Exp Brain Res (2000) 135:204–214
DOI 10.1007/s002210000524

RESEARCH ARTICLE

Joel Lomelí · Luis Castillo · Pablo Linares
Pablo Rudomin

Effects of PAD on conduction of action potentials within segmental and ascending branches of single muscle afferents in the cat spinal cord

Received: 1 February 2000 / Accepted: 14 July 2000 / Published online: 10 October 2000

Abstract In anesthetized and paralyzed cats under artificial respiration, we examined the extent to which primary afferent depolarization (PAD) might affect invasion of action potentials in intraspinal axonal and/or terminal branches of single muscle afferents. To this end, one stimulating micropipette was placed at the L6 spinal level within the intermediate or motor nucleus, and another one at the L3 level, in or close to Clarke's column. Antidromically conducted responses produced in single muscle afferents by stimulation at these two spinal levels were recorded from fine lateral gastrocnemius nerve filaments. In all fibers examined, stimulation of one branch, with strengths producing action potentials, increased the intraspinal threshold of the other branch when applied at short conditioning testing stimulus intervals (<1.5–2.0 ms), because of the refractoriness produced by the action potentials invading the tested branch. Similar increases in the intraspinal threshold were found in branches showing tonic PAD and also during the PAD evoked by stimulation of group I afferent fibers in muscle nerves. It is concluded that during tonic or evoked PAD, axonal branches in the dorsal columns and myelinated terminals of muscle afferents ending deep in the L6 and L3 segmental levels continue to be invaded by action potentials. These findings strengthen the view that presynaptic inhibition of muscle afferents produced by activation of GABAergic mechanisms is more likely to result from changes in the synaptic effectiveness of the afferent terminals than from conduction failure because of PAD.

J. Lomelí · P. Linares · P. Rudomin (✉)
Departamento de Fisiología, Biofísica y Neurociencias,
Centro de Investigación del Instituto Politécnico Nacional,
Apartado Postal 14-740, México D.F. 07000, México
e-mail: rudomin@fisio.cinvestav.mx
Tel.: +52-5-7477099, Fax: +52-5-7477109

L. Castillo
Centro Básico, Universidad de Aguascalientes,
Aguascalientes, México

Key words Primary afferent depolarization · Presynaptic inhibition · Conduction failure · Action potentials · Spinal cord

Introduction

Recent investigations (Lomelí et al. 1998) have indicated that stimulation of cutaneous afferents, as well as stimulation of the motor cortex and of the bulbar reticular formation, may differentially inhibit the primary afferent depolarization (PAD) elicited in pairs of segmental (L6) and ascending (L3) branches belonging to the same muscle spindle afferent. In many fibers the PAD elicited in one branch was almost completely abolished by these conditioning stimuli, leaving the PAD in the other branch practically unaffected. Spinal block could reverse the asymmetry in the inhibition of the PAD. This suggested that the intraspinal arborizations of the afferent fibers are not obligatory routes for information transmission, but are instead dynamic systems that can be centrally modulated to convey information to selected neuronal targets (Eguibar et al. 1997; Lomelí et al. 1998).

The consequences of PAD on the synaptic effectiveness of muscle afferents have not been fully elucidated. Presynaptic inhibition appears to involve local modulation of transmitter release by means of activation of $GABA_A$ receptors (for review see Rudomin and Schmidt 1999), via axoaxonic synapses made by GABAergic interneurons with the intraspinal terminals of the afferent fibers (Maxwell et al. 1990; Lamotte d'Incamps et al. 1998; Maxwell and Riddell 1999). Several investigators have suggested in addition that during PAD there can be conduction failure at branch points of the intraspinal arborizations of afferent fibers (Henneman et al. 1984; Luscher 1998), either because of the shunt associated with the increased chloride conductance (Redman 1990; Segev 1990; Walmsley et al. 1995; Walmsley and Nicol 1998) or because of sodium channel inactivation produced by the sustained depolarization (Graham and Redman 1994). This view has received strong support by

the recent observations of Wall and colleagues (see Wall 1994, 1995) who showed that, in the rat spinal cord, impulses fail to propagate in the long-range reach of myelinated fibers caudally in the dorsal columns, probably because of a tonic GABA-operated Cl^- shunt operating in the terminals. Suppression of this tonic mechanism by $GABA_A$ antagonists relieved impulse blockade (Wall and McMahon 1994; Wall 1995).

Previous studies made by Curtis et al. (1995, 1997) have indicated that stimulation of afferent fibers in the peripheral nerve leaves a state of relative refractoriness in their central terminals, suggesting that they are invaded by the action potentials initiated in the periphery (see also Curtis 1998). We have used a similar approach to examine the extent to which action potentials initiated by direct stimulation of one branch may invade another branch of the same fiber ending three segments away, and whether this invasion is impaired during PAD. A preliminary communication of these findings has been published in abstract form (Castillo et al. 1998).

Materials and methods

General procedures

Guidelines contained in NIH publication 85–23 revised in 1985, on the principles of laboratory animal care were followed throughout. Briefly, experiments were carried out on nine adult cats initially anesthetized with pentobarbitone sodium (40 mg/kg body weight, i.p.). After the surgical procedures, the animals were paralyzed with i.v. pancuronium bromide, (0.3 mg/kg initial dose, subsequently 10% solution i.v. at 0.3 mg/h) and artificially respired. Tidal air volume was adjusted to have a 4% CO_2 concentration in the expired air. Deep anesthesia was maintained during the recording sessions by i.v. injections of pentobarbitone sodium (3 mg/h). Adequacy of anesthesia was assessed by verifying that the pupils were constricted, that mean arterial blood pressure was stable (between 100 and 120 mmHg), and that there was no response to noxious stimulation of the skin.

The lumbosacral and low thoracic spinal segments were exposed and the left L5 to S1 ventral roots sectioned. The posterior biceps and semitendinosus (PBSt), sural (SU), and superficial peroneus (SP) nerves were dissected, sectioned, and their central ends prepared for stimulation. In all experiments the PBSt nerve was stimulated with trains of four shocks at 300 Hz, 1.3–3.2 times the threshold of the most excitable fibers (×T), applied 25 ms before the intraspinal threshold testing pulse. The SU and SP nerves were stimulated with single pulses 1.55–7.1×T and 1.96–8.75×T, respectively, applied 55 ms before the intraspinal threshold testing pulse.

The central end of the lateral gastrocnemius (LG) nerve was divided into fine filaments to enable recording of antidromic responses of single muscle afferents in response to intraspinal microstimulation (Jankowska et al. 1981; Rudomin et al. 1983; Quevedo et al. 1997). Exposed tissues were covered with mineral oil to prevent desiccation and kept at constant temperature (37°C) by means of radiant heat.

Measurement of intraspinal threshold

One glass micropipette (filled with NaCl 2 M, 1.2–1.8 MΩ) was inserted within the intermediate or motor nucleus region in the L6 spinal segment and another micropipette in the L3 segment aiming at Clarke's column (Fig. 1A). Positioning of the micropipette at the L6 level was aided by recording the orthodromic extracellular field potentials produced by stimulation of the available muscle nerves and, when searching the motor nuclei, the antidromic field potentials produced by stimulation of the central end of the sectioned L6 ventral rootlet. In the case of Clarke's column at the L3 level, in addition to the orthodromic field potentials, we recorded the antidromic responses produced by stimulation of the spinocerebellar tract in the ipsilateral dorsolateral fascicle at low thoracic level (see Walmsley and Nicol 1991).

