 0.7 m/s; this was the maximum speed the cat performed before the injection and is exemplified by the regularity in the raw EMG data (Fig. 14A). However, at a 15° downhill tilt, the cat remained incapable of walking on the treadmill (Fig. 14B). There was an important asymmetry in stepping that led to turning and slipping of its hindquarters to one side. This is illustrated by the disrupted and disorganized pattern of EMG activities in Fig. 14B. In the 15° uphill incline, the cat also presented an asymmetry of stepping, but this was less dramatic than during the 15° downhill (Fig. 14C). At that time, cats had major difficulties to walk over the rungs of the ladder. The cats could not correctly place their hindfeet on the rung; sometimes they were placed too far or just before. Indeed, the cats had to constantly readjust their foot placement before the weight transfer, otherwise they slipped. If the cats readjusted their foot placement, it usually took them four to five corrections before the paw contacted the rung. However, the cats were able to stand without slipping or falling from the ladder if their hindpaws were correctly placed over the rungs. This is in contrast to the effects observed after clonidine, when the cats had seemingly no problems with their foot placement but could easily slip over the ladder’s rungs.

**SPINAL CAT.** The ability of yohimbine (400–1,600 mg/100 g) to modulate the spontaneous locomotion was assessed in the late spinal state, at a time when cats showed a stable locomotor...
In summary, high doses of yohimbine (800–1,600 µg/100 µl) in intact cats caused major walking difficulties characterized by asymmetric stepping, stumbling, and a poor lateral stability. Small doses (400 µg/100 µl) induced slight effects such as abduction of one of the hindlimbs with an increase of the step duration. In the spinal cat, yohimbine had no effect even at large doses. Similar results were found when comparing the effects of yohimbine in intact and spinal cats. In conclusion, yohimbine had a pronounced effect on the locomotor pattern in intact cats, while its effects were minimal in spinal cats.

DISCUSSION

The purpose of this study was to compare the effects of the α2 agonist clonidine and the antagonist yohimbine in the same
cats, initially in the intact state and after spinalization, to
determine how the effects of these drugs vary according to the
animal’s condition.

Clonidine injection on locomotion in intact and spinal cats

The differential effects of clonidine in intact versus spinal
cats can only be observed in the late spinal cat, when the cat
was capable of hindlimb locomotion on the treadmill. Whereas
activation of $\alpha_2$-adrenoceptors in the intact cat decreased step
cycle duration and sometimes burst duration in the spinal cat,
the step cycle duration, in particular the swing phase, increased
after clonidine together with an augmented burst duration and
amplitude. In both intact and spinal cats, activation of $\alpha_2$
-adrenoceptors by clonidine seemed to modulate essentially the
timing of the muscles and to a much lesser extent the output
amplitude. As proposed before, clonidine may exert an effect
primarily on interneurons that coordinate the timing between
flexor and extensor muscles (Chau et al. 1998a).

In the intact cat, the small transient effects seen after
clonidine or NA on locomotion indicate that animals can
readily compensate for the imbalance of one system, namely in
this case an over stimulation of receptors. Furthermore, in the
intact cat after clonidine, the regularity of walking was im-
proved, as reflected by a much more homogeneous and better-
organized EMG pattern. This finding was also observed in the
same animal after spinalization as well as in other studies
where clonidine improved the characteristic walking pattern
that had deteriorated and had become irregular due to a lack of
training (Rossignol et al. 1995). This improved regularity of
walking documented after clonidine in both intact and spinal
conditions may be the manifestation of changes occurring in the
properties of rhythm generation circuits. It had been shown
that L-DOPA and clonidine induce plateau potentials in spinal
motoneurons in acute complete spinal cats (Conway et al.
1988). It has been proposed that the role of the plateau poten-
tials was to facilitate, shape, and time the propagation of motor
rhythm (Kiehn et al. 1996). Thus this could lead to the more
regular and constant locomotor pattern seen after clonidine. It
is also possible that the regularization of the rhythm by
clonidine in the intact condition was due to side effects such as
drowsiness. In this state, the cat could be less disturbed by
external stimuli and therefore reduce the irregularity. However,
this could not explain the improvement of regularity docu-
mented in the spinal condition after clonidine.

