Contribution of Hind Limb Flexor Muscle Afferents to the Timing of Phase Transitions in the Cat Step Cycle

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SUMMARY AND CONCLUSIONS

1. In this investigation, we tested the hypothesis that muscle spindle afferents signaling the length of hind-leg flexor muscles are involved in terminating extensor activity and initiating flexion during walking. The hip flexor muscle iliopsoas (IP) and the ankle flexors tibialis anterior (TA) and extensor digitorum longus (EDL) were stretched or vibrated at various phases of the step cycle in spontaneously walking decerebrate cats. Changes in electromyogram amplitude, duration, and timing were then examined. The effects of electrically stimulating group I and II afferents in the nerves to TA and EDL also were examined.

2. Stretch of the individual flexor muscles (IP, TA, or EDL) during the stance phase reduced the duration of extensor activity and promoted the onset of flexor burst activity. The contralateral step cycle also was affected by the stretch, the duration of flexor activity being shortened and extensor activity occurring earlier. Therefore, stretch of the flexor muscles during the stance phase reset the locomotor rhythm to flexion ipsilaterally and extension contralaterally.

3. Results of electrically stimulating the afferents from the TA and EDL muscles suggested that different groups of afferents were responsible for the resetting of the step cycle. Stimulation of the TA nerve reset the locomotor step cycle when the stimulus intensity was in the group II range (2–5 ×T). By contrast, stimulation of the EDL nerve generated strong resetting of the step cycle in the range of 1.2–1.4 ×T, where primarily the group Ia afferents from the muscle spindles would be activated.

4. Vibration of IP or EDL during stance reduced the duration of the extensor activity by similar amounts to that produced by muscle stretch or by electrical stimulation of EDL at group Ia strengths. This suggests that the group Ia afferents from IP and EDL are capable of resetting the locomotor pattern generator. Vibration of TA did not affect the locomotor rhythm.

5. Stretch of IP or electrical stimulation of TA afferents (5 ×T) during the flexion phase did not change the duration of the flexor activity. Stimulation of the EDL nerve at 1.8–5 ×T during flexion increased the duration of the flexor activity. In none of our preparations did we observe resetting to extension when the flexor afferents were activated during flexion.

6. We conclude that as the flexor muscles lengthen during the stance phase of gait, their spindle afferents (group Ia afferents for EDL and IP, group II afferents for TA) act to inhibit the spinal center generating extensor activity thus facilitating the initiation of swing.

INTRODUCTION

It has been demonstrated that the spinal cord alone, in the absence of afferent feedback, is capable of generating the rhythmic alternation of flexor and extensor activity that forms the basis of locomotion (Grillner and Zangger 1984). However, for the locomotor output to be adapted to suit the external environment it must be modulated by afferent feedback regarding the state of the moving limbs. Spontaneously stepping decerebrate (Shik et al. 1966) and stepping spinal cats (Forsberg and Grillner 1973) can adjust their locomotor rhythm to match the speed of the treadmill belt. The duration of the stance phase can vary greatly, being long at slow speeds and short at faster speeds. In contrast, the duration of the swing phase remains relatively constant over a large range of speeds (for review, see Grillner 1981). These observations suggest that afferent signals play an important role in the timing of the stance phase.

At the present time, there are two proposals regarding the afferent control of the timing of the stance phase to swing phase transition during the locomotor step cycle. One proposal is that an unloading of extensor muscles at the end of stance phase is necessary for the swing phase to begin. Duyens and Pearson (1980) observed in decerebrate walking cats that by loading the triceps surae muscle group of one limb, as the other three limbs freely stepped on a moving treadmill, the flexor activity could be prevented. Only when the extensor force was reduced below 40 N did flexor activity resume. More recently, it has been demonstrated that extensor group Ib afferent input from the force-sensitive Golgi tendon organs can inhibit ongoing flexor activity and reset the locomotor rhythm to extension in fictive spinal preparations (Conway et al. 1987). Furthermore, input from these afferents can entrain the locomotor rhythm (Conway et al. 1987; Pearson et al. 1992) and facilitate activity in extensor muscles (Pearson and Collins 1993). These observations have led to the conclusion that input from Ib afferents functions to maintain extensor activity while a limb is loaded during the stance phase of walking. However, a recent investigation has demonstrated that activation of extensor muscle spindle group Ia afferents during fictive locomotion in decerebrate cats also is capable of producing a prolongation in extensor activity (Guertin et al. 1995).

The second proposal is that hip extension during stance is a critical signal for the initiation of swing. Grillner and Rossignol (1978) observed in chronic spinal cats stepping on a moving treadmill that by blocking the extension of a limb they could prevent the transition to swing from taking place. If the limb then was allowed to extend, flexion typically occurred when the hip reached an angle of >90 degrees. Sinusoidal movements of the hip have been shown to entrain the fictive locomotor rhythm in spinal and decerebrate cats. During entrainment, the onset of flexor bursts...
occurs near the end of the imposed hip extension (Andersson and Grillner 1983; Kriellaars et al. 1994). The recent work of Kriellaars et al. (1994) demonstrated that afferents from the hip joint capsule were not required for the entrainment and indicated that the afferents from the muscles acting around the hip joint were responsible. The critical muscles and the specific afferents have not been determined.

