Sensory Integration in Presynaptic Inhibitory Pathways During Fictive Locomotion in the Cat

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Ménard, Ariane, Hugues Leblond, and Jean-Pierre Gossard. Sensory integration in presynaptic inhibitory pathways during fictive locomotion in the cat. J Neurophysiol 88: 163–171, 2002; 10.1152/jn00615.2001. The aim of this study is to understand how sensory inputs of different modalities are integrated into spinal cord pathways controlling presynaptic inhibition during locomotion. Primary afferent depolarization (PAD), an estimate of presynaptic inhibition, was recorded intra-axonally in group I afferents (n = 31) from seven hindlimb muscles in Lc–S1 segments during fictive locomotion in the decerebrate cat. PADs were evoked by stimulating alternatively low-threshold afferents from a flexor nerve, a cutaneous nerve and a combination of both. The fictive step cycle was divided in five bins and PADs were averaged in each bin and their amplitude compared. PADs evoked by muscle stimuli alone showed a significant phase-dependent modulation in 20/31 group I afferents. In 12/20 afferents, PAD reduction was obtained in 4/5 bins i.e., for most of the duration of the step cycle. These effects were seen in group I afferents from all seven muscles. On the other hand, we found that different cutaneous nerves had quite different efficacy: the superficial peroneal (SP) being the most efficient (85% of trials) followed by Saphenous (60%) and caudal sural (44%) nerves. The results indicate that cutaneous interneurons may act, in part, by modulating the transmission in PAD pathways activated by group I muscle afferents. We conclude that cutaneous input, especially from the skin area on the dorsum of the paw (SP), could inhibit presynaptic inhibition in some group I afferents during perturbations of stepping (e.g., hitting an obstacle) and could thus adjust the influence of proprioceptive feedback onto motoneuronal excitability.

INTRODUCTION

Sensory feedback can adapt the timing and magnitude of the motor activities of stepping to the physical constraints of the environment (cf. Rossignol 1996). Presynaptic inhibition of primary afferent terminals may be the initial mechanism allowing the selection of relevant sensory information in the adaptation of stepping movements in several species (cf. Nusbaum et al. 1997). Presynaptic inhibition is known to be induced by GABAergic spinal interneurons that depolarize primary afferent (PAD) terminals and to decrease transmitter release (Eccles et al. 1961; Nicoll and Alger 1979; Schmidt 1971). Other mechanisms and neurotransmitter systems have also been reported to contribute to presynaptic inhibition (cf. Rudomin and Schmidt 1999). There is also cumulating evidence for an important role of proprioceptive feedback in humans (Dietz et al. 1985; Sinkjaer et al. 1996, 2000; Stephens and Yang 1998) and for presynaptic inhibition of IAs in the control of walking patterns (Faist et al. 1996; Morin et al. 1984; Simonsen and Dyhre-Poulsen 1999; Stein and Capaday 1988; Yang and Whelan 1993).

Sensory inputs are particularly potent in activating the interneurons of the PAD pathways. When various muscle and cutaneous afferents activate PAD interneurons projecting to terminals of afferents of their own sensory modality, presynaptic inhibition could constitute a negative feedback suppressing trivial inputs (Schmidt 1971). However, the giving and the receiving primary afferents can also feature different modalities. Indeed, PAD interneurons can receive inputs from multiple peripheral sources, which may be excitatory or inhibitory (Rudomin and Schmidt 1999; Schmidt 1971). A convergence of multimodal sensory inputs could be an efficient way to modulate presynaptic inhibition. It is thus important to learn more about the interaction between different sensory afferent systems on PAD pathways.

Of particular interest to us are the convergence of inputs from cutaneous and muscle afferents of large diameter because they are the first to reach spinal cord networks and the regulation of transmission in muscle group I afferents, which synaptic action is of prime importance for the adjustment of motoneuronal excitability and stepping patterns. However, only very limited information is available on how presynaptic inhibition is regulated by sensory interaction. In the anesthetized cat (Enriquez et al. 1996), of the 12 group IA fibers studied, stimulation of cutaneous sural afferents decreased the PAD evoked by stimulating a hip flexor nerve [posterior biceps-semimembranosus (PBSm)] in 11 afferents and increased it in one. Among the 13 group IB fibers tested, sural conditioning stimuli decreased the PAD evoked by PBSm in 8 and increased it in 5. Another investigation on the soleus H reflex in humans reported that activation of the low-threshold receptors of the...
skin of the distal foot reduced presynaptic inhibition of soleus IA afferents and even more efficiently during plantar flexion (Iles 1996).