The effects of clonidine in the early and late spinal cat
reported in this study are in close agreement with previous
results (Barbeau et al. 1987; Chau et al. 1998a). These authors
have previously shown that clonidine can initiate locomotion in
the early spinal cats and in a few week after the spinalization
(i.e., late spinal cats), modulate the locomotor pattern by in-
creasing the cycle duration and flexor burst duration while the
mean EMG amplitude tended to increase or remain the same in
flexor and decrease in extensors. However, in the present
experiment, some increase in extensor bursts amplitude was
sometimes seen after high doses of clonidine (Fig. 4B). This
may result from activation of $\alpha_1$-adrenergic receptors at high
doses of clonidine (Kehne et al. 1985; Timmermans and van
Zwieten 1982) since their stimulation was found to increase the
output amplitude of extensor muscles to a much greater extent
than following the $\alpha_2$-noradrenergic receptor stimulation
(Chau et al. 1998a). These results, in both intact and spinal cat,
are also in contrast to findings in cats subjected to ventral and
ventrolateral spinal lesions in which clonidine had a detrimen-
tal effect on walking (Brustein and Rossignol 1999). In these
partial spinal cats, clonidine caused major reduction in weight
support of the hindlimbs, an increase in swaying of the hind-
quarters, stumbling, and falling.

The differential effects of clonidine in the intact cat com-
pared with complete and partial spinal cats could in part be
attributed to a difference in targets, alterations in the sensitivity
traces are often disrupted and not well organized. 

\[ \text{during slopes, the EMG containing terminals, leading to a loss of presynaptic} \]

\[ \text{degeneration of all descending nerve fibers including NA-} \]

\[ \text{receptors. After a complete spinal cord transection, there is a} \]

\[ \text{become normal in the late spinal cat, there may be modifica-} \]

\[ \text{tion in the efficacy of the effector transducing mechanisms} \]

\[ \text{downstream of the binding sites so that the receptor and effec-} \]

\[ \text{tor complex may operate more efficiently than under control} \]

\[ \text{condition. In the case of cats with partial lesions, presynaptic} \]

\[ \text{\( \alpha_2 \)-adrenergic receptors may still be present on the spared NA de-} \]

\[ \text{scending terminals and could thus contribute to the observed} \]

\[ \text{detrimental effect (Timmermans and van Zwieten 1982).} \]

**Effects of yohimbine on locomotion in intact versus spinal cats**

In this study, the specific \( \alpha_2 \)-antagonist yohimbine was used to selectively block \( \alpha_2 \)-adrenergic receptors (Goldberg and Robertson 1983). Yohimbine has been shown previously to completely antagonize the behavioral effect of clonidine on spinal locomotion (Barbeau et al. 1987). In the present study, yohimbine did not affect the locomotion in spinal cats even if \( \alpha_2 \)-adrenoceptors remain after spinal cord transection (Giroux et al. 1999b). Following a complete spinal cord transection in the cat, NA contents below the lesion decrease dramatically (Roulet et al. 1993) and antagonists mediate their effects only in presence of NA or NA agonists; this may explain the lack of effects of yohimbine in the spinal cat. Thus spontaneous spinal locomotion probably does not depend on the activation of NA receptors since spinal cats can walk in the absence of noradrenergic descending pathways, and blockade of \( \alpha_2 \)-noradrenergic receptors with yohimbine does not prevent spinal locomotion. The expression of spinal locomotion must depend on other neurotransmitter systems located within the spinal cord. In contrast, blockade of \( \alpha_2 \)-noradrenergic receptors in the intact cat had a detrimental effect on locomotion. Thus the NA descending system probably contributes to the modulation of this spontaneous locomotion.

The \( \alpha_2 \)-adrenoceptors are autoreceptors on NA nerve terminals that diminish neurotransmitter release; their blockade by antagonist (e.g., yohimbine) will increase NA release (Reimann and Schneider 1989). Therefore this increased NA may be responsible for the detrimental effect of yohimbine in the intact cat. This interpretation is less likely since NA administration in the intact cat (unpublished observations) had no such effect on locomotor patterns. In Xenopus laevis, neuromodulators such as NA and serotonin (5-HT) act presynaptically on glycnergic terminals of commissural interneurons mediating reciprocal inhibition during swimming (McDearmid et al. 1997). If the same control exists in higher vertebrates, yohimbine may interfere with the presynaptic control of glycnergic release. This may cause alterations of interlimb coordination, leading to the effect observed after yohimbine administration in the intact cat, such as asymmetry of stepping and turning of the hindquarter on one side.