In the present set of experiments, we tested a hypothesis related to the latter of these two proposals, namely that afferent signals generated by the lengthening of the hind-leg flexor muscles during stance promote the onset of flexor burst activity associated with swing. Ramp stretches of the hip flexor iliopsoas (IP) and ankle flexors tibialis anterior (TA) and extensor digitorum longus (EDL) were applied at various phases of the step cycle and the resultant changes in electromyographic (EMG) amplitude, duration, and timing were examined. We predicted that if stretch sensitive receptors in these flexor muscles are involved in the initiation of swing then stretch during the stance phase would reduce the duration of extensor activity and promote an earlier onset of flexor activity. The effect of Vibrating the flexor muscle and electrically stimulating the nerves to TA and EDL also were examined in an attempt to identify which muscle afferents influence the timing of the stance-to-swing transition.

METHODS

Eighteen adult cats (2.0−4.0 kg) were used in this series of experiments. All procedures were carried out with approval from the University of Alberta Health Sciences Animal Welfare Committee. Each cat was anesthetized with halothane and the trachea was cannulated for continued administration of the anesthetic. One of the carotid arteries was ligated, and a cannula to monitor the blood pressure was placed in the other. A cannula was inserted in one jugular vein for the administration of fluids and drugs. After the above initial procedure, one of two different experimental preparations was used. The procedure for each will be described separately.

Decerebrate locomotion with a hind leg immobilized (11 animals)

After the insertion of the various cannulae, the following nerves were transected in the right hind leg: saphenous, sartorius, obturator, hamstrings, superficial peroneal, deep peroneal, sural, and distal tibial. The patellar tendon of the right hind leg was cut and a portion of the calcaneum removed to free the triceps surae muscle group. The insertion of IP in the right leg was freed by removing a portion of the lesser trochanter of the femur. A thread was attached to the detached distal tendon of IP, passed between the posterior biceps and semitendinosus (ST) muscles and led from the animal through a cut in the skin of the posterior thigh (Fig. 1A). The TA and EDL tendons were also cut and tied to threads to allow later attachment to a muscle puller. Electrodes for recording EMGs were sutured into the muscle bellies of IP, TA, ST, vastus lateralis (VL), and medial or lateral gastrocnemius (MG or LG) of the right hind leg and into IP and/or ST and VL and/or LG of the left hind leg.

In three experiments, the FDI, nerve of the freely stepping left hind leg was implanted with a stimulating nerve cuff [see Wheelan et al. (1995) for details of these stimulating cuffs]. A recording cuff was implanted around the sciatic nerve to monitor the strength of stimuli to the EDL nerve.

To fix the back of the animal during the experimental procedure, a piece of stainless steel was secured by dental acrylic to the L6-S1 spinous processes (pins were placed in the base of these processes to attach the acrylic to the spine). The animal then was held by the back and by a stereotaxic head holder over a motor-driven treadmill and positioned so that limbs were in contact with the surface of the treadmill. The right hind leg was fixed above the treadmill surface at the knee and ankle. The angle of the hip, knee and ankle joints were held at ~90-deg angles (Fig. 1A). This preparation will be referred to as the immobilized or fixed limb (FL in the figures) preparation.

Decerebration was performed by transecting the brain stem at a 50-deg angle from the anterior edge of the superior colliculus and removing the brain. The anesthetic then was discontinued. About 1 h after decerebration, 8 of the 11 animals began to walk spontaneously with the three free legs on the moving treadmill (speed 0.25–0.5 m/s). Rhythmic activity in the right hind leg was coordinated with the stepping movements in the three stepping limbs. Spontaneous locomotion lasted between 1 and 4 h. When spontaneous locomotion was no longer reliably generated, an attempt was made to induce locomotion by electrical stimulation (15 Hz, 0.5-ms duration, 30–100 μA) of the mesencephalic locomotor region (MLR; coordinates P2, L4, H6) (Shik et al. 1966).

Decerebrate quadrupedal locomotion (7 animals)

In this preparation, the hind limbs were not extensively denervated and the patella and Achilles tendon were kept intact. This
allowed both hind legs to freely step on the treadmill. This preparation will be referred to as the quadrupedal (Q in the figures) preparation. In five of the experiments, the IP insertion of the right hind leg was freed and attached to a muscle puller (see above). The TA and EDL muscles could not be stretched in this preparation. In three animals, a stimulating nerve cuff was implanted around the cut TA nerve and a recording cuff was placed on the sciatic nerve. In one of the three animals with a TA nerve cuff, the EDL nerve of the contralateral hind leg was implanted similarly with a stimulating nerve cuff and a recording sciatic cuff. This allowed a direct comparison of the two nerves in the same animal.

**Activation of the flexor muscle afferents**

Flexor muscle afferents were activated either by stretching (7–8 mm for IP, 5–6 mm for TA and EDL, 50–100 ms rise time) or vibrating (150 Hz, 80 μm) individual muscles or pairs of muscles. The muscles were held at a background tension of 100 g. Postmortem dissection in two animal revealed that the lesser trochanter of the femur, which is the insertion point of the IP muscle, moves 10–12 mm as the hip joint is moved from flexion to extension. This is similar to the 9 mm length change of IP during the step cycle calculated by Goslow et al. (1973) for the shortest portion of the IP muscle. The natural change in length of TA and EDL during locomotion is ~6 mm (Prochazka et al. 1989). Therefore, we believe that the amplitude of stretch imposed during walking was within physiological parameters.