Sensory integration in spinal cord pathways is best described in anesthetized cats, but its operation during locomotion, or any other movements, remains obscure. We know that transmission in PAD pathways activated by sensory inputs (Gossard and Rossignol 1990; Gossard et al. 1991; Nusbaum et al. 1997; Rossignol et al. 1997) or supraspinal inputs (Leblond et al. 1999) is modulated by the central pattern generator (CPG) for locomotion and by the CPG for scratching (Côté et al. 2000). Our previous work (Ménard et al. 1999) showed that the amplitude of PAD in muscle group I afferents, evoked by muscle nerve stimulation, is modulated in a phase-dependent manner during fictive locomotion. Based on these observations, the present study thus focuses on the more complex problem of sensory interaction in PAD pathways during fictive locomotion and whether this interaction changes with different cutaneous nerves. Some of these results have been previously reported in abstract form (Ménard et al. 1998).

**METHODS**

**Surgical procedures**

Thirty-four adult cats of either sex (2.5–4.5 kg) were used for these experiments, which were conducted in compliance with the Guide for the Care and Use of Experimental Animals (Canada) and the Ethics Committee of Université de Montréal. Details of the preparation can be found in a previous publication (Ménard et al. 1999). In brief, the animals were anesthetized with a mixture of halothane (2–3%) oxygen (50%), and nitrous oxide (50%) during the time of the surgery, which included insertion of cannulae for monitoring blood pressure or for injection in the internal carotid arteries. Animals were anesthetized with a mixture of halothane (2–3%) oxygen (50%), and nitrous oxide (50%) during the time of the surgery, which included insertion of cannulae for monitoring blood pressure or for administration of fluids, nerve dissection, lumbar laminectomy (L2–L4) and craniotomy. Cats were then decerebrated with a transection at the preoccipital postmammillary level and removal of all rostral brain tissue (Leblond et al. 2000; Ménard et al. 1999).

The following muscle and cutaneous nerves from the left hindlimb were dissected: PBSt, semimembranous-anterior biceps (SmAB), lateral gastrocnemius-soleus (LGS), medial gastrocnemius (MG), plantaris (Pl), flexor digitorum longus and flexor hallucis longus together (FDHL), tibialis anterior (TA), extensor digitorum longus (EDL), superficial peroneus (SP), caudal cutaneous sural (CCS), saphenous (Saph). All these nerves were dissected free but not cut except for PBSt, SmAB, and CCS. The nerve Saph was mounted in a polymeric cuff electrode.

After decerebration, anesthesia was discontinued, and the cats were paralyzed with pancuronium bromide (Pavulon; 0.2 mg/kg supplemented every 45 min) and artificially ventilated (pCO₂ maintained around 4%). Pools filled with warm mineral oil were constructed around the spinal cord and around hindlimb nerves, which were mounted on bipolar electrodes for recording (electroneurogram, ENG) and stimulation. Fictive locomotion was either occurring spontaneously following decerebration (n = 20), evoked by the stimulation of mesencephalic locomotor region (MLR) (n = 5), or induced by injection of nialamide (50 mg/kg) and L-dihydroxyphenylalanine (LDOPA) (150 mg/kg) in decerebrate/spinal cats at T₁₃ (n = 9) (Jankowska et al. 1967a,b).

**Recording and stimulation**

Stimulus intensity was expressed as a multiple of the threshold (T) for the most excitable fibers in the nerve that is the stimulus strength required to just evoke a deflection in the cord dorsum potential. Figure 1 is a schematic illustration of the neuronal circuitry investigated in this study. Stimulation of a muscle afferent activates primary afferent depolarization (PAD) interneurons (PAD INS) contacting terminals of a group I muscle afferent impaled by a micropipette. Cutaneous and muscle afferents are stimulated electrically to see how they interact when PAD interneurons are driven by the central pattern generator (CPG) for locomotion.