Stimulating pulses (400 μs duration, 2–20 μA) were delivered at 1 Hz through each micropipette, via different current pulse generators. The position of the L3 and L6 micropipettes was adjusted until each of them produced antidromic responses of a single afferent fiber in the ipsilateral LG nerve filament. Interaction, because of refractoriness, between the antidromic responses produced by the L3 and L6 microstimulation was taken as evidence that both of them were produced by activation of two branches belonging to the *same* afferent fiber (see Quevedo et al. 1997; Lomelí et al. 1998; Figs. 3B, C, 5A).

After performing the collision tests, stimulating pulses were delivered at 1 Hz in alternation through each micropipette. The antidromic action potentials produced by these stimuli were passed through separate window discriminators and the intensity of the stimuli delivered through each micropipette was independently adjusted, by means of different digital controller units, to produce, in each case, antidromic responses with a constant probability (set to 0.5; see Lomelí et al. 1998). The current pulses were measured in the return path to ground, separately integrated, and recorded in different channels of a penwriter. With this technique, PAD produced by conditioning volleys appears as a reduction of the intraspinal threshold of the afferent terminals, and inhibition of PAD as a threshold increase (Rudomin et al. 1983).

Histology

At the end of the experiment the animal was killed with a pentobarbital overdose and perfused with 10% formalin, and the spinal cord removed leaving the two stimulating micropipettes in place. After complete fixation and dehydration, the lumbosacral cord segment containing the excitability testing micropipettes was placed in a solution of methyl salicylate for clearing. Subsequently, the spinal cord was cut transversally. Sections containing the tips of the glass micropipettes were photographed and used to determine the location of the stimulated sites.

Results

Conduction velocity and peripheral threshold

Altogether we were able to examine with detail the intraspinal threshold changes of nine pairs of L3 and L6 branches from single afferent fibers. Fig. 2A shows the location of the stimulated sites within the L3 and L6 spinal levels. Each symbol indicates a different afferent fiber. Five L3 terminals were stimulated within or close to Clarke's column, three L6 terminals were stimulated within the ventral horn, and four within or just dorsal to the intermediate nucleus.

Figure 2B shows the relationship between the latency of the antidromic responses produced in the same afferent fiber by microstimulation at L3 and L6. It may be seen that in all fibers the antidromic latency of the responses produced by stimulation at the L3 level was longer than the latency of the responses produced by stimulation at the L6 segmental level (0.2–1.4 ms), mostly because of the time taken by the action potential to travel

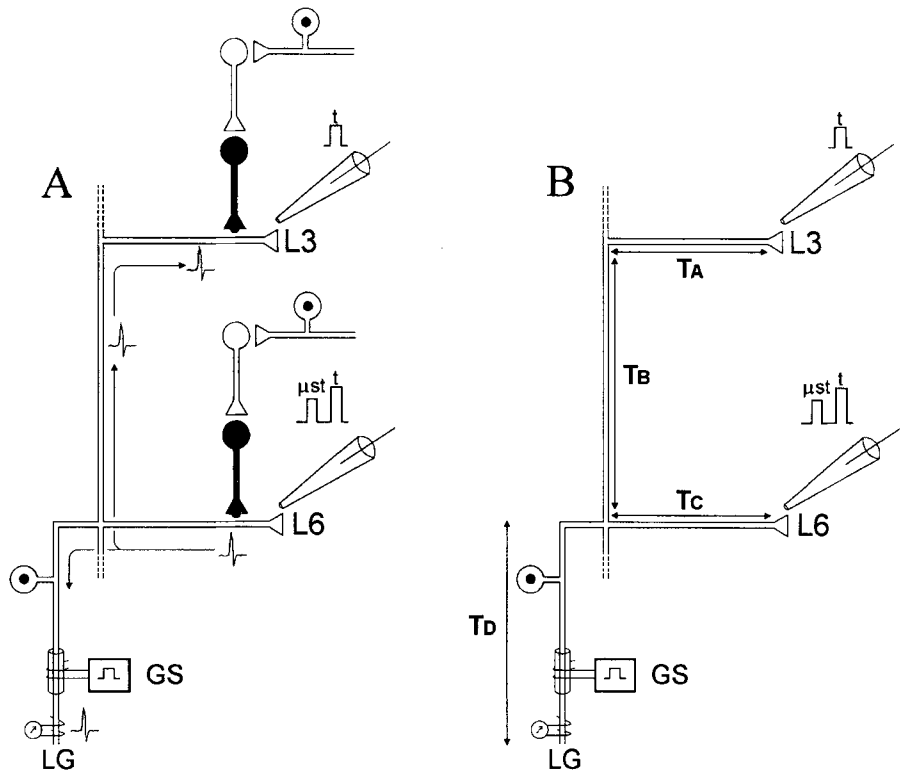

Fig. 1A,B Diagram of the experimental array. **A** A pair of glass micropipettes was placed within the spinal cord to activate two branches of the same afferent fiber ending at the L3 and L6 segmental levels. Threshold changes of both branches were determined by alternate application of a test stimulating pulse (*t*) delivered at 1 Hz. The intensity of the pulses was automatically changed by means of a digital computer in order to produce antidromic responses of the afferent fiber with a constant probability. To test for refractoriness an additional current pulse (*μst*) was passed through one of the micropipettes (L6 in the diagram) at various conditioning-testing time intervals, while exploring the threshold changes produced in both branches. The strength of the conditioning stimulus was above that required to produce antidromic responses in a single lateral gastrocnemius (*LG*) afferent fiber in the peripheral nerve. **B** Same as **A**, but with indications of the conduction times in the peripheral and intraspinal segments of the explored afferents. *GS* Gastrocnemius soleus nerve, T_A, T_B, T_C, T_D time taken by action potential to travel along the intraspinal and peripheral branches of the fiber, as indicated

along the spinal cord from the L3 to the L6 segmental level (see below).

Mean conduction velocities of the examined fibers were calculated by dividing the total conduction distance by the antidromic latency. The conduction distance comprised the distance from the point of insertion in the spinal surface of the stimulating micropipette to the recording site in the peripheral nerve, plus the intraspinal conduction distance, estimated from the location of the micropipette relative to the cord surface. These values varied from 37.2 to 81.4 m/s when measured from L3 and from 34.5 to 81.0 m/s when measured from L6, while the peripheral threshold of the fibers varied from 1.04 to 1.73×T (Fig. 2C).

Three afferent fibers with mean conduction velocities above 60 m/s had peripheral thresholds in the low range (between 1.05 and 1.15×T) which best fulfilled criteria of group I afferents. The peripheral thresholds of the five afferent fibers with mean conduction velocities below 60 m/s varied between 1.04 and 1.73×T, still below those for group II afferents (Riddell et al. 1995), so that they might be likewise classified as group I afferents on the basis of these thresholds, although the possibility that these were group II afferents should be left open.

To exclude the possibility that the low conduction velocity of some fibers was due to impaired peripheral conduction of action potentials, because of the time elapsed after the nerve section (12–20 h), we compared the latencies of the antidromic responses of the afferent fibers (produced by intraspinal microstimulation at L3 or L6) with the latency of the presynaptic spike of the group I field potential recorded at the same site following stimulation of the whole gastrocnemius soleus (GS) nerve. This latency was adjusted to compensate for differences in conduction distance between the GS stimulating electrode, and the site of recording of the antidromic spikes in the LG nerve filament (see Fig. 1A). This was done assuming that the conduction velocity in the peripheral nerve was the same as the mean conduction velocity, estimated as described above.