Muscle afferents also were activated using electrical stimulation (150–200 Hz, 200- to 500-ms trains) of the nerves to the TA or EDL muscles. The strength of the electrical stimulation applied to the nerves was measured as a multiple of the threshold strength, from a single shock, required to evoke the smallest observable potential in the sciatic nerve. The onset of the stimuli, electrical or stretch, occurred after a preset delay from the onset of EMG activity in ipsilateral VL or LG to examine the influence during extension or from the onset of activity in IP to examine the effects during flexion. The time of the stimulus onset was controlled via a Transduction DT12821 interface card installed into a Zenith 386 microcomputer. Custom written programs were used to recognize the onset of EMG bursts, to control the parameters of stretch and vibration, and to trigger the trains of electrical stimulation.

**Data analysis**

The data were recorded on VHS tape using a Vetter 4000A PCM recording unit. The set-up allowed us to record eight channels of data. Due to the limitation of available channels we were not able to simultaneously record all of the implanted EMGs. On occasions some of the implants did not generate a clear EMG and were therefore not used. Furthermore, due to changes in the viability of preparations it was not always possible to investigate all of the flexor muscles or nerves prepared for the experiment.

A hard copy of the data subsequently was displayed on a Gould ES1000 electrostatic chart recorder. Selected sequences were digitized and stored on disc using the Axotape data acquisition system (Axon Instruments). Custom written software capable of reading and displaying the Axotape data files was used to average and display the EMGs and to determine the cycle period and burst durations before and during the applied stimulus. The duration of extension was calculated as the time between the onset of extensor EMG activity and the onset of the following flexor burst. Any change in extensor EMG duration was calculated as the percent difference of perturbed step cycle compared to the preceding unperturbed step cycle \[100 \times (a - b)/a, \text{ see Fig. 1B}\]. Statistical analyses using Student’s t-test were performed to determine significant differences \((P < 0.05)\).

**RESULTS**

In 7 of the 11 preparations where one hind leg was immobilized, strong spontaneous stepping movements were evoked in the three unrestrained limbs in response to the moving treadmill belt. MLR stimulation was required to generate a stepping pattern in one animal. Three animals failed to generate any useful stepping sequences either spontaneously or in response to MLR stimulation.

Periods of spontaneous locomotion were observed in five of the seven decerebrate quadrupedal walking preparations. Stimulation of the MLR was used in an attempt to prolong locomotor activity after spontaneous stepping had ceased and to generate a locomotor rhythm in the two animals that did not demonstrate any spontaneous locomotion. In all quadrupedal preparations, MLR stimulation did not produce any coordinated locomotion.

**Flexor muscle stretch during stance phase**

In the immobilized limb preparations, an imposed stretch of either IP, TA, or EDL midway through the stance phase (100–200 ms after LG or VL onset) caused a reduction in extensor activity. There was a distinct inhibition in the amplitude of the EMG 20–40 ms after the onset of the stretch (Fig. 1B). Regardless of whether hip flexor (IP) or ankle flexor (TA, EDL) muscles were stretched, the magnitude of the bursts in the knee (VL) and the ankle (LG) extensor muscles was less than normal during the plateau phase of the stretch. Along with the decrease in extensor EMG amplitude, there was also a shortening of the duration of the extensor burst activity as compared with the normal burst duration. Similar results were obtained when the IP muscle was stretched in the quadrupedal walking preparation. However, the decrease in extensor EMG amplitude and burst duration was not as apparent during flexor stretch in the quadrupedal preparation as it was in the immobilized limb preparation.

Data summarizing the reduction in the duration of extensor activity resulting from stretch of the individual flexor muscles are presented in Fig. 2. In all experiments, stretch of each flexor muscle alone produced a significant reduction in the duration of the extensor activity \((P < 0.05)\). In this and subsequent figures, the data from fixed hind-limb preparations are identified with the prefix FL and the quadrupedal preparations by the prefix Q. In Fig. 2C, where the IP muscle is being stretched, note that the reduction of the extensor duration observed in the quadrupedal preparation, while still significant when compared with the normal extensor burst, was not as pronounced as the reduction observed for a similar stretch in the immobilized limb preparation (Fig. 2C). On average, stretch of the IP muscle in the four immobilized limb preparations reduced the extensor duration by 13%, whereas the reduction during quadrupedal locomotion was 5%.

Associated with the reduction in the duration of extensor bursts was an earlier onset of flexor activity (Figs. 1B and
Thus the stretch of the flexor muscles brought about an earlier extensor-to-flexor (stance-to-swing) transition. Although the onset of the flexor activity was earlier than normal, the latency from the onset of the stretch was still large (range 200–500 ms). This long delay in the onset of the flexor bursts was associated with activity in the contralateral flexors; the ipsilateral flexor burst could not be advanced by flexor muscle stretch to the extent that it overlapped significantly with the contralateral flexor burst (although a small overlap sometimes occurred, i.e., Fig. 4B). Figure 3C illustrates the relationship between the onset of the ipsilateral flexor burst (value Y in Fig. 3A) and the onset of the contralateral extensor activity (value X in Fig. 3A) after stretch of IP and TA muscles in a fixed-limb preparation. The latter value indicates the termination of contralateral flexor activity. The plot in Fig. 3C shows that the onset of the flexor burst after the muscle stretch typically occurred after the onset of the contralateral extension phase and hence after the termination of the contralateral flexor activity. The wide variation of the latency to the onset of ipsilateral flexor activity, apparent in Fig. 3C, was due to the variability in the effect on the contralateral flexor bursts. In some trials, there was a marked reduction in the duration of contralateral flexor bursts, as indicated by the reduced interval between contralateral extensor bursts illustrated in Fig. 3A. These trials were associated with the shortest latencies to the onset of ipsilateral flexor bursts (clustered around 300 ms in Fig. 3C).

