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RESEARCH ARTICLE

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Tonic differential supraspinal modulation of PAD and PAH of segmental and ascending intraspinal collaterals of single group I muscle afferents in the cat spinal cord

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Abstract We compared in the anesthetized cat the effects of reversible spinalization by cold block on primary afferent depolarization (PAD) and primary afferent hyperpolarization (PAH) elicited in pairs of intraspinal collaterals of single group I afferents from the gastrocnemius nerve, one of the pairs ending in the L3 segment, around the Clarke's column nuclei, and the other in the L6 segment within the intermediate zone. PAD in each collateral was estimated by independent computer-controlled measurement of the intraspinal current required to maintain a constant probability of antidromic firing. The results indicate that the segmental and ascending collaterals of individual afferents are subjected to a tonic PAD of descending origin affecting in a differential manner the excitatory and inhibitory actions of cutaneous and joint afferents on the pathways mediating the PAD of group I fibers. The PAD-mediating networks appear to function as distributed systems whose output will be determined by the balance of the segmental and supraspinal influences received at that moment. It is suggested that the descending differential modulation of PAD enables the intraspinal arborizations of the muscle afferents to function as dynamic systems, in which information transmitted to segmental reflex pathways and to Clarke's column neurons by common sources can be decoupled by sensory and descending inputs, and funneled to specific targets according to the motor tasks to be performed.

Keywords Primary afferent depolarization · Presynaptic inhibition · Muscle afferents · Spinal reflexes

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Introduction

Rudomin et al. (1974) reported that stimulation of cutaneous afferents often increases the intraspinal threshold of group I muscle afferents (primary afferent hyperpolarization or PAH) and leads to presynaptic facilitation. Although it was proposed that PAH could be due to direct hyperpolarization of the afferent terminals (Chan and Barnes 1972), the prevailing view has been that PAH results from inhibition of a tonic primary afferent depolarization (PAD), at least for group I afferents (see Burke and Rudomin 1977; Rudomin and Schmidt 1999). This view has been supported by the finding that stimulation of cutaneous and joint afferents, as well as stimulation of supraspinal structures such as the motor cortex, bulbar reticular formation and raphe nuclei, reduced the PAD elicited in functionally identified muscle spindle and tendon organ afferents (see Rudomin et al. 1986; Enríquez et al. 1996).

Although there is a substantial amount of information supporting the view that cutaneous afferents are subjected to a tonic PAD (Carpenter et al. 1963a; Lundberg 1964, 1982; Lidierth and Wall 1998), the information pertaining to tonic PAD of group I muscle afferents is rather scarce (see Rudomin and Schmidt 1999). Quevedo et al. (1993) have reported that, unlike the dorsal root potentials (DRPs) produced by stimulation of cutaneous and articular afferents, the DRPs produced by stimulation of group I fibers from the posterior biceps and semitendinosus nerves (PBSt) are depressed rather than facilitated during reversible spinalization (see also Carpenter et al. 1963a, 1963b), which may be attributed to the suppression of a tonic descending facilitation acting on the pathways mediating the PAD of group I afferents. In addition Quevedo et al. (1993) found that spinalization affected the magnitude of the PAD produced by PBSt stimulation in single group I gastrocnemius soleus (GS) fibers ending in the intermediate zone of the L6 segment, as well as the magnitude of the inhibition of PAD that follows stimulation of cutaneous and joint nerves.

Recently we made a thorough study of the PAD patterns of pairs of L3 and L6 collaterals of single group I afferents and found that these patterns can be changed in a differential manner following stimulation of peripheral and descending pathways (Rudomin et al. 2004). The present report describes effects of a reversible cold block of impulse conduction in the thoracic spinal segments in the same population of afferent fibers. It provides further evidence for a tonic differential PAD of L3 and L6 collaterals of group I afferents that is modulated by stimulation of cutaneous and articular nerves, as well as by stimulation of the motor cortex. Some of the observations presented in this paper were reported in a preliminary publication (Lomelí et al. 1998).

Methods

Preparation

The data included in this report were obtained from the same series of experiments as those reported by Rudomin et al. (2004), which may be consulted for details. Guidelines contained in Principles of Laboratory Animal Care (NIH publications 85–23, revised in 1985) were followed throughout. Adult cats of either sex (2.8–4.0 kg) were anesthetized with pentobarbitone sodium 40 mg/kg i.p. The carotid artery, radial veins, trachea and urinary bladder were cannulated. A solution of 100 mM of sodium bicarbonate with glucose 5% was given intravenously (0.055 ml/min) to prevent acidosis (Jankowska and Riddell 1995). When necessary, additional doses of 2 mg/kg of pentobarbitone sodium were applied intravenously to maintain a deep level of anesthesia during the surgical procedures, tested by assessing that withdrawal reflexes were absent, that the pupils were constricted and that arterial blood pressure was between 100 and 120 mmHg.