Intra-axonal recordings of PADs were performed with glass micropipettes (10–25 MΩ) filled with K⁺ acetate (3 M) and inserted into the dorsal columns. Only axons with ≥40 mV spike, a membrane potential no less than −40 mV, and a stable DC signal were accepted for study. Axons were identified as group I muscle afferents according to specific physiological criteria (Gossard 1996; Ménard et al. 1999): threshold for activation (<2.0 T), the ability to follow electrical stimulation of the muscle nerve at high frequency (>500 Hz) with a short and constant latency and the absence of a prepotential on the evoked spike, and conduction velocity as estimated by dividing the nerve length, measured at the end of the experiment, by the latency of the response. When possible, group IA and IB fibers were identified by their distinct firing responses to a twitch contraction evoked by a direct electrical stimulation in different loci in the (curarized) muscle belly (Matthews 1972). A clear pause in the firing rate of the axon suggests that it is a IA fiber, whereas a sudden increase in firing rate suggests that it is a IB fiber. To investigate the interaction between muscle and cutaneous afferent inputs (question mark in Fig. 1), we compared the PAD amplitude before, and when preceded by, stimulation of cutaneous afferents (CCS, SP, and Saph) given at T and S (2 and 3 p, 300 Hz). Because the maximal effect produced by PAD interneurons occurs ~20 ms (Eccles et al. 1962, 1963), the cutaneous stimulus preceded the muscle stimulus by such an interval. Similar stimulation protocols have been frequently used in previous work on PAD pathways (e.g., Enriquez et al. 1996; Jiménez et al. 1988; Lund et al. 1965; Rudomin et al. 1983, 1986; Willis et al. 1976) also because it excludes from the PAD measurement the important extracellular field potential evoked by some cutaneous nerves (see Fig. 3A). All the stimuli were delivered alternatively allowing the comparison within the same locomotor episode. More specifically, the muscle stimulus alone was followed by the combined cutaneous and muscle stimuli and, once every other, by the cutaneous stimulus alone (Fig. 2). Extracellular fields evoked by the same stimuli were recorded with the micropipette...
Data collection and analysis

Data suited for analysis were digitized and analyzed with interactive custom-made software (Spinal Cord Research Center, University of Manitoba, Winnipeg, Canada). The fictive step cycle, defined as two successive bursts of ENG activity in flexors, was normalized and divided into five equal portions or “bins.” Responses to the different stimuli occurring in similar bins were respectively averaged together (cf. Fig. 3A). The retained runs had averages of ≥10 responses per bin in a majority of bins. Phase plots of the integrated amplitude of the averaged responses were constructed (cf. Menard et al. 1999). The amplitude of PADs, of PADs preceded by cutaneous input, and cutaneous responses (PAD or primary afferent hyperpolarization, PAH), were plotted in the same phase plot as separate curves (e.g., Fig. 3B). Averaged rectified ENG activities of a flexor and an extensor nerve were aligned with the plot to identify the fictive step cycle’s different phases (e.g., Fig. 3B), which were also simply illustrated as F and E boxes for clarity in Figs. 4 and 5.

At first, we performed the Kolmogorov-Smirnov-Liliefors statistical test, and it confirmed that some values were nonparametric. Subsequently, the Mann-Whitney U test was used to compare in each bin the amplitude of PADs evoked by muscle stimuli alone to PADs preceded by the cutaneous input. A significant (P < 0.05) difference in PAD amplitude is indicated by a star in phase plots and by ▲ and in the results illustrated in Fig. 6. In Fig. 6, ▼ indicates a reduction of PAD amplitude by the cutaneous input, whereas ▲ indicates an increase in PAD amplitude. To determine if there was a significant phase-dependent modulation in PAD amplitude, the same statistical tests were used to compare the largest PAD amplitude with values of every other bin. When a significant difference was found, the bin with the maximal PAD amplitude was represented in the