In the same experiment as illustrated in Fig. 3A, we were able generate longer extensor bursts and create a larger period of overlap between the contralateral and ipsilateral extensors by slowing the speed of the treadmill. This created the opportunity to time the flexor muscle stretch to occur.
when the contralateral limb was not in flexion (Fig. 3B).
In the absence of contralateral flexor activity, the mean latency to the onset of ipsilateral flexor burst was reduced to 95 ± 10 ms (mean ± SD). In Fig. 3C, this value, represented by an asterisk, is plotted against a contralateral extensor latency of 0 (we chose this value because the contralateral extensors were active at the time of the stretch). Note that this minimum latency corresponds to the y-axis intercept of the regression line of the data obtained from trials represented by Fig. 3A (the onset of ipsilateral flexor and contralateral extensor activity when the stretch occurs during contralateral flexor activity).

Thus it appears that at least two opposing factors can influence the timing of the transition from extension to flexion. Stretch-sensitive flexor muscle afferents facilitate the activation of the flexor muscles, whereas the activity in the contralateral flexor burst generating system prevents their activation.

Electrical stimulation of afferents from ankle flexors during stance phase

The stretches we used to reset the locomotor rhythm would have activated many groups of afferents: group Ia and II afferents from muscle spindles, group Ib afferents from Golgi tendon organs, and group III stretch-sensitive afferents. To gain information as to which of these groups of afferents might contribute to resetting, we electrically stimulated the nerves supplying TA and EDL (no attempt was made to place stimulating electrodes on the IP nerves). Based on threshold measurements of identified afferents from the TA muscle (Jack 1978), we assumed that any effects just above threshold could be attributed to group Ia afferents, effects beginning in the range 1.3–1.5 xT could be attributed to group Ib afferents, and any effects of group II afferents would be seen in the range 2–5 xT.

Electrical stimulation of either the TA or EDL nerves (trains of 400–600 ms at 200 Hz) starting approximately midway through an extensor burst reduced the duration of the extensor bursts and promoted an earlier onset of flexor burst activity and contralateral extensor activity (Figs. 4 and 5). However, there was a clear and consistent difference between TA and EDL nerves in the stimulus strength required to produce this resetting. For the EDL nerve, shortening of the extensor bursts was observed at stimulus strengths of 1.2, xT and the effect was almost maximal at about 1.6 xT (Fig. 5, A–C). By contrast, TA nerve stimulation only produced shortening of the extensor bursts at strengths >3 xT, and the effect progressively increased as the stimulus strength was increased from 4 to 5 xT (Fig. 5, D–F). The average magnitude of the reduction in extensor burst duration produced by electrical stimulation of the EDL nerve (2 xT) or the TA nerve (5 xT) was usually in the range of 10–20%. Thus in general the effects produced by electrical stimulation of the nerves were comparable with the effects produced by muscle stretch (compare with data shown in Fig. 2). The only exception was observed in one quadrupedal walking preparation in which EDL nerve stimulation at low strengths produced exceptionally large (~40%) decreases in extensor duration (Fig. 5C). In the contralateral leg of

![FIG. 4. Electrical stimulation of EDL nerve (A) and TA nerve (B) resets locomotor rhythm in a manner similar to that produced by stretching muscles. Onset of hip flexor (IP) burst occurred earlier than normal—the arrows indicate where bursts would have occurred had rhythm not been modified by stimulus. A slight reduction in duration of the contralateral flexor EMG (cIP) also occurred. Note that stimulus threshold required for such resetting was different for 2 nerves. Traces for A and B are taken from 2 different Q preparations.

the same animal, electrical stimulation of the TA nerve had the usual modest effect over the range of 2–5 xT (Fig. 5F).

Vibration of flexor muscles

The fact that very low strength electrical stimuli to the EDL nerve promoted the onset of flexor bursts and reduced extensor burst duration suggested that these effects were produced largely by activation of group Ia afferents. To test this possibility we vibrated the EDL muscle in immobilized limb preparations (n = 4) to selectively activate the group Ia afferents (Brown et al. 1967). As expected bursts of vibration (150 Hz, 80 μm, 600 ms) delivered midway through the extensor bursts promoted an earlier onset of ipsilateral flexor activity (Fig. 6A).

Similar effects also were observed when the IP muscle was vibrated (Fig. 6B). As in the experiments where we stretched IP, the magnitude of the reduction produced by vibration in the immobilized limb preparation was distinctly greater than that observed in the quadrupedal preparation.

An unexpected observation was that vibration of the TA muscle also promoted an earlier onset of flexor activity (Fig. 6C). Electrical stimulation of the TA nerve indicated that group I afferents from TA had no influence on the locomotor rhythm. One explanation for this apparent contradiction is that the vibration was spreading to the nearby innervated EDL muscle. This was demonstrated in one preparation by cutting the TA nerve and then vibrating or stretching the denervated TA muscle. Vibration continued to reduce the extensor duration after the nerve was cut (Fig. 6C), whereas stretch no longer did (data not shown). Subsequent cutting
of the EDL nerve abolished all effects of TA or EDL vibration on the rhythm (Fig. 6C). Apart from indicating that the effect of vibration of TA was mediated by afferents in the EDL nerve, this observation showed that the effects of vibration of TA and EDL were not mediated by spread to more proximal muscles.