The left posterior biceps and semitendinosus (PBSt), sural (SU), superficial peroneus (SP), and posterior articular (PAN) nerves were dissected free, sectioned and their central ends mounted on bipolar electrodes for stimulation. The left lateral (LG) and/or medial (MG) gastrocnemius nerves (GS when together) were dissected up to their fine branches in the muscle and sectioned.

After the nerve dissection, S1–L1 and T12–T10 spinal segments were exposed by laminectomy. The cat was fixed to a rigid metal frame, the dura mater opened and the left S1–L4 ventral roots sectioned. The right motor cortex and the brain stem were exposed by craniotomy. Partial removal of the cerebellum allowed exposure of the floor of the fourth ventricle.

After the main dissection, the animals were paralyzed with pancuronium bromide (Pavulon, initial dose 0.3 mg/kg; subsequently 0.3 mg was applied every 45 min) and maintained under artificial ventilation. A bilateral pneumothorax was performed and end tidal volume was adjusted to maintain expiratory CO_2 level at about 4%. Mean blood pressure was usually between 80 and 120 mmHg. When necessary a solution of etilefrine (1:10 in saline; Effortil, Boehringer-Ingelheim) or dextran (10%) was infused intravenously at a rate of 3 ml/h to keep the blood pressure within this range. Adequacy of anesthesia was ensured with supplementary doses of anesthetic (2 mg/kg in a hour) and by verifying that blood pressure was constant when applying noxious stimulation and that the pupils were fully constricted. To prevent desiccation of the exposed tissues, pools were made with the skin flaps, filled with paraffin oil and maintained between 36° and 37°C by means of radiant heat.

Segmental and supraspinal stimulation

Ag/AgCl stimulating ball electrodes were placed on the surface of the right (contralateral) motor cortex, in the region of the posterior sigmoid gyrus somewhat laterally to the hindlimb representation of group I afferents. The indifferent electrode was inserted in the occipital muscle. Stimuli to the cortex were trains of anodal pulses. A pair of silver ball electrodes was placed over the surface of the spinal cord at a low lumbar level on the left side to stimulate the dorsolateral fasciculus (DLF). Stimulating tungsten electrodes were also placed in the left bulbar reticular formation (RF). The usual location of the RF electrode was 4 mm rostral, 2 mm lateral to the obex, and 3–5 mm in depth.

In this series of experiments, the PBSt nerve was stimulated with trains of four pulses at 300 Hz, 1.3–2×T strength, applied 25 ms before the threshold testing pulse, and the SU and SP nerves with single pulses 1.3–12.5×T, applied 35 ms before the testing pulse. The PAN, motor cortex and RF were stimulated with trains of eight pulses at 700 Hz applied 75 ms before the threshold testing pulse. Stimulus strengths were 3–15×T for joint afferents, 6–10 V for cortical surface and 50–300 μA for RF stimulation.

Measurement of the intraspinal threshold of single afferents

The experiments aimed to measure the intraspinal threshold changes produced by stimulation of peripheral nerves and supraspinal structures in pairs of collaterals belonging to the same afferent fiber. To this end two stimulating micropipettes were inserted into the spinal cord. One was placed in the region of Clarke's column at the L3 level, and the other within the intermediate nucleus at the L6 level (for details of electrode placement see Rudomin et al. 2004).

Once in position, the two recording micropipettes were connected to separate computer-controlled stimulators that generated constant current pulses (0.4 ms, 1–25 μA) at 1 Hz and were displaced vertically until each of them produced all-or none antidromic responses in the same LG or MG nerve filament. Tests for refractoriness were made to ensure that the antidromic responses were due to

activation of the same afferent fiber (see Figs. 4A, B, 7A, B).

The occurrence of PAD was examined in each collateral by measuring the intraspinal threshold changes produced by conditioning stimulation of sensory nerves or supraspinal structures. To this end, independent current pulses were applied in alternation through each micropipette and their strengths were automatically adjusted by the computer to produce, in each case, antidromic responses of the afferent fiber with a probability of 0.5 (see Eguibar et al. 1997; Quevedo et al. 1997). The current pulses were delivered once per second through each micropipette, separately integrated, and the obtained values maintained until the next cycle to display a continuous recording of the fiber's threshold. These values were digitized and stored to allow subsequent calculations of mean values (see below). During PAD less current is required for antidromic firing of the afferent fiber. During inhibition of background PAD, the stimulating current must be increased (Madrid et al. 1979).