As shown in Fig. 2D, there was a relatively good match between the latency of the antidromic responses of the afferents with mean conduction velocities above 60 m/s and the adjusted latency of the group I presynaptic field potential recorded at the same site. The differences in both latencies varied between 0.01 and 0.2 ms for the L3 and L6 terminals (mean 0.01±0.3 ms, n=8). In contrast, the five fibers with conduction velocity below 60 m/s, responded antidromically to pulses at L3 or L6

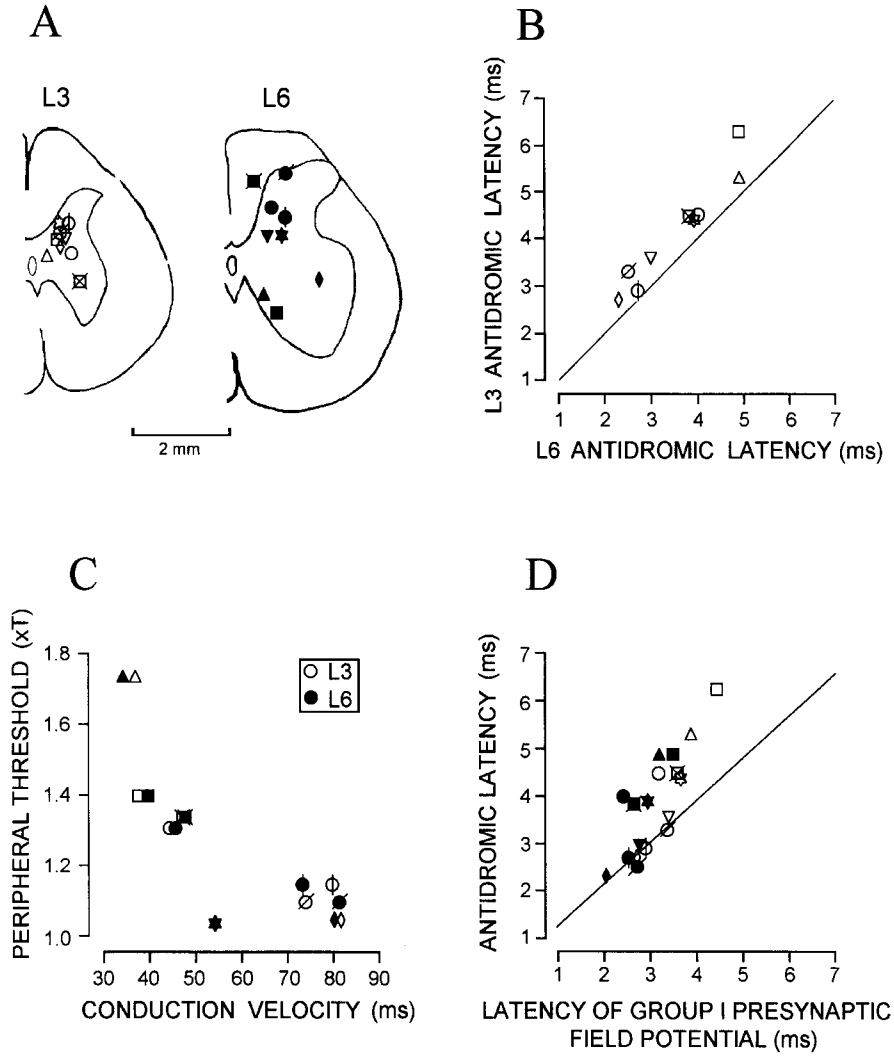

Fig. 2 **A** Histological reconstruction of the threshold-testing sites at the L3 and L6 spinal levels. Pairs of branches of the same afferent fiber are indicated with the same symbol in this and other figures. **B** Relation between the antidromic latencies of individual afferents produced by stimulation at L3 and L6. **C** Relation between mean conduction velocities and peripheral threshold. **D** Plot of the latency of the L3 and L6 antidromic responses of individual afferents versus the adjusted onset latency of the LG nerve group I presynaptic field potential recorded at the site of threshold testing. *Continuous lines* in **B** and **D** have a slope of 1. In **B–D**, *open symbols* L3 branches, *filled symbols* L6 branches

with a latency that was 0.7–1.9 ms longer than the latency of the corresponding group I field potential (mean 1.3±0.5 ms, n=10). Clearly, this was not due to deterioration of the peripheral axons of these fibers because, in this case, the latency of the group I presynaptic field potential should have also increased. The observed latency differences could reflect a lower conduction velocity along the relatively fine intraspinal branches of afferent fibers with peripheral conduction velocities still within the group I range, or else to low conduction velocities along the whole central and peripheral segments of presumed group II afferent fibers (see Discussion).

Patterns of PAD

PBSt conditioning stimulation with trains 1.3–3.2×T reduced the intraspinal threshold of the L3 and L6 branches of the nine fibers examined because of PAD. In eight fibers, the threshold reduction relative to control was slightly larger in the L6 than in the L3 terminals (81.26±13.8% for L3 and 77.8±11.86% for L6), but these differences were not statistically significant (however, see Quevedo et al. 1997; Lomelí et al. 1998). Only in one case (Fig. 5) was stimulation of the PBSt nerve with pulses in the group I range found to produce a strong PAD when testing at the L3 level and almost no effect at the L6 level. This was most likely because at L6 the stimulating micropipette was within the dorsal columns (Fig. 2A *crossed solid square*).

The effects produced by stimulation of cutaneous nerves were more variable. In seven fibers these stimuli produced no threshold changes, but were able to inhibit the PBSt-induced PAD of both collaterals in 6/7 fibers and to inhibit the PBSt-PAD in the L3 collateral and facilitate the PAD in the L6 collateral in 1/7 fibers. SU and SP stimulation reduced the threshold of both collaterals in one fiber and increased the threshold of both collaterals in another fiber (see Fig. 5).

Tests for refractoriness

Conduction of action potentials was inferred by testing the relative refractoriness produced in a given branch by action potentials initiated in the other branch. To this end