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PADs were recorded in a total of 86 muscle afferents. Because of the variability in PAD responses and fictive stepping patterns, only the best sequences of recordings were kept for analysis which included extensive statistical tests. Adequate stimulated sequences of PADs were retained from 31 muscle group I afferents from seven muscles (6 LGS, 5 Pl, 9 FDHL, 5 TA, 2 EDL, 2 PBSt, and 2 SmAB) recorded during fictive stepping occurring spontaneously (n = 8), induced by MLR stimulation (n = 2) or following L-DOPA injection in spinal cats (n = 3). No detectable differences were found between decerebrate and spinal preparations although this may be due to the limited number of cats. Figure 2 gives an example of the recorded signals obtained during an episode of spontaneous fictive locomotion that can be seen as alternating ENG activities in an extensor (SmAB) and a flexor (TA) nerve. It also shows the intra-axonal recording of a group I afferent from SmAB in which responses were evoked by the interleaved stimuli falling at random times along the fictive step cycle. The resulting axonal responses are presented with a shorter time scale as traces titled 90° above the intra-axonal signal. The PADs (arrow) produced in response to PBSt nerve stimulation (3 p, 2 T, 300 Hz) are traces in black while responses to the stimulation of cutaneous nerve SP (1 p, 2 T) alone are traces in light gray and responses to the combined stimuli are traces in dark gray.

Figure 3 shows a representative example of the most common effect produced by cutaneous input on PADs during the different phases of the fictive step cycle. Traces in black are axonal responses evoked by PBSt (3 p, 2 T, 300 Hz) stimuli, traces in dark gray, responses evoked by muscle stimuli preceded by a SP stimulus (1 p, 2 T) and traces in light gray, responses to the SP stimuli alone. For each bin, the three traces are superimposed. It is quite obvious that the SP stimulus decreased the amplitude of PADs (in dark gray) as compared with controls (in black) in each of the five bins. Note that the SP volley evoked an important negative potential field (trough) at the beginning of the axonal recording (cf. Lund et al. 1965; Mendell 1972), but because it preceded the PAD by 20 ms, this field was completely excluded from the measurement of the PAD amplitude. The integrated amplitude (area under the averaged trace) was measured for each of the evoked PAD, averaged for each bin and compared in a phase plot (see METHODS) as three different curves (with the corresponding color) as shown in Fig. 3B. From these curves, we can follow the changes in PAD (or PAH) amplitude over the normalized cycle that begins with the onset of the flexor phase as depicted by the averaged ENG activity in TA nerve under the graph. First, it is clear that the amplitude of PADs (top curve in black) was modulated over the step cycle (cf. Ménard et al. 1999). It reached a maximum at the beginning of the flexor phase (circle) and decreased (2nd–4th bins) during most of the extensor phase (represented by the ENG activity in MG). Second, the amplitude of the SP-conditioned PADs (curve in dark gray) was clearly decreased as compared with PADs as evidenced by the position of this curve under the control one (in black). A significant decrease in PAD amplitude caused by SP is indicated by a star in all five bins. Note that the epoch (bin) for maximal PAD amplitude (circles in 1st bin) was not modified by the additional cutaneous input. Because the cutaneous-related reduction in PAD is more or less constant throughout the cycle, the patterns of phase-dependent modulation are quite similar in both cases. Third, the bottom curve in light gray shows that the cutaneous stimuli alone evoked PAHs (Mendell 1972; Rudomin et al. 1974) in the first two bins with a maximum amplitude (circle) occurring in the second. The same cutaneous stimuli were able to evoke large dorsal root potentials (Gossard and Rossignol 1990; Gossard et al. 1990; Schmidt 1971) in a Lc dorsal rootlet (not shown) and thus was able to evoke PADs in other types of afferents. However, in this Pl group I fiber, the SP stimulus decreased the PAD amplitude at the beginning of the step cycle. Note that the patterns of CPG-dependent modulation of PAD (in black) amplitude and of PAH amplitude (in light gray) are quite different. Note also that, even though PAHs are evoked only in the first two bins, that the cutaneous stimuli decreased PAD amplitude significantly in all five bins.

When possible (see METHODS), group I afferents were further...
characterized as IA \((n = 8)\) or IB \((n = 4)\) fibers. In Fig. 4, we compare the action of SP input in a IA and a IB fibers from the same muscle (LGS) during fictive locomotion. The patterns of phase-dependent modulation in PAD amplitude (evoked by PBSt; 3 p, 2 T, 300 Hz) are quite similar for both fibers (curves in black) with a maximum occurring in the first bin (circles). Also, in both fibers, the amplitude of PADs was significantly reduced (curves in dark gray) by the SP stimulus (1 p, 2 T). Even though there are slight differences in the magnitude of PAD reduction from bin to bin in the two fibers, the overall pattern is the same, i.e., a significant decrease in PAD amplitude in all five bins (as indicated by stars) that was especially important during the extensor phase when it becomes close to zero. Also, the occurrence of maximal PAD amplitude remained in the first bin (circles). Note that the SP stimulus alone evoked small PAHs mostly during the first two bins in both types of fibers. Overall, cutaneous input decreased PAD amplitude in 4/8 IAs and in 3/4 IBs.