Due to the characteristics of the control system there was a phase lag between the changes in the antidromic firing of the fiber and the change of the threshold current to its new value. The fluctuations in the stimulating current are mostly due to the automatic adjustment of the control system in order to maintain a constant firing probability of the collateral under study. Mean thresholds were calculated from the digitized data points by setting two cursors in the region where the threshold changes had already attained a steady value (see Rudomin et al. 1980).

The threshold changes (ΔT) produced by a given conditioning stimulus were calculated relative to the resting threshold of the fiber (R_τ), according to the expression $\Delta T = (C_\tau/R_\tau) \times 100$, where C_τ is the threshold attained during the application of the conditioning stimuli. Using percentage threshold changes to measure PAD allowed comparison of effects produced by conditioning volleys on different collaterals, since they appear not to depend on the distance between the stimulating micropipette and the tested afferent terminal (see Fig. 1 in Eguibar et al. 1997). Activation of pathways that inhibit the PAD results in a relative increase in the threshold of the fiber to a new steady value, I_τ. The percentage increment in the threshold due to the inhibition may be calculated by $\Delta I = (I_\tau - C_\tau)/(R_\tau - C_\tau) \times 100$.

Spinal block

To block impulse conduction in the thoracic spinal cord, a silver-plated thermode was placed over the surface of the exposed spinal cord at the low thoracic level. The thermode was connected by thermally isolated tubes to reservoirs with warm (40°C) and cold (−19°C) circulating fluid. The thermode had an attached thermocouple that allowed measurement of the temperature of the cord surface below the cooling chamber. The cooling thermode was isolated with Vaseline jelly from the pool of oil. Switching to the cold mixture allowed gradual cooling of the spinal cord surface (between 0 and −3°C). The effectiveness of this procedure to block impulse conduction was assessed by recording in the lumbosacral spinal cord the descending volleys in the cord dorsum produced by constant stimulation of the ipsilateral RF and/or of the contralateral motor cortex. This cooling device was used in previous studies and proved to produce a fully reversible spinal block (see Quevedo et al. 1993).

Histology

At the end of the experiment the animal was put to death with a pentobarbital overdose, perfused with 10% formalin, and the spinal cord removed, leaving the shafts of the two stimulating micropipettes in place. After complete fixation and dehydration, the lumbosacral cord segment containing the micropipettes was placed in a solution of methyl salicylate for clearing. Subsequently, the spinal cord was cut transversally to obtain sections containing the electrodes. The tracks of the microelectrodes were drawn with a camera lucida (Wall and Werman 1976).

Results

General features of the afferent fibers

The present series of observations was performed on 42 fibers. Data obtained from these fibers before the spinal block were included in the data base used to analyze the PAD patterns of muscle afferents described in the preceding paper (Rudomin et al. 2004).

As discussed by Rudomin et al. (2004), classification of the afferent fibers as from muscle spindles or tendon organs only on the basis of their PAD patterns can be somewhat misleading, because both types of afferents display similar PAD patterns, although in different proportions within the population (see Enríquez et al. 1996). In the present series of experiments, the conduction velocity (CV) of individual afferents was calculated using the conduction distance measured from the site of micropipette insertion at L3 or L6 to the recording site in the nerve filament divided by the conduction time of the antidromic responses. This gives overall conduction velocities that are usually lower than peripheral conduction velocities because they include intraspinal conduction times.

Figure 1 shows an inverse relationship between the peripheral threshold of the fiber and the conduction velocity (see also Jack 1978 and Jankowska et al. 1993). The overall conduction velocity of the fibers varied from 37 to 97.7 m/s and from 37.4 to 106 m/s when using data from the L3 and L6 collaterals, respectively (means 68.5 ±13.5; 68.4±15.2 m/s). Their peripheral thresholds were lower than 2.5×T, most of them below 2.0×T. According to the criteria outlined above, we have considered fibers with peripheral thresholds below 2×T as group I (see Riddell et al. 1995), but the possibility that fibers with overall