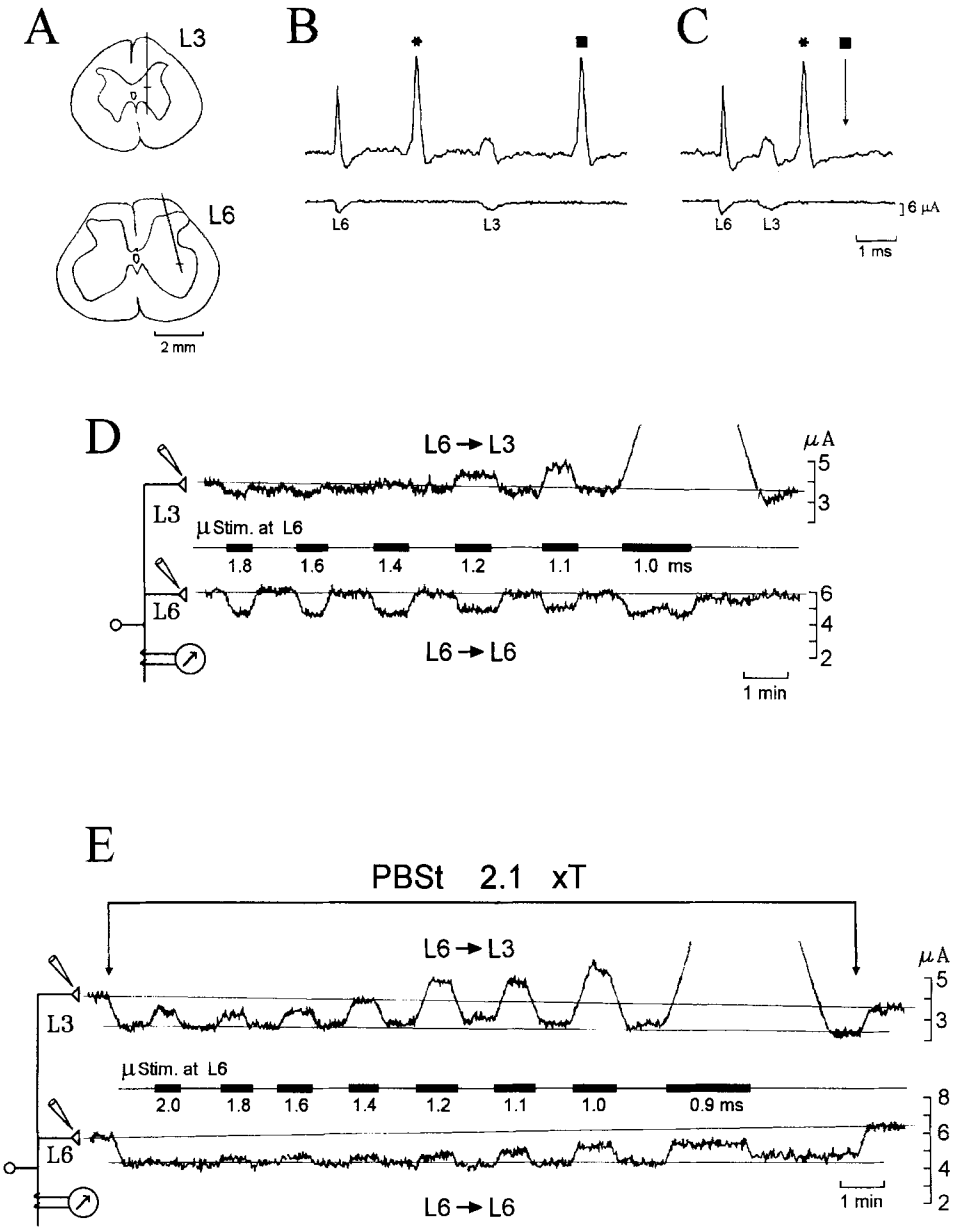

Fig. 3A–E Invasion of L3 and L6 branches of a single group Ia afferent during posterior biceps and semitendinosus (PBSt)-induced primary afferent depolarization (PAD). **A** Histological reconstruction of electrode tracks. **B,C** Interaction between antidromic spikes produced by intraspinal stimulation at L3 and L6. **D** Intraspinal threshold changes produced in the L3 and L6 branch by action potentials initiated in the same fiber by intraspinal microstimulation at L6. **E** Same as **D**, but during the PAD produced by stimulation of the PBSt nerve, as indicated. Mean L6 conduction velocity 80.4 m/s; peripheral threshold 1.05 times the threshold of the most excitable fibers (×T). Some of the records used for this figure have been published in a review article (Rudomin 1999; reproduced with permission of Elsevier)

a single pulse was applied through one of the two micropipettes at various time intervals before the pulses delivered to measure the intraspinal threshold (see Fig. 1A). Stimulus strength was above that required to produce antidromic action potentials in the peripheral nerve filament. We examined the refractoriness of five L3 terminals produced by conditioning stimulation at L6, and of six L6 terminals by stimulation at L3. In four fibers we could test refractoriness of both the L3 and L6 terminals. Altogether, 18 tests for refractoriness before and during PAD were made in the five L3 terminals and 13 tests in the six L6 terminals. In all cases we could demonstrate refractoriness of one terminal following stimulation of the other terminal at short conditioning test (C-T) time stimulus intervals, as described below.

Figure 3 shows the results obtained in one fiber classified as Ia because it had an L6 mean conduction velocity of 80.4 m/s, a peripheral threshold within the group I range (1.05×T), was antidromically activated from the ventral horn at L6 (Fig. 3A), and had a type A PAD pattern characteristic of muscle spindle afferents. Namely, stimulation of group I afferents from flexors produces PAD while stimulation of cutaneous and descending inputs produces no PAD but instead inhibits the PAD elicited by muscle afferents (Rudomin et al. 1986).

Conditioning pulses applied to the L6 branch 1.6 and 1.8 ms before the L3 testing pulses slightly reduced the intraspinal threshold of the L3 branch (Fig. 3D *upper trace*), while at shorter C-T time intervals the threshold of the L3 branch increased gradually. When the time interval between the L6 and L3 pulses was of 1 ms it was not possible to produce an antidromic response in the L3 branch, even with the highest stimulus strengths (17 μA; see also Fig. 4A *circles*). This increase in threshold is assumed to result from the relative refractoriness produced by the action potential invading the L3 branch at the site

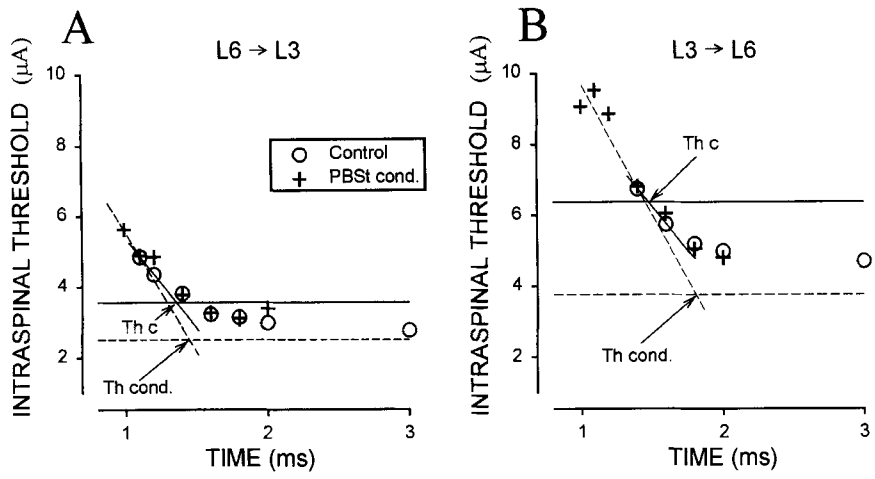

Fig. 4A,B Threshold recovery curves of L3 and L6 terminals of a single Ia afferent fiber. Same pair of branches as in Fig. 3. **A** Time course of threshold recovery of the L3 terminal by conditioning pulses applied through the L6 micropipette. *Abscissa* Time interval between conditioning and test pulses, *ordinates* threshold changes in μA. *Continuous horizontal line* shows resting threshold of the L3 terminal and *interrupted line* threshold reduction because of the PBSt-induced PAD. Estimates of threshold cross-over point are indicated by *arrows* (Th_C control, Th_{COND} during PBSt conditioning). **B** Time course of threshold recovery of the L6 terminal by conditioning pulses applied through the L3 micropipette. Same format as in **A**

of excitability testing (Curtis et al. 1995, 1997; Curtis 1998).