Another explanation for the detrimental effects of yohimbine in intact cats is that it may interfere with spinal interneurons that receive influences from descending pathways controlling posture and balance. Various brain stem and cerebellar regions have been so far implicated in the control of posture. Lesions of the lateral and superior vestibular nucleus, or of the associated fastigial nuclei of the cerebellum, produced severe disturbances of posture and balance (Carpenter et al. 1959; Modianos and Pfaff 1976). Rats with such lesions had pronounced tremor and abnormal head posture and exhibited an asymmetrical trunk posture; the latter manifested by the tilting of the
head and splaying of the limbs on one side of the body. Other descending pathways from the brain stem reticular formation influence postural tonus and also have an effect on posture and movement (Luccarini et al. 1990; Mori 1987; Takakusaki et al. 1994). In spinal cats, the transection destroys all descending fibers, including vestibulo- and reticulospinal pathways; thus the experimenter has to provide equilibrium and posture by gently steering the tail and maintaining the cat straight on the treadmill.

Cutaneous excitability

In both intact and spinal cats, clonidine caused a decrease in the fast paw shake responses as well as an increase in the threshold for electrical stimulation. This decrease in cutaneous reflex excitability was in line with previous studies in chronic spinal cats, showing that clonidine reduced dramatically cutaneous reflex responses (Barbeau et al. 1987; Chau et al. 1998a). Also, in fictive preparations, α2 agonists have been reported to reduce fast paw shake response, i.e., high-frequency synchronous activity of flexor and extensor muscle (Pearson and Rossignol 1991). Short latency transmission from afferents has been found to be depressed after L-DOPA and clonidine (Andén et al. 1966; Grillner 1973) as well as by stimulation of the mesencephalic locomotor region, known to trigger locomotion in the decerebrate cat (Grillner and Shik 1973). In awake, nonanesthetized monkeys, Corboz et al. (1991) showed that a α2 agonist reduced the EMG response of the flexor reflex induced by stimulation of cutaneous afferents, and this could be prevented by the administration of yohimbine, suggesting that it is mediated by an α2-adrenoceptor. Other studies have reported that NA agonists, including clonidine, depress transmission from group II muscle afferents in cats (Bras et al. 1990; Schomburg and Steffens 1988). Furthermore, stimulation of the locus coeruleus and subcoeruleus in decerebrate cats depressed interneuronal pathways involved in the reflex action of group II afferents on motoneurons (Jankowska et al. 1993), and NA was found to modulate ascending information originating from skin and muscles afferent (Jankowska et al. 1997).

In intact and spinal cats, clonidine seems to affect cutaneous reflex excitability in a similar way; the threshold for electrical stimulation in both conditions increased three to four times
after its administration. However, the time course of its effect seems to be shorter in the intact cat; i.e., cutaneous responses return to normal after 2–3 h in the intact cat while in the spinal animal some of these effects are still present 6 h later. This difference in time course could be due to the effective inactivation mechanisms or better clearance mechanisms in intact cats. Although yohimbine did not affect dramatically the cutaneous reflex excitability in both intact and spinal cats, it was
found to reverse the clonidine’s decreased cutaneous reflex response (Barbeau et al. 1987).

Slopes and walking on ladder

After clonidine, all cats were able to walk either on 15° slopes (uphill or downhill) or on a horizontal ladder. On the ladder, they could correctly place their feet on the rungs but their paws often slipped off. The decrease in the cutaneous excitability after clonidine may be responsible for such sliding. In contrast, following yohimbine administration the intact cats could not place correctly their hindfeet on the rungs and had major difficulty walking on slopes despite normal cutaneous excitability, suggesting effects on spinal neurons that receive descending commands. Indeed, it has been shown that destruction of the motor cortex, or interruptions of the corticospinal tract, produced no major change on level walking but lead to the inability of cats to walk on a wire mesh or on horizontal bars (Eidelberg and Yu 1981). Moreover, walking on the ladder was impossible after inactivation of the motor cortex by tetrodotoxin or after cortical lesions (Beloozerova and Sirota 1993a). Thus it is conceivable that blocking α2-adrenoceptors at the spinal level may have interfered with spinal neurons that receive inputs from descending supraspinal tracts, such as the corticospinal that control the accuracy of locomotor movements. On the other hand, the corticospinal tract seems to be less important during walking on slopes since the inactivation of the motor cortex did not impede the performance of cats walking on an incline surface (Beloozerova and Sirota 1993b). Also, discharges of cortical neurons and pyramidal tract fibers during locomotion up a 10° incline were the same as during locomotion at a horizontal level (Armstrong and Drew 1984). Yohimbine may act on interneurons that receive other descending projections, such as the reticulospinal or vestibulospinal pathways; the cells of origin of these tracts have been shown to be modulated with EMG activity during locomotion on an inclined surface (Matsuyama and Drew 1996) or even during level walking (Drew et al. 1986).