The analysis of all group I afferents pooled together revealed that cutaneous volleys could significantly modify the amplitude of PADs evoked by muscle stimuli in at least one part (bin) of the step cycle in 17/31 (55%) of group I afferents. The most common effect (13/17) was a decrease in the PAD amplitude (by 35% on average), whereas it was increased by 17% on average in the others (4/17). The analysis further indicated that PADs evoked by muscle stimuli showed a phase-dependent modulation of their amplitude in 20/31 (65%) muscle group I afferents, thus supporting the idea that the transmission in PAD pathways is phasically modulated by the CPG for locomotion (cf. Ménard et al. 1999). Also, in 12 of these 20 afferents, there was a significant phase-dependent modulation of PAH amplitude \((n = 9)\) or of PAD amplitude \((n = 3)\) in response to cutaneous volleys.

We further inquired whether different cutaneous nerves, innervating different skin areas of the hindlimb, produced specific interaction patterns with muscle input in PAD pathways. We thus combined the muscle stimuli with the stimulation of two to three different cutaneous nerves within the same
axonal recording and succeeded to do so in 11 group I afferents. In Fig. 5, A and B, we compare the effects of CCS and Saph stimulation on the PAD evoked by PBSt (3 p, 2 T, 300 Hz) in a LGS group I afferent. The organization of phase plots is the same as in Fig. 3B. First, there was a phase-dependent modulation of PAD amplitude with the minimum occurring in flexion (1st bin) and the maximum occurring in extension (circle). Second, both CCS (Fig. 5A) and Saph (Fig. 5B) stimulation significantly decreased the amplitude of PADs in all five bins (as indicated by the stars). Except for a small difference in the bin for maximal PAD, the two cutaneous nerves had a similar effect, i.e., a clear decrease in PAD amplitude in all five bins. Both CCS and Saph stimuli evoked PAHs in that axon, but statistical analysis showed a significant phase-dependent modulation only with Saph stimulus with a maximum PAD amplitude occurring in the third bin (circle).

Again, note that the patterns of CPG-dependent modulation of PAD amplitude (in black) and of PAH amplitude (in light gray) were quite different. Figure 5, C and D, shows another comparison of cutaneous action on PADs recorded in a group I afferent from SmAB, a hip extensor. The amplitude of PADs evoked by PBSt (3 p, 2 T, 300 Hz) is phasically modulated along the fictive step cycle with a maximum occurring at the beginning of flexion (circle) that progressively decreased during extension (E). Both CCS (Fig. 4C) and SP (Fig. 4D) stimuli induced a similar dramatic decrease in PAD amplitude in all five bins (stars) and did not change the moment for maximal PAD amplitude (circles). Note the different patterns in phase-dependent PAH modulation evoked by CCS and SP stimuli (curves in light gray).

Overall, cutaneous inputs alone were able to evoke axonal responses in 12/13 trials for SP (92%; 7/12 were PAHs), in 11/17 trials for CCS (65%; 6/11 were PAHs) and, in 8/10 trials for Saph (80%; 5/8 were PAHs). The same inputs could change significantly the PAD amplitude, in at least one bin, in 17/31 afferents (55%) of group I afferents (10 decerebrate cats and 3 spinal cats). In Fig. 6 are grouped only the trials where cutaneous input did change PAD amplitude significantly (all evoked by PBSt stimuli). They are organized according to muscle of origin (abbreviated on the left) and according to the cutaneous nerve; CCS in A, SP in B, and Saph in C. Each box in Fig. 6 represents the effect of a cutaneous volley on the amplitude of PAD during the normalized step cycle divided in five bins with the gray box representing flexion and the white one, extension. For each trial, a significant modification in PAD amplitude in a particular bin is indicated by an arrowhead (which corresponds to a star in phase plots). A downward (empty) arrowhead indicates a reduction of PAD amplitude by the cutaneous input (as in Fig. 3–5) while an upward (black) arrowhead indicates an increase in PAD amplitude. First, as seen in Figs. 3–5, it is clear that, in the vast majority of trials (23/28), there are downward arrowheads indicating that the most common effect of cutaneous inputs was a decrease in PAD amplitude. Second, this decrease is also observed for a majority of bins (4/5 bins) in most trials (16/28). However, detailed comparisons between the effects of the three cutaneous nerves indicate some subtle differences. The potency for modifying PAD amplitude was greater for SP (85% of trials) as compared with CCS (44%) or Saph (60%). Also the number of bins being affected was larger for SP (4.4 bins) than for Saph (4.2) or CCS (3 bins).