Conditioning stimulation of the PBSt nerve reduced the intraspinal threshold of the L3 terminal to 62.5% of control (Fig. 3E *upper trace*). This is a fairly large effect when compared with the mean percentage changes produced in Ia GS fibers by PBSt conditioning with group I strength (see Rudomin et al. 1981, 1983, 1986). Action potentials generated by stimulation of the L6 terminals during PAD increased the threshold of the L3 terminals throughout the whole range of explored C-T time intervals (1–2 ms; Figs. 3E *upper trace*, 4A *crosses*). This suggests that PAD was unable to prevent impulse conduction from the L6 to the L3 terminals at the testing site.

In this fiber we could also examine the threshold changes produced in the L6 terminals following conditioning action potentials initiated by stimulation at L3. The results obtained also indicated that at short C-T time stimulus intervals the threshold of the L6 terminals increased because of refractoriness (Fig. 4B *circles*). Conditioning stimulation of the PBSt nerve produced a rather large threshold reduction because of PAD (to 55.2% of control). Yet, refractoriness produced by the invading action potentials was not prevented (Fig. 4B *crosses*).

It may be noticed in the lowest trace of Fig. 3D that the conditioning stimulus applied through the L6 micropipette reduced the intraspinal threshold of the L6 terminals throughout the whole range of explored C-T stimulus time intervals. This could be due to the phase of supernormal excitability left by the conditioning action potential (Rudin and Eisenman 1953; Blight and Someya 1985; Curtis et al. 1995) and/or to the PAD generated by direct activation of last-order GABAergic interneurons (Jankowska et al. 1981; Quevedo et al. 1997). The failure of the conditioning action potential to increase the threshold of the L6 terminals at the shortest C-T time stimulus intervals is somewhat puzzling. It is possible that the duration of the relative refractoriness of the L6 terminals was shorter than 1 ms and thus may not have been detected at this interval (see Curtis et al. 1995), or else that the action potentials produced by the intraspinal microstimulation at L6 were generated at the first or other nodes of Ranvier, but failed to invade the unmyelinated portions of the L6 terminals, where the PAD was generated.

During the PAD elicited by PBSt stimulation, the threshold of the L6 terminals appeared to be increased above the already lowered threshold at all C-T time intervals (Fig. 3E *lower trace*). However, this increase in threshold was relative because the threshold changes measured in μA appeared to be the same as those observed in the absence of the PBSt-induced PAD. It thus seems that in this case there was very little interaction between the PBSt-induced PAD and the action potentials, probably because both processes affected different regions of the terminal arborizations. So far, this behavior was observed only in one of the nine fibers examined and, although very suggestive, it may not necessarily represent a general finding. However, it is interesting to mention that Cattaert and El-Manira (1999) also found that PAD may not necessarily interact with the action potentials conducted in the intraganglionic arborizations of sensory neurons of invertebrates.

Figure 5 illustrates data obtained from a fiber with a rather low mean conduction velocity (47.6 m/s) and a peripheral threshold of 1.37×T. Stimulation of the PBSt nerve with a train of pulses 1.36×T reduced the intraspinal threshold of the L3 terminals (to 92.5% of control), practically without affecting the threshold at L6, most likely because the testing electrode was placed within the dorsal columns (see Fig. 2A). The effects produced by pulses of 1.8×T were confined to the L3 terminals while pulses of 2.7×T, already maximal for group I affer-

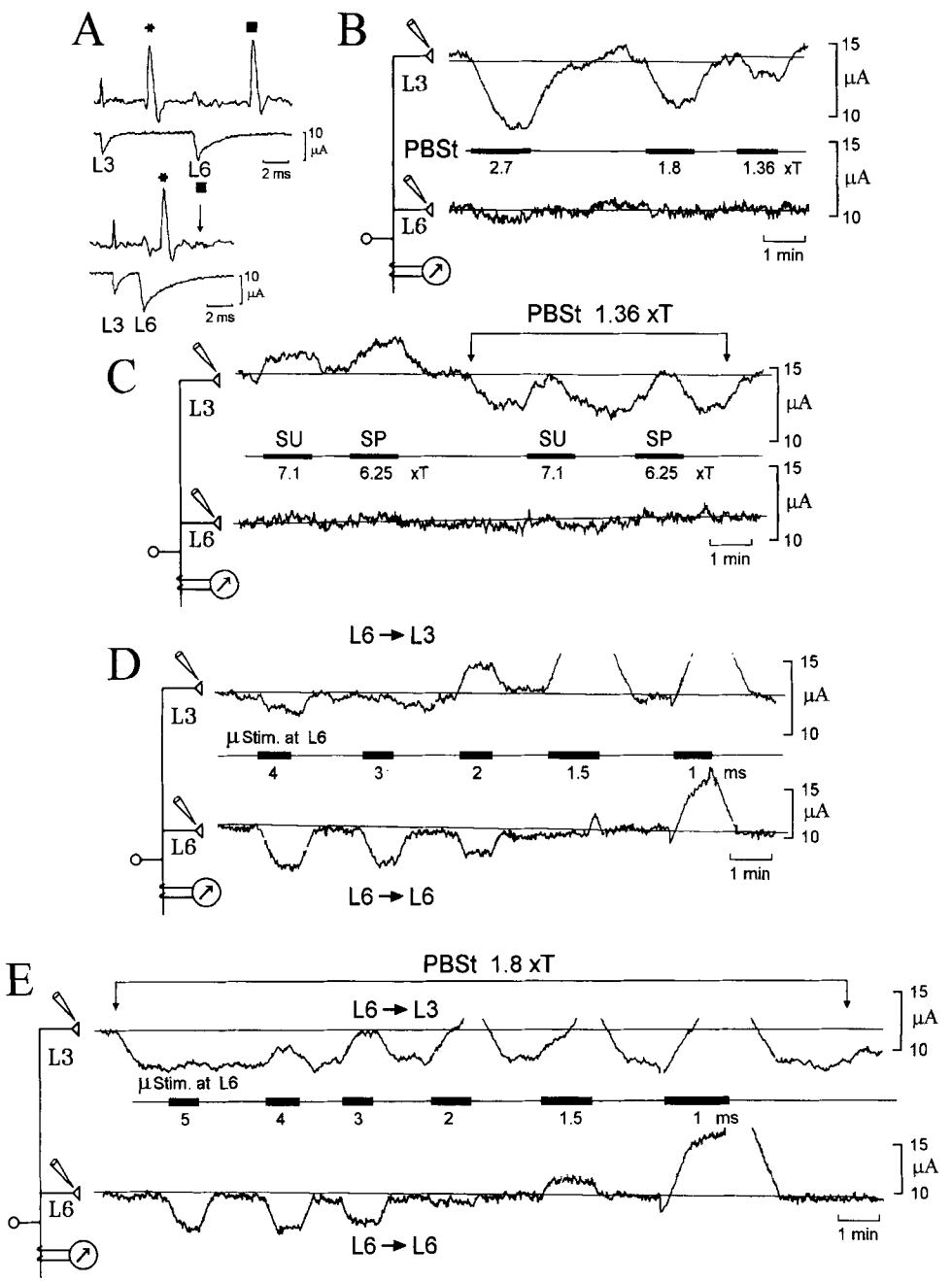

Fig. 5A–E Conduction of action potentials in an L3 branch subjected to tonic PAD. **A** Interaction between antidromic action potentials produced by L3 and L6 microstimulation. **B** Effects of graded stimulation of the PBSt nerve on the intraspinal threshold of the L3 and L6 branches, as indicated. **C** Effects produced by stimulation of cutaneous nerves on the resting threshold and during the PBSt-induced PAD. Note increase in resting threshold due to inhibition of a tonic PAD in the L3 branch and inhibition of the PBSt-induced PAD. **D** Intraspinal threshold changes produced in the L3 and L6 branches by action potentials initiated by stimulation at L6. **E** Same as **D**, but during the PAD produced in the L3 branch by stimulation of the PBSt nerve, as indicated. L6 mean conduction velocity 47.5 m/s; peripheral threshold 1.37×T. *SU* Sural nerve, *SP* superficial peroneus nerve