Intrathecal cannula

There are many advantages to use an intrathecal cannula for drug delivery such as allowing for the immediate action of drugs as well as reducing systemic side effects. In the present study, we also found that such delivery system can remain in place and be effective for a long period of time, i.e., 2 yr (see Fig. 2, cat NG2). This system can be quite reliable since the results were reproducible for a given drug in the same animal and on different days (Fig. 2).

The location of the cannula’s tips does not account for the turning of the hindquarters on one side, particularly as seen following yohimbine injection, or for the intensity of drug effects. In fact, the cannula of cat NG3 terminated on the left side of the spinal cord while the cannula’s tip was found on the right side in the cat NG5, and both cats turned on the same side. Furthermore, cat NG3 was found to have the most rostral cannula’s location (L2 segment), a region removed from the motoneuron pool responsible for locomotion; in this animal, clonidine and yohimbine effects were comparable to those observed in the two other cats. This was confirm with a study on localized applications of drugs at specific lumbar segments in the spinalized cats (Marcoux and Rossignol 2000). These authors found that clonidine topically applied or micro-injected in the upper lumbar segment L3–L4 was sufficient to trigger locomotion, and this locomotor activity could be blocked or prevented by yohimbine. Together, these findings suggest that future pharmacological therapies should focus to these specific spinal segments.

Conclusions

The present study indicates that the stimulation of the noradrenergic receptors are not essential for the control of locomotion in the spinal cat because the spinal cat can recover hindlimb locomotion in absence of NA descending pathways, and the blockade of NA receptors does not perturb spinal locomotion. Therefore it is proposed that other classes of receptors still present after the spinal transection such as excitatory amino acids (EAA) may also be involved in the activation of locomotion (Chau et al. 1994; Douglas et al. 1993). We are currently investigating the glutamatergic system, in particular the N-methyl-D-aspartate (NMDA) receptors, on receptors binding and on the expression of locomotion in the intact and chronic spinal cat. Preliminary evidence suggests that glutamatergic receptors are not downregulated several months after spinalization when compared with other receptors (Giroux et al. 1999b).

In contrast, in the intact state, the NA descending system plays a crucial role, since blockade of α2-adrenoceptors results in a marked disruption of walking. One possible role of the NA system is that in the spinal cord both descending 5-HT and NA act as modulators that enhance the effects of excitatory inputs to motoneurons. It has been demonstrated that iontophoretic applications of 5-HT or NA on lumbar motoneurons does not cause action potentials but rather dramatically potentiates the action of glutamate-evoked action potentials (White and Newman 1980). The modulatory role of monoamines was later confirmed in rat spinal cord preparations, where 5-HT and NA modulate the NMDA-induced oscillatory activity of spinal cord motoneurons and interneurons (Cazalets et al. 1990; MacLean et al. 1998). If this applies to humans, then a strategy of combining drugs that could interact, i.e., NA or 5-HT agonists with other drugs acting on glutamatergic receptors, may be beneficial. Therefore knowledge on the neuropharmacology of the spinal cord injury could be of great importance to provide a pharmacological basis for a rational choice of therapeutic agents in the management of patients suffering from impaired motor function after chronic spinal cord lesions.

We gratefully acknowledge J. Provancher and F. Lebel for assistance during surgeries, experiments, analyses, and preparation of the illustrations. We also thank P. Drapeau and G. Messier for programming, C. Gagner for electronic surgeries, experiments, analyses, and preparation of the illustrations. We also thank P. Drapeau and G. Messier for programming, C. Gagner for electronic surgeries, experiments, analyses, and preparation of the illustrations. J. Faubert for help during surgery, and J. Lavoie for histological assistance.

This work was supported by the Canadian Institutes for Health Research and the Spinal Cord Research Foundation (SCRF). N. Giroux was sponsored by studentships from the Neuroscience Network of Centers of Excellence of Canada, from the Rick Hansen Man in Motion Foundation (SJ-06), and from the Groupe de Recherche sur le Système Nerveux Central (GRSNC) of the Fonds Pour la Formation de Chercheurs et l’Aide à la Recherche.

REFERENCES

INTRATHECAL INJECTION OF CLONIDINE AND YOHIMBINIE