**Entrainment of the locomotor rhythm**

The observation that the locomotor rhythm could be reset by stretching flexor muscles (Fig. 3A) suggested that the rhythm could be entrained by repetitively stretching these muscles. Previous studies have shown that rhythmic movements of the hip joint are capable of entraining the fictive locomotor rhythm (Andersson and Grillner 1983; Kriellaars et al. 1994). However, in those experiments there was no phasic afferent feedback except for that resulting from the imposed movement of the hip. It was therefore of interest to examine whether the locomotor rhythm could be entrained in the presence of afferent feedback from the other three freely stepping limbs. The effect of rhythmically stretching the hip and ankle flexor muscles was investigated in three of the immobilized limb preparations in which a regular locomotor pattern was generated for long periods of time.

Provided that the frequency of the entraining stretch (a repeating saw-toothed pattern) was no more than 15% greater than the natural locomotor rhythm, the rhythm in the immobilized test limb could be entrained for periods of up to 40 s. No entrainment was observed when the frequency of the stretch was set lower than the natural rhythm. An example of entrainment is illustrated in Fig. 7 where IP and TA were stretched together. In Fig. 7B, the phase of onset of the flexor activity within the stretch cycle is plotted against time. Periods of entrainment are indicated by the regions where the phase of flexor onset remained relatively constant. This form of entrainment, where the onset of EMG activity occurs at a preferred phase and then eventually drifts away from the imposed rhythm, has been referred to as relative coordination (Horsmann et al. 1983). The progressive increase in phase, observed when the rhythm was not entrained indicates that the frequency of the imposed saw-tooth stretch was higher than that of the endogenous rhythm. Similar plots were obtained in another preparation for stretch of IP alone and in this and one other preparation when IP and EDL were stretched together. In none of our preparations was the rhythm sufficiently sustained and regular to test all combinations of flexor muscles and to compare the strength of entrainment with different combinations.

The timing of the locomotor activity during entrainment was such that the onset of the flexor EMG corresponded to a point near the end of the imposed stretch (see Fig. 7A). This indicates that the stretch of the flexor muscles was acting to bring about the initiation of flexor burst activity. Consistent with this was the observation that the duration of the extensor bursts during entrainment was reduced compared with the duration of extensor bursts when the rhythm was not entrained. Thus the reduction in the extensor burst duration created a slightly shorter step cycle period, which accounts for the increase in the frequency of the stepping rhythm. These observations are in accord with those of Andersson and Grillner (1983) and Kriellaars et al. (1994).

Rhythmic stretching of the flexor muscles of the immobilized limb also entrained the stepping pattern of the contralateral hind leg (Fig. 7B, bottom). This occurred despite the
stimulation of the nerves to TA, sartorius, and semitendinosus at 5 xT during flexor activity shortened the duration of activity in flexor nerves and evoked activity in ipsilateral extensor nerves (Perreault et al. 1995).

To examine this issue further, the IP muscle was stretched (2 quadrupedal preparations), or the TA (1 fixed limb and 3 quadrupedal preparations) or EDL (1 fixed limb and 1 quadrupedal preparation) nerve was stimulated electrically, during flexor activity. Stimulation of the EDL nerve at 1.4–2 xT during flexor activity increased the duration of the flexor bursts (measured from the IP burst) in both quadrupedal (Fig. 9A) and fixed limb experiments, though not to the 600-ms duration of the stimulus trains. However, with higher levels of stimulation (5 xT), the ipsilateral flexor EMG activity did last for the duration of the applied nerve stimulation (Fig. 9B). We did not test whether this duration could be extended further. The prolongation of the flexor burst duration was matched by a larger interval between contralat-

fact that the rhythm in the intact contralateral leg (and the rhythm of the forelegs) was strongly coupled to the speed of the moving treadmill belt. The rate of stepping in the contralateral leg increased during entrainment due primarily to a decrease in the duration of the extensor (stance) phase.

In one immobilized limb preparation, we were able to entrain the locomotor rhythm by repetitively vibrating the IP muscle (Fig. 8). When the repetition rate of the bursts of vibration was slightly higher than the natural stepping cadence, entrainment could last for substantial periods (~30 s). In accordance with our observations on the effect of repetitively stretching IP (Fig. 7), the timing of the flexor EMG bursts was such that during entrainment activity was initiated near the end of vibration. It should be noted that nearby hip and trunk muscles were not denervated. It is therefore likely that some afferents in these surrounding muscles also were excited by the vibration.

**Stimulation of flexor muscle nerves during swing phase**

Based upon the current observation that stretch-sensitive afferents from flexor muscles can facilitate the termination of extensor activity and facilitate the onset of flexor activity, we would predict that activation of flexor muscle afferents during flexion would facilitate and prolong flexor activity. However, in recent experiments, it has been reported that
cles are stretched during stance, one possible source for the
Grillner and Rossignol 1978). Because the hip flexor mus-

eral flexor bursts, indicating an increase in the contralateral
extensor burst durations.

Stretch of IP during flexor activity failed to produce any
change in the step cycle rhythm in any preparation: the du-
ration of flexor activity was not increased nor was there a
noticeable increase in the EMG amplitude (data not shown).
There was a similar absence of effect in response to electrical
stimulation of the TA nerve at 2–5 xT in three of the four
experiments. In the fourth preparation, there was no effect
at 2 xT. However, when TA was stimulated at 5 xT, the
duration of the flexor activity increased, though not for the
duration of the 600-ms stimulation. On average the duration
of flexor activity increased by 13 ± 12% of normal (data
not shown).