ents, further reduced the intraspinal threshold of the L3 terminals (to 64.9% of control) and had a mild effect at L6 (to 93.5% of control; Fig. 5B). Quite interestingly, stimulation of the SU and SP nerves with relatively high strengths (7.1 and 6.25×T, respectively) increased very clearly the threshold of the L3 terminals (to 108% and 112.5% of control) practically without changing the threshold at L6, and also inhibited the PAD produced in the L3 terminals by PBSt stimulation (Fig. 5C).

The increase in the resting threshold of the L3 terminals by cutaneous volleys has been previously observed in muscle spindle afferents and has been attributed to inhibition of a tonic PAD (see Lomelí et al. 1998). This provided an excellent opportunity to examine the invasion of action potentials in a tonically depolarized terminal, which is important because Wall and collaborators (1994, 1995) have proposed that this tonic PAD may explain conduction failure in the long-range afferents that project caudally in the rat spinal cord.

Stimuli applied through the L6 micropipette, with strengths above those required to produce antidromic action potentials in the peripheral axon of the afferent fiber, reduced threshold of the L3 terminals when applied 4 ms before the test pulse. At a shorter time interval (2 ms) there was instead a small threshold increase, probably because of refractoriness. At even shorter C-T time stimulus intervals (1.5 and 1 ms) it was not possible to activate the L3 terminals any further and produce an antidromic action potential (Fig. 5D *upper trace*). These findings suggest that the L3 branch was invaded by ac-

tion potentials, even though it was subjected to a tonic PAD. Refractoriness of the L3 terminals due to action potentials elicited at L6 was also observed during the PAD produced by PBSt stimulation, as illustrated in Fig. 5E (*upper trace*).

Conditioning stimulation through the L6 micropipette applied 2–5 ms before the test pulse also reduced the threshold of the L6 terminals, while at shorter C-T stimulus time intervals (1 ms) the threshold was increased (Fig. 5D *lower trace*). Similar changes were observed during conditioning stimulation of the PBSt nerve (Fig. 5E *lower trace*). Since the L6 electrode was within the dorsal columns (Fig. 2A), the period of supernormal excitability can be attributed to spike negative afterpotentials in the afferent fiber (see Rudin and Eisenman 1953; Blight and Someya 1985), rather than to activation of last-order GABAergic interneurons, while the threshold increase would be due to the relative refractoriness left by the action potential (Curtis et al. 1995).

Threshold cross-over times

Curtis et al. (1995) have used the cross-over times of the intraspinal threshold recovery curves of afferent fibers to provide an indirect indication of the duration of the orthodromic action potentials invading these terminals. The arrow on the continuous line in Fig. 4A indicates the estimated threshold cross-over time (Th_C) of the L3 terminal following a stimulus applied to the L6 terminal (1.36 ms). During the evoked PAD, this value (Th_{COND}) remained essentially unchanged (1.43 ms; Fig. 4A *arrow on interrupted line*). For the L6 terminal of the same fiber following stimulation at L3, the estimated cross-over times were of 1.47 and 1.94 ms before and during PAD, respectively (Fig. 4B).

A more accurate calculation of the threshold cross-over times requires subtraction of the time (T) taken by the action potential to travel from one branch to the other (see Curtis et al. 1995, 1997), where $T=T_A+T_B+T_C$ (see Fig. 1B). If $T_{L3}=T_A+T_B+T_D$ and $T_{L6}=T_C+T_D$, it then follows that $T>(T_{L3}-T_{L6})$, because $T_{L3}-T_{L6}=T_A+T_B-T_C$. We can have a better approximation of T by assuming: (a) that the conduction velocity was uniform through both intraspinal terminals, which may not be entirely justified because there is evidence that conduction velocity can be significantly slower in the intraspinal arborizations of the afferent fibers (see Discussion; Fern et al. 1988), (b) that the collaterals from the dorsal columns to the site of excitability testing had a straight trajectory that may be estimated from the depth of the stimulating micropipette, (c) that the conduction time was nearly the same for both the L3 and L6 intraspinal collaterals, and (d) that utilization time was of 0.2 ms (see Gustafsson and Jankowska 1976).

With these assumptions, the time required for the action potential to travel from the L6 to the L3 branch in the fiber of Figs. 3 and 4 was of 0.89 ms. After subtracting this value, the cross-over time was reduced to 0.47 ms. During PAD the corrected cross-over time would be of 0.54 ms. In the case of the L6 terminal of the same fiber, the corrected cross-over time would be reduced to 0.58 ms, and during PAD would be increased to 1.05 ms.

The L3 and L6 terminals of fibers with mean conduction velocities above 60 m/s had a mean cross-over time of 0.83±0.34 ms (n=5), and of 0.92±0.22 ms during PAD. Clearly, these are still overestimates, because the conduction velocity in the intraspinal terminals can be slower than in the peripheral axon. On the other hand, the four terminals in L3 and the five terminals in L6 of the fibers with conduction velocities below 60 m/s appear to have a more prolonged threshold recovery time than the faster conducting fibers after being invaded by action potentials. The mean cross-over time derived from the corresponding recovery curves was 3.08±1.35 ms (n=9), and of 4.06±1.05 ms during PAD.

The minimal C-T time interval at which it was possible to produce an antidromic action potential was reduced during PAD in seven branches by 0.1–0.3 ms (mean 0.22±0.1 ms; see Fig. 3D, E) and was increased by 0.2 ms in one branch. The shortening of the critical C-T time interval during the PBSt-induced PAD had no clear relation with the resting threshold of the branch, nor with the magnitude of the PAD produced by PBSt conditioning. It may have been produced by activation of the afferent fibers at a more distal node of Ranvier because of the increased stimulus strength required to activate the fiber during the relative refractoriness. Another explanation might be that the PAD induced an acceleration of action potentials.

Discussion

Conduction of action potentials in intraspinal terminals

As stated in the Introduction, one of the main purposes of this investigation has been to examine the extent to which PAD affects conduction of action potentials within the intraspinal terminals of single muscle spindle afferents. We found that in all the nine examined fibers, action potentials generated in one branch increased the intraspinal threshold of the other branch at short C-T time stimulus intervals (<1.5–2.0 ms). In no case PAD appeared to prevent the threshold increase during the relative refractoriness produced by a preceding action potential.