Also illustrated in 22 trials of Fig. 6 are the occurrence of the maximal PAD amplitude before (indicated by ○) and following (●) cutaneous conditioning, are indicated, ○, when the bin of maximal PAD amplitude that did not change because of the cutaneous input.
DISCUSSION

The present work looked at the integration of synaptic inputs from low-threshold cutaneous afferents, muscle afferents and from the CPG for locomotion in presynaptic inhibitory pathways to muscle group I afferents. It was found that PAD amplitude during fictive stepping is significantly influenced by the combination of different sensory feedback. We will first see how the results may be integrated into the known PAD interneuronal circuitry and second, how they could contribute to the sensory control of motoneuronal excitability during stepping.

Overall, cutaneous volleys could significantly modify the PAD evoked by muscle stimuli in at least one part (bin) of the step cycle in 17/31 (55%) group I afferents. Clearly, the most common effect (n = 13/17) was a decrease in the PAD amplitude (by 35% on average). Note that the absence of effects in 16 afferents was often due to the CCS stimulation, which turned out to be less potent, and modified a smaller number of bins, than the SP or Saph nerves. However, the sural nerve was the most commonly used in PAD studies in the anesthetized cat. In a previous study measuring the excitability of afferent terminals in response to supraspinal and/or peripheral inputs in the anesthetized cat, the use of sural nerve stimulation was critical to classify group IA and IB terminals in response to supraspinal and/or peripheral inputs in the anesthetized cat, the use of sural nerve stimulation was critical to classify group IA and IB fibers into three distinct types of PAD patterns (Rudomin et al. 1986). However, more recent studies from the same group (Enriquez et al. 1996; Jiménez et al. 1988), using intra-axonal recordings of MG group IA and IB fibers, revealed important overlaps between IAs and IBs, and they showed that the sural stimulation could either evoke or inhibit PAD with the latter being the most frequent in IAs. Our very limited sample (n = 12) of identified IAs and IBs indicates that cutaneous volleys decreased the PBSt-PAD in 4/8 IAs and in 3/4 IBs. Also, in the rest of our group I fibers, which must have included both IAs and IBs, an inhibition of PAD was the most frequent finding. A larger sample of functionally identified IAs and IBs is needed to determine if this dominant inhibitory effect is due to locomotor circuitry. Lund et al. (1965) reported that the monosynaptic EPSP in extensor motoneurons, which was presynaptically inhibited by PBSt stimuli, could regain its original size when preceded by the stimulation of the sural nerve (also Ashby et al. 1987; Rudomin et al. 1975). Such removal of presynaptic inhibition of IA-excitatory postsynaptic potential by cutaneous input is consistent with our findings. Also, we found that cutaneous input alone could evoke a phase-dependent modulation of PAHs (n = 9) or PADs (n = 3) in group I afferents during fictive locomotion that has never been reported before (see following text).

Gating of PAD pathways during fictive locomotion

Whether cutaneous responses were transmitted through PAD interneurons activated by PBSt or independently transmitted to axonal terminals is impossible to determine with certainty at this point. If the latter is true, one would expect linear summation (or subtraction) of axonal responses evoked by the two sensory inputs. However, in most cases, the pattern of phase-dependent modulation in PAHs (or PADs) evoked by cutaneous stimuli cannot explain the overall (4/5 bins) reduction of PBSt-evoked PAD. This can be appreciated in all illustrated phase plots. For example, in Fig. 3B, the SP stimuli evoked small PAHs only in the first two bins of the cycle and no detectable responses in the last three bins and, yet, when combined to PBSt stimuli, there was a significant reduction in PAD amplitude in all five bins. If both responses had been independently transmitted to the axonal terminals, one would have expected PAHs to subtract some of the PAD amplitude only during the first two bins. Also, the magnitude of PAD reduction in a given bin appears to be much larger than the PAH amplitude evoked in that same bin (e.g., Figs. 3–5). Indeed, linear summation analysis (not shown) revealed that the amplitude of PAHs evoked by cutaneous stimuli was much smaller than the amplitude of PAD reduction evoked by the combined stimuli. However, we believe analysis based on linearity is difficult to interpret during fictive stepping because of the involvement of many nonlinear intrinsic cellular properties (e.g., Hultborn and Kiehn 1992). Therefore the previous observations are taken as clear indications (not evidence) that convergence of cutaneous and muscle inputs onto common PAD pathways is the best explanation for our results.