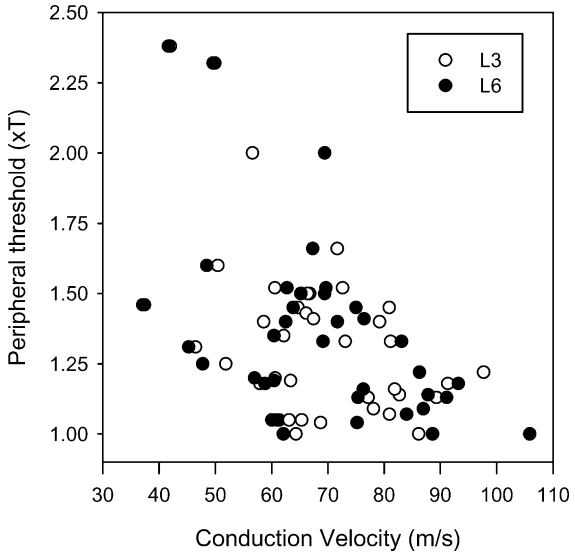

Fig. 1 Conduction velocity versus peripheral threshold. The conduction velocity (CV) of individual afferents was calculated from the conduction distance divided by the conduction time of the antidromic responses produced by intraspinal stimulation at L3 (*open circles*) and at L6 (*filled circles*). The peripheral threshold of each fiber was obtained from the strength of the peripheral nerve stimulus required to block the antidromic response recorded from the same nerve

conduction velocities below 70 m/s were group II should be left open (Lomelí et al. 2000).

Effects of spinal block on resting intraspinal threshold

Previous investigations from our laboratory (Quevedo et al. 1993) have shown that spinal block could increase the inhibitory actions of cutaneous and joint afferents on the PAD of group I fibers. This effect was attributed to the suppression of a tonic descending modulation exerted on the pathways mediating inhibition of PAD. We have now examined the effects induced by spinal cold block on the resting threshold of pairs of L3 and L6 collaterals belonging to the same fiber. Figure 2 provides one example of the effects of cold-block reversible spinalization. Cooling the thoracic spinal cord to block conduction increased the threshold of the L3 collateral and decreased the threshold of the L6 collateral of this particular fiber. These threshold changes were maintained throughout the whole time of spinalization. Rewarming the spinal cord reversed the effects of the cold block.

Spinal block produced a *transient* blood pressure drop, of 10–15 mmHg. We were concerned about the extent to which these changes in blood pressure contributed to the observed changes in baseline threshold by displacing the nervous tissue relative to the threshold testing micropipettes. The vertical line labeled 1 in Fig. 2 has been aligned with the onset of the intraspinal threshold change in the L3 collateral. It should be noted that the threshold changes *preceded* the onset of the blood pressure drop, by 18 s in this case. Vertical line 2 was placed at a time when the blood pressure had regained its control level. If blood pressure changes were responsible for the shifts in baseline threshold, we would expect that at that time the threshold of both collaterals would be shifted to the values attained before the cold block. Instead, the threshold changes kept the same level until the removal of the cold block (indicated by vertical line 3). Both collaterals recovered their original thresholds, although in this case the time course of the recovery resembled that of the blood pressure rise.

Data on the effects of cold block spinalization on the resting threshold of the L3 and L6 collaterals that seemed not to be due to blood pressure changes were obtained in 20 out of the 42 fibers examined. Figure 3A, B shows the effects of cold block spinalization on the resting threshold of the L3 and L6 collaterals. The changes in resting threshold are expressed as percentage relative to control. They were plotted in increasing order and displayed sequentially. Values smaller than 100% indicate reduction and values above 100% indicate increase in resting threshold. We found that spinalization increased the threshold of 8/20 L3 and of 12/20 L6 collaterals and reduced the threshold of 6/20 L3 and of 4/20 L6 collaterals. Figure 3C shows the effects of reversible spinal cold block on the resting threshold of pairs of collaterals of the same afferent fiber. Spinalization increased the threshold of both collaterals in 6/20 fibers and reduced the threshold in 2/20 fibers. In the other fibers the threshold was increased in one collateral and was either unchanged or reduced in the other collateral.

The large variability of the effects produced by spinal block on the resting threshold of the L3 and L6 collaterals could be due to the use of data obtained from afferent fibers with different levels of tonic PAD (see Lomelí et al. 1998). But even so, spinalization appears to increase the resting intraspinal threshold of a substantial number of L3 as well as L6 collaterals.

Effects of spinalization on the phasic PAD produced by muscle afferents

Figures 4 and 7 provide two particular examples of the effects produced by spinalization on the PBSt-induced PAD. In both cases the PAD produced by PBSt stimulation in the L3 and L6 collaterals appeared not to be significantly changed by spinalization. However, as shown below, in other fibers this procedure clearly modified the PBSt-induced PAD. Altogether we examined the effects of spinal block on the phasic PBSt-induced PAD in 42 pairs of L3 and L6 collaterals of single fibers.

The filled circles in Fig. 5A show the percentage threshold changes relative to resting threshold of the L3 collaterals produced by stimulation of the PBSt nerve *before the spinal block,* ranked