In this regard, it should be pointed out that the method allows detection of an action potential at some distance from the microelectrode, but it is not certain that the action potential further propagated along the branch up to the microelectrode site. Nonetheless, our observations indicate that the mean intraspinal threshold cross-over times of the five branches of fibers with the highest mean conduction velocities was of 0.8±0.3 ms, which places them closer to the values reported by Curtis et al. (1995) for terminals (0.8–1.2 ms). It thus seems reason-

able to conclude, at least for those fibers with conduction velocities above 60 m/s, that PAD produces no conduction block along the relatively fine myelinated intraspinal terminals of Ia and possibly also of Ib afferents ending either at the L3 or L6 level.

Relief of an already established conduction block within the examined portions of the afferent fibers also seems not to play a significant role in the differential inhibition of the PBSt-induced PAD in the terminal arborizations of the muscle spindle afferents. Most likely, the differential inhibition of PAD results from changes in synaptic effectiveness within those sets of neurons mediating the PAD of individual or small groups of collaterals (Quevedo et al. 1997). The question of whether or not there is conduction block during PAD in the more caudal branches of muscle spindle afferents, as in the long-range cutaneous fibers of the rat (Wall 1994; Wall and McMahon 1994), remains open.

The mean cross-over times of nine endings from fibers with low mean conduction velocity was 3.1±1.3 ms. These values are clearly larger than those reported by Curtis et al. (1995) for group I afferents. Since these endings had a relatively high activation threshold (4.0–20 μA), it could be argued that the threshold recovery curves were more prolonged because the excitability testing micropipette was relatively far from the terminals. On the other hand, it is possible that these were indeed slow-conducting fibers with a higher intraspinal threshold and longer lasting action potentials, but this must be left as an open issue. What is clear, however, is that even with all of the above limitations, there were no signs of conduction block during PAD elicited in at least the myelinated intraspinal segments of the afferents with low mean conduction velocity.

Functional identity of the analyzed fibers

The experimental paradigm used in this study required recording of antidromic action potentials from the central end of peripheral nerve filaments. This precluded use of responses to muscle stretch and/or muscle contraction to identify the afferent fibers according to their receptor type, as was done in other studies (Jiménez et al. 1988; Enríquez et al. 1996).

As discussed in the Results, the data presented in this study were obtained from nine pairs of branches of single muscle afferents. Their peripheral thresholds varied between 1.04 and 1.73×T, which places them in the group I range (Jack 1978). On the other hand, their mean conduction velocities varied between 34.5 and 81.4 m/s, which could comprise group I as well as group II afferents.

Analysis of the PAD patterns of the afferent fibers indicates that at least four, and probably five, of the afferents with the lowest mean conduction velocities (L6 conduction velocity 34.5–54.6 m/s) and three of the afferents with the highest mean conduction velocities (L6 conduction velocity 68.0–81.4 m/s) had the type A PAD pattern characteristic of most group Ia afferents. Namely, they were depolarized by PBSt but not by cutaneous afferents which instead inhibited the PBSt-induced PAD (Rudomin et al. 1986). This could be an argument suggesting that these fibers were group I and not group II because, as shown by Riddell et al. (1995), group II fibers are strongly depolarized by cutaneous and by group II inputs, and the only fiber that was depolarized by stimulation of cutaneous nerves had a mean conduction velocity of 81.4 m/s and a peripheral threshold of 1.1×T and could be either Ia or Ib (Enríquez et al. 1996). However, considering the uncertainties in the classification of the examined afferents as group I or group II, the restricted set of nerves utilized to define the PAD patterns, and the variety of PAD patterns that are displayed muscle spindle and tendon organs (Enríquez et al. 1996), the possibility that some of the fibers with low mean conduction velocity presently analyzed were in fact group II and had type A PAD pattern must be left open for future studies.

Some functional implications

One of the purposes of this investigation was to examine the extent to which impulses generated in one branch of muscle spindle afferents are able to invade another branch several segments away, and if PAD elicited in these branches can produce conduction block. In this regard, it must be pointed out, as discussed above, that with the approach employed in the present study, it was not possible to exclude the possibility that PAD blocked conduction of action potentials in the unmyelinated intraspinal terminals of the afferent fibers. However, even in these circumstances, because of the local character of PAD elicited in different collaterals of individual afferents (Quevedo et al. 1997; Lomelí et al. 1998), conduction block and/or reduction of the synaptic effectiveness would be expected to occur only in those collaterals subjected to PAD (Cattaert et al. 1992; Nusbaum et al. 1996; Cattaert and El-Manira 1999).

The dorsal root reflexes generated in some collaterals during PAD, for example during fictive locomotion (see Dubuc et al. 1985, 1988; Dueñas et al. 1990; Rossignol et al. 1998), would still propagate to those branches not subjected to PAD, and activate second-order neurons, in the same manner as during stimulation of the peripheral nerves (Gossard 1995; Nusbaum et al. 1996). This could allow a differential activation of neurons subserved by a common sensory input, thus complementing the segmental and descending differential inhibition of PAD observed in pairs of collaterals of individual muscle spindle afferents (Eguibar et al. 1997; Lomelí et al. 1998).

A closely related question is the influence that the dorsal root reflexes produced in individual afferents during PAD may have on the spike trains generated in the receptor itself. Gossard et al. (1999) found that anti-

dromic action potentials in Ia afferents are able to reset the discharges of muscle spindles and even to reduce their frequency of discharge in a cumulative manner. The functional relevance of this mechanism will depend on the degree of synchronization of the dorsal root reflexes generated in the whole population of afferent fibers, which can be rather high, as suggested by the observations of Kerkut and Bagust (1995) and Bagust et al. (1989) in the isolated spinal cord of the adult rat (see also Rudomin and Dutton 1969).

Acknowledgements We thank C. León for technical assistance and J.E. Velázquez and P. Reyes for skillful programming. This work was partly supported by NIH grant NS 09196, CONACyT 41739, 3908 N, and by Sistema Nacional de Investigadores, México. J.L. had support from CONACyT and from the National Polytechnic Institute; L.C. had support from CONACyT.

References

Bagust J, Kerkut GA, Rakkeh NIA (1989) The dorsal root reflex in isolated mammalian spinal cord. Comp Biochem Physiol 93:151–160

Blight AR, Someya S (1985) Depolarizing afterpotentials in myelinated axon of mammalian spinal cord. Soc Neurosci Abstr 15:1–12

Castillo L, Lomelí J, Linares P, Rudomin P (1998) Does PAD block spike invasion to axonal branches of individual group I afferents? Soc Neurosci Abstr 24:1152

Cattaert D, El Manira A (1999) Shunting versus inactivation: analysis of presynaptic inhibitory mechanisms in primary afferents of the crayfish. J Neurosci 19:6079–6089

Cattaert D, El Manira A, Clarac F (1992) Direct evidence for presynaptic inhibitory mechanisms in crayfish sensory afferents. J Neurophysiol 67:610–624

Curtis DR (1998) Two types of inhibition in the spinal cord. In: Rudomin P, Romo R, Mendell L (eds) Presynaptic inhibition and neural control. Oxford University Press, New York, pp 150–161

Curtis DR, Gynther BD, Beattie DT, Lacey G (1995) An electrophysiological investigation of group Ia afferent fibres and terminations in the cat spinal cord. Exp Brain Res 106: 403–417

Curtis DR, Gynther BD, Lacey G, Beattie DT (1997) Baclofen: reduction of presynaptic calcium influx in the cat spinal cord in vivo. Exp Brain Res 113:520–523