On the other hand, if PAH evoked by cutaneous stimuli was due to a direct inhibition of PAD interneurons activated by the PBSt stimuli, one could have expected both responses to follow a similar pattern of phase-dependent modulation (cf. Burke 1999). As mentioned before, this was clearly not the case and we believe that, when muscle (PBSt) afferents were not stimulated, the PAHs evoked by cutaneous stimuli alone were probably due to the inhibition of different PAD pathways that were driven by other segmental, descending, and/or CPG inputs. In previous studies of PAD circuitry in the anesthetized cat, cutaneous action is always described as being mediated through pathways activated by muscle and/or descending inputs (Rudomin et al. 1983, 1986). Predominance of cutaneous excitation over inhibition or vice versa would then depend on the nature of the descending and other segmental influences received by each set of PAD interneurons (Enriquez et al. 1996). Actually, PAHs were only observed in preparations with an important background excitability in spinal cord networks (Mendell 1972) that could explain the low incidence of PAH observed in anesthetized cat (Rudomin et al. 1974). In a recent study (Gosgnach et al. 2000), a background level of PAD in group IA afferents (see Baev and Kostyuk 1982) has been proposed to explain a tonic decrease in monosynaptic EPSPs size during MLR-evoked fictive locomotion in the decerebrate cat. Moreover, previous studies clearly showed that fictive stepping is also accompanied by a phasic (CPG-related) PAD in many afferents (Dubuc et al. 1988; Gossard et al. 1989, 1991). We believe that, in the absence of muscle stimuli, the phasic modulation of responses evoked by cutaneous input alone, as seen in this study, is best explained by changes in the activity of CPG-related PAD pathways.

Finally, we observed that cutaneous inputs decreased PAD amplitude for most of the duration of the step cycle (4/5 bins) in 8/13 afferents (57% of trials; 16/28) and did not change the moment (bin) of maximal PAD amplitude in them (68% of trials; 15/22). These observations suggest that a proportion of cutaneous inhibitory interneurons impinging onto PAD pathways is tonically active during fictive stepping. This, in turn, suggests that a cutaneous input triggered any time in the step cycle would be able to reduce efficiently the level of presynaptic inhibition in a number of group I afferents without changing the pattern of phase-dependent modulation. We also observed that the different cutaneous nerves had different
potency to modify PAD transmission. Indeed, the overall potency to modify PAD was greater for SP (85%) than for CCS (44%) or Saph (60%) nerves, and the effect of SP was generally a removal of PAD (9/11 trials). Further differences may have escaped our intra-axonal recordings. Indeed, recent work (Eguibar et al. 1997; Loneli et al. 1998; Quevedo et al. 1997) showed that different collaterals of the same afferent may be controlled by different PAD interneurons in the anesthetized animal. It is not known if such differential control exists during locomotion or any other movement, but if it does, it could exert a focal control of transmission that may also be phase dependent. The different efficacies reported in our results do suggest that sensory feedback from different skin areas during real movements could regulate differently the transmission from group I afferents (mono- and oligosynaptic) in proprioceptive pathways to motoneurons. It has been estimated that feedback from muscle afferents contributes for 35–50% of the excitability drive to motoneurons in extensors during the stance phase in walking decerebrate cats (Hiebert and Pearson 1999; Misiaszek et al. 2000). Therefore by modulating presynaptic inhibition, a relatively modest cutaneous stimulation (as used in our experiments) would be able to modify dramatically the motoneuronal excitability. For example, on hitting an obstacle, the activation of the dorsum of the paw (innervated by SP) would remove presynaptic inhibition of group I afferents that, in turn, would increase the excitatory drive to motoneurons (Aimoneetti et al. 2000; Iles 1996) involved in the ensuing corrective movements.

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