In none of the preparations did we observe the resetting
to extension when flexor afferents were stimulated during
flexor activity, as reported by Perreault et al. (1995). How-
ever, in one preparation, we did observe a brief inhibition
of IP activity just after onset of stimulation (1

DISCUSSION

Previous studies have shown that movements around the
hip joint can reset and entrain the fictive locomotor pattern
in spinal and decerebrate cats (Andersson and Grillner 1983;
Kriellaars et al. 1994). The characteristics of resetting and
entrainment have suggested that afferent signals arising from
hip muscles during extension could act to trigger the transi-
tion from stance to swing in a walking animal (see also
Grillner and Rossignol 1978). Because the hip flexor mus-
cles are stretched during stance, one possible source for the
afferent signal is stretch sensitive receptors in these muscles.
One of the main findings of the present study was that im-
posed stretches of the IP muscle during stance produced an
earlier onset of flexor activity (Figs. 1–3). We also found
that stretches of the ankle flexors TA and EDL produced
similar effects on the timing of flexor burst activity (Fig.
2). The general conclusion of this study is that feedback
from stretch-sensitive afferents in leg flexor muscles during
stance acts to facilitate the transition to flexor activity associ-
ated with the swing phase of the locomotor cycle.

Action of flexor afferents during extension

In general, the effects of stretching the hip and ankle flexor
muscles on the timing of the locomotor rhythm were modest.
Typically, the reduction in the duration of the extensor phase
in which a stretch was applied was between 10 and 20% (Fig.
2). We believe that the magnitude of the reduction in
the extensor phase usually was limited by strong mutual
inhibition between the flexor half-centres (for review of half-
center hypothesis for locomotion, see Lundberg 1980) of
the central rhythm generator (Orsal et al. 1990). In most
instances, the stretches were applied during the flexion phase
of the contralateral leg (Fig. 3A). Thus the ipsilateral flexor
burst could not be generated immediately after the onset of
the stretch because of inhibition from the contralateral flexor
half-center. Consistent with this conclusion is that the timing
of the onset of the ipsilateral flexor bursts was correlated
strongly with the termination of the contralateral flexion
phase (Fig. 3C). Furthermore, when stretches were applied
during the contralateral extensor phase there was a much
earlier onset of ipsilateral flexor activity and a corresponding
greater reduction in extensor duration (Fig. 3, B and C).

Theoretical considerations also support this conclusion. As-
suming that the step cycles for each limb are 180 deg out

FIG. 8. Entrainment of locomotor rhythm by vibration of IP muscle in
FL preparation. A: sample rectified and filtered EMG traces and imposed
vibrations of IP muscle showing parameters measured to calculate phase
of onset of flexor EMG activity. a: cycle period of entraining vibration, b:
time of onset of ipsilateral IP activity relative to onset of vibration. B: plot
showing phase of onset of ipsilateral flexor EMG bursts during 2 periods
of entrainment. Note that onset of flexor EMG activity during entrainment
occurred near end of vibration. This is similar to the timing of entrainment
when the muscles were stretched in another preparation (see Fig. 7).

FIG. 9. Electrical stimulation of flexor muscle afferents during the
flexion phase can prolong the duration of flexor activity. A: stimulation of
EDL at 2 xT increased flexor duration, although not for full duration of
stimulus train. B: stimulation at 5 xT prolonged duration of flexor activity
for duration of stimulus. Note the short period of inhibition of the flexor
activity just after onset of stimulation (1).
The extensor half-center (pathway 1 in Fig. 10) and that the flexor stretch-sensitive afferents act by inhibiting the onset of flexor bursts evoked during the contralateral extensor phase (95 ms; Fig. 3C). These observations suggest that the flexor burst duration, when the stimulus onset occurs during stretch of the TA muscle group, is no change in flexor duration of the contralateral leg). This value is close to the average reduction in extensor burst duration observed during stretch of IP, TA, and EDL in our immobilized limb preparations (Fig. 2B). The strong inhibitory influence of the contralateral flexor system also has been documented in another study that showed that flexion responses to unexpected perturbations during locomotion cannot be initiated if the contralateral limb is in swing phase (Gorassini et al. 1994). However, the inhibitory action between flexor burst generators can not be considered to be absolute. In the present study, while a synchronous activation of flexors from both hind limbs never occurred, the early flexor activity in the test limb did occasionally overlap with the end of flexor activity in the contralateral limb (see Fig. 4B). This could represent a transient change from the alternating gait of walking to an in-phase gait such as galloping (Wetzel and Stuart 1976).

An unresolved issue is the mechanism by which the stretch-sensitive flexor afferents influence the locomotor rhythm generators. One clue is that stretch of the flexor muscles and electrical stimulation of flexor nerves produced a sustained inhibition of extensor activity with a latency of -30 ms. This latency is much less than the latency to the onset of flexor bursts evoked during the contralateral extensor phase (95 ms; Fig. 3C). These observations suggest that the flexor stretch-sensitive afferents act by inhibiting the extensor half-center (pathway 1 in Fig. 10) and that the earlier onset of flexor activity is a secondary consequence of less inhibition from the extensor to the flexor half-center. Of course, this does not exclude the possibility of a direct excitatory action on the flexor half-center (pathway 2 in Fig. 10). In addition to influencing the timing of the rhythm in the ipsilateral leg, stretching the flexor muscles and electrically activating flexor muscle afferents usually reduced the duration of contralateral flexor phase (Figs. 3 and 4). The simplest explanation for this is an inhibitory connection to the contralateral flexor half-center (pathway 3 in Fig. 10). Alternatively, the earlier onset of ipsilateral flexor activity may prematurely terminate the contralateral flexor bursts by the inhibitory pathway from the ipsilateral to contralateral flexor half centers (pathway 4 in Fig. 10).