Dubuc R, Cabelguen JM, Rossignol S (1985) Rhythmic antidromic discharges of single primary afferents recorded in cut dorsal root filaments during locomotion in the cat. Brain Res 359: 375–378

Dubuc R, Cabelguen JM, Rossignol S (1988) Rhythmic fluctuations of dorsal root potentials and antidromic discharges of single primary afferents during fictive locomotion in the cat. J Neurophysiol 60:2014–2036

Dueñas SH, Loeb GE, Marks WB (1990) Monosynaptic and dorsal root reflexes during locomotion in normal and thalamic cats. J Neurophysiol 63:1467–1476

Eguibar JR, Quevedo J, Rudomin P (1997) Selective cortical and segmental control of primary afferent depolarization of single muscle afferents in the cat spinal cord. Exp Brain Res 113: 411–430

Enríquez M, Jiménez I, Rudomin P (1996) Changes in PAD patterns of group I muscle afferents after peripheral nerve crush. Exp Brain Res 107:405–421

Fern R, Harrison PF, Riddell JS (1988) The dorsal column projection of muscle afferent fibres from the cat hindlimb. J Physiol 401:97–113

Gossard JP (1995) Do antidromic discharges in primary afferents have postsynaptic effects? Soc Neurosci Abstr 21:414

Gossard JP, Bouyer L, Rossignol S (1999) The effects of antidromic discharges on orthodromic firing of primary afferents in the cat. Brain Res 825:132–145

Graham B, Redman SJ (1994) A simulation of action potentials in synaptic boutons during presynaptic inhibition. J Neurophysiol 71:538–549

Gustafsson B, Jankowska E (1976) Direct and indirect activation of nerve cells by electrical pulses applied extracellularly. J Physiol (Lond) 258:33–61

Henneman E, Lüscher HR, Mathis J (1984) Simultaneously active and inactive synapses of single Ia fibres on cat spinal motoneurones. J Physiol (Lond) 352:147–161

Jack JJB (1978) Some methods for selective activation of muscle afferents fibers. In: Porter R (ed) Studies in neurophysiology. Cambridge University Press, Cambridge, pp 155–176

Jankowska E, McCrea D, Rudomin P, Sykova E (1981) Observations on neuronal pathways subserving primary afferent depolarization. J Neurophysiol 46:506–516

Jiménez I, Rudomin P, Solodkin M (1988) PAD patterns of physiologically identified afferent fibres from the medial gastrocnemius muscle. Exp Brain Res 71:643–657

Kerkut GA, Bagust J (1995) The isolated mammalian spinal cord. Prog Neurobiol 46:1–48

Lamotte d'Incamps B, Destombes J, Thiesson D, Hellio R, Lasserre X, Kouchtir-Devanne N, Jami L, Zytnicki D (1998) Indications of GABA-immunoreactive axoaxonic contacts on the intraspinal arborization of a Ib fiber in the cat: a confocal microscope study. J Neurosci 18:10030–10036

Lomelí J, Quevedo J, Linares P, Rudomin P (1998) Local control of information flow in segmental and ascending collaterals of single afferents. Nature 395:600–604

Luscher HR (1998) Control of action potential invasion into terminal arborizations. In: Rudomin P, Romo R, Mendell L (eds) Presynaptic inhibition and neural control. Oxford University Press, New York, pp 126–137

Maxwell DJ, Riddell JS (1999) Axoaxonic synapses on terminals of identified group II muscle spindle afferent axons in the cat spinal cord. Eur J Neurosci 11:2151–2159

Maxwell DJ, Christie WM, Short AD, Brown AG (1990) Direct observations of synapses between GABA-immunoreactive boutons and muscle afferent terminals in lamina VI of the cat's spinal cord. Brain Res 53:215–222

Nusbaum MP, El Manira A, Gossard J-P, Rossignol S (1996) Presynaptic mechanisms during rhythmic activity in vertebrates and invertebrates. In: Stein PSG, Grillner S, Selverston AI, Stuart DG (eds) Neurons, networks and motor behaviour. MIT Press, Cambridge, pp 237–253

Quevedo J, Eguibar JR, Lomelí J, Rudomin P (1997) Patterns of connectivity of spinal interneurons with single muscle afferents. Exp Brain Res 115:387–402

Redman S (1990) Quantal analysis of synaptic potentials in neurons of the central nervous system. Physiol Rev 70:165–198

Riddell JS, Jankowska E, Huber J (1995) Organization of neuronal systems mediating presynaptic inhibition of group II muscle afferents in the cat. J Physiol 483:443–460

Rossignol S, Beloozerova I, Gossard JP, Dubuc R (1998) Presynaptic mechanisms during locomotion. In: Rudomin P, Romo R, Mendell L (eds) Presynaptic inhibition and neural control. Oxford University Press, New York, pp 385–397

Rudin DO, Eisenman G (1953) After-potential of spinal axons in vivo. J Gen Physiol 36:643–657

Rudomin P (1999) Selectivity of presynaptic inhibition: a mechanism for independent control of information flow through individual collaterals of single muscle spindle afferents. Prog Brain Res 123:109–117

Rudomin P, Dutton H (1969) Effects of muscle and cutaneous afferent nerve volleys on excitability fluctuations of Ia terminals. J Neurophysiol 32:158–169

Rudomin P, Schmidt RF (1999) Presynaptic inhibition in the vertebrate spinal cord revisited. Exp Brain Res 129:1–37

Rudomin P, Engberg I, Jiménez I (1981) Mechanisms involved in presynaptic depolarization of group I and rubrospinal fibers in cat spinal cord. J Neurophysiol 46:532–548

Rudomin P, Jiménez I, Solodkin M, Dueñas S (1983) Sites of action of segmental and descending control of transmission on pathways mediating PAD of Ia- and Ib-afferent fibers in cat spinal cord. J Neurophysiol 50:743–769

Rudomin P, Solodkin M, Jiménez I (1986) PAD and PAH response patterns of group Ia- and Ib-fibers to cutaneous and descending inputs in the cat spinal cord. J Neurophysiol 56:987–1006

Segev I (1990) Computer study of presynaptic inhibition controlling spread of action potentials into axonal terminals. J Neurophysiol 63:987–998

Wall PD (1994) Control of impulse conduction in long range afferents. Eur J Neurosci 6:1136–1142

Wall PD (1995) Do nerve impulses penetrate terminal arborizations? A presynaptic control mechanism. Trends Neurosci 18: 99–103

Wall PD, McMahon SB (1994) Long range afferents in rat spinal cord. III. Failure of impulse transmission in axons and relief of the failure after rhizotomy of dorsal roots. Philos Trans R Soc Lond B Biol Sci 343:211–223

Walmsley B, Nicol MJ (1991) The effects of Ca^{2+}, Mg^{2+} and kynurenate on primary afferent synaptic potentials evoked in cat spinal cord neurones in vivo. J Physiol 433:409–420

Walmsley B, Nicol MJ (1998) Action potential propagation along primary afferents and presynaptic inhibition in Clarke's column of the spinal cord. In: Rudomin P, Romo R, Mendell L (eds) Presynaptic inhibition and neural control. Oxford University Press, New York, pp 138–149

Walmsley B, Graham B, Nicol MJ (1995) Serial E-M and simulation study of presynaptic inhibition along a group of Ia branch in the spinal cord. J Neurophysiol 74:616–623