Identity of afferents promoting onset of flexor bursts

A number of observations indicate that afferents arising from the spindles of the flexor muscles were responsible for reducing extensor duration and promoting the early onset of flexor activity. First, the reduction in extensor duration produced by EDL during electrical stimulation occurred at low strengths (1.2 xT). These stimulus levels would activate preferentially primary (group Ia) spindle afferents. Consistent with this was the observation that vibration of EDL also promoted an earlier onset of flexor activity, with the magnitude of the effect being similar to that produced by stretch (Figs. 2 and 4). Second, vibration of IP also promoted an earlier onset of flexor bursts, and repetitive bursts of vibration to this muscle could entrain the locomotor rhythm (Figs. 4 and 8). These observations suggest that the group Ia muscle spindle afferents from IP and EDL were generating the resetting effects.

For TA, however, our data indicate that the secondary (group II) muscle spindle afferents must also be activated to promote the early onset of flexor activity when TA is stretched. First, resetting of the locomotor rhythm by group Ia afferents alone can be excluded because vibration of TA had no effect on the timing of the rhythm (Fig. 6C) and no effects on timing were observed when the TA nerve was stimulated to maximally activate group I afferents (~2 xT) (Jack 1978) (Fig. 5, D–F). Second, group II muscle spindle afferents are progressively recruited over a range of stimulus strengths from 2–5 xT, which corresponds to our observation of a progressively larger influence of TA nerve stimulation as the strength was increased over this range (Fig. 5, D–F). Although these observations suggest that the activation of the secondary group II muscle spindle afferents are responsible for TA's ability to reset the locomotor rhythm, they do not preclude the possibility that the primary group Ia spindle afferents may have a subthreshold effect. Therefore, we conclude that during stretch of the TA muscle group II muscle and possibly group I afferents are responsible for promoting the transition from extension to flexion.

The reason why the effects of TA and EDL stimulation are mediated by different sets of afferents is unclear in that both muscles have a similar function in flexing the ankle joint, with EDL having the additional role of extending the phalanges (toes). However, it has been shown that the primary (group Ia) muscle spindle afferent discharge that oc-
curs at the end of stance phase is larger for EDL than for TA (Loeb and Duysens 1979). It also has been shown that the EDL nerve has a slightly greater divergence of monosynaptic connectivity onto other synergistic ankle flexors than the TA nerve (Eccles et al. 1957). It has been suggested that divergence of the monosynaptic reflex is mirrored by a parallel divergence onto interneurons mediating reciprocal inhibition of antagonistic motoneuronal pools (Baldissera et al. 1981; Hultborn and Udo 1972). On the basis of these observations, the Ia afferents from EDL also might have a greater inhibitory effect on interneurons of the extensor half-center.

Whether or not spindle afferents (group Ia and/or II) are solely responsible for the resetting effects of flexor muscle stretch is unknown. However, we consider it unlikely that input from the flexor muscle group Ib afferents, from force-sensitive Golgi tendon organs, is involved. In our experiment, the stretch of the flexor muscles was applied during the period of extensor activity, i.e., at a time when the flexor muscles were inactive. Because group Ib afferents respond primarily to forces produced in actively contracting muscles (Houk and Henneman 1967), it is unlikely that the flexor group Ib afferents would have been activated strongly by the muscle stretches.

Another possibility is that higher threshold group III afferents could facilitate the generation of flexor bursts because some are known to be stretch sensitive (Mense and Meyer 1985), and these afferents are usually included in the flexor reflex afferents system (McCre 1992). However, the observation that the magnitude of resetting was similar when flexor muscles were stretched and when flexor nerves were stimulated at strengths below that necessary to activate group III afferents suggests that the effect of these afferents during stretch is probably minimal.

Action of flexor afferents during flexion

The action of flexor muscle afferents on the timing of the locomotor rhythm has been examined in relatively few studies. Conway et al. (1987) reported that stimulation of the group I afferents from flexor afferents (exact nerves unspecified) did not produce phase shifts of the locomotor rhythm. A recent and more detailed investigation of flexor afferents also reported that stimulation of the group I afferents from flexor nerves during flexor activity did not alter the fictive locomotor rhythm (Perreault et al. 1995). This is consistent with our observation that stimulation of group I afferents from TA and that stretch of the IP muscle had no effect on flexor burst duration. However, in the present investigation we did observe that stimulation of EDL group I afferents during flexor activity consistently prolonged the duration of the flexor bursts (Fig. 9A). The effect of stimulating afferents in the EDL nerve was not investigated in previous studies.

A major finding of Perreault et al. (1995) was that stimulation of the group II afferents from TA, sartorius, and semitendinosus during flexor activity consistently reset the locomotor rhythm to extension. We were surprised to observe that stimulation of TA nerve group II afferents, in three of four experiments, resulted in no change in the duration of flexor activity, and that in one experiment stimulation of TA nerve at 5X prolonged the flexor burst in other flexor muscles. Furthermore, stimulation of EDL group II afferents during flexion produced a larger prolongation of flexor burst duration than that seen when the group I afferents were stimulated alone: typically the flexor burst was prolonged until the end of the stimulus train (Fig. 9B). Thus in summary, our data in decerebrate walking animals indicate that flexor muscle afferents have either no effect or an excitatory effect on flexor burst generation.

At present, we cannot account for the difference between our results and those of Perreault et al. (1995). However, it may be related to differences in the experimental preparations. The preparation used by Perreault et al. (1995) was immobilized and locomotion was evoked solely by electrical stimulation of the mesencephalic locomotor region. Interestingly, in one of our preparations, we did observe a transient inhibition of flexor activity in response to stimulating the EDL nerve at 5 X (Fig. 9B). This may reflect the inhibitory pathway described by Perreault et al. It is possible that in our walking preparations this inhibitory pathway is weaker and can be overwhelmed by excitation mediated by other pathways.

Integration of proprioceptive signals

The primary objective of this investigation was to establish whether stretch-sensitive afferents in the hind-leg flexor muscles could be involved in triggering the transition from stance to swing. Although our data support this proposal, it is clear that other processes also regulate the timing of the swing phase onset. As mentioned in the introduction, the most prominent of these is the load carried by the extensor muscles. Numerous studies now have shown that activation of group I afferents in extensor muscles during stance inhibits the generation of flexor burst activity and promotes extensor activity (Conway et al. 1987; Guertin et al. 1995). In particular, there is evidence that a necessary condition for swing to be initiated is an unloading of the leg extensor muscles (Conway et al. 1987; Pearson and Collins 1993). Muscle loading is signalled mainly by feedback from the force-sensitive Golgi tendon organs. Therefore, for the stretch-sensitive afferents in the flexor muscles to have a facilitatory action in promoting swing, the load carried by the leg must be below some critical level.

Consistent with this idea was our finding that stretching the IP muscle during quadrupedal walking produced a smaller reduction in the duration of extensor activity than that produced when the leg was immobilized (Fig. 2C). During quadrupedal locomotion, the extensor muscles of the test limb were being phasically loaded. As stated above, force feedback during locomotion promotes extensor activity. Thus for any reduction in extensor duration to occur, the inhibition produced by the stretch-sensitive afferents from the flexor muscles must be greater than the excitation produced by loading of the extensor muscles. In the fixed-leg preparation, the latter was minimal because the tendinous insertion of the quadiceps and triceps surae muscle groups had been cut. It follows therefore that in this preparation activation of the flexor afferents would be expected to have
a more potent effect in reducing the extensor duration, as indeed we observed.

Additional support for this conclusion is that stretching the ankle extensors, or electrically stimulating the nerves to these muscles, in an immobilized leg preparation (thereby artificially supplying force feedback) (see Hiebert et al. 1995) reduces the influence of flexor muscle afferents on the locomotor rhythm (unpublished observations). A key issue for future research will be to evaluate quantitatively the interaction between these two systems in regulating the stance-to-swing transition, rather than examining each system in isolation.

Figure 10 summarizes the factors we have discussed that can influence the timing of the transition from extension to flexion during locomotion: inhibition of the extensor half-center (1) and excitation of the flexor half-center (2) by stretch-sensitive afferents in flexor muscles, mutual inhibition of the flexor half-centres (3), crossed inhibition of the contralateral flexor half-center (4), and indirect inhibition of the flexor half-center by the Golgi tendon organs of extensor muscles reinforcing the extensor half center (5). Clearly the scheme illustrated in Fig. 10 provides only a partial summary for the mechanisms that normally function to regulate the initiation of swing. It also must be considered that cutaneous afferents may contribute to the control of phase transitions in the step cycle. There are numerous examples of the cutaneous afferent input modifying the locomotor pattern (Andersson et al. 1978; Drew and Rossignol 1987; Duysens 1977; Duysens and Pearson 1976; Wand et al. 1980).

Also not illustrated in Fig. 10 are the possible connections to and contributions of supraspinal structures. Given the long latency of the inhibition of the extensor EMG (20–40 ms) and the even longer latency to the onset of the flexor activity (95 ms; see Fig. 3C) the activation of supraspinal structures could be involved in generating the effects documented in the present study. For example, in decerebrate animals signals from the reticular formation and red nucleus can strongly influence the flexor burst generating system (Armstrong 1986) and in intact animals descending signals from the motor cortex have also been shown to facilitate flexion (Armstrong 1986; Drew 1991, 1993).

Finally, the overall role of peripheral feedback in the regulation of the walking pattern may be very dependent on the type of preparation used (i.e., fictive locomotion compared to decerebrate walking compared with intact walking). We already have examples of different responses produced in different preparations. One example from this study is that stimulation of the ankle flexors during flexor activity can prolong the duration of the flexor bursts, whereas stimulation in a fictive preparation generates a resetting to extensor activity (Perreault et al. 1995). Another is the absence of effect of extensor group la afferents on the locomotor rhythm in spinal animals (Conway et al. 1987; Pearson et al. 1992) and a marked effect on the fictive rhythm in decerebrate preparations (Geurtin et al. 1995).

An important problem now is to establish relative weighting of phase-dependent afferent inputs in regulating the locomotor cycle. Interestingly, the same need is being addressed by control engineers, as they attempt to control increasingly complex robotic systems, including neural prostheses (Driankov et al. 1993; Popovic 1993). Rule-based control systems have been very successful in this regard, for example in quantifying the relative importance of different types of sensor. The identification of the sensorimotor rules governing step cycle transitions such as those described in this paper may facilitate the development of these rule-based control systems (Prochazka 1995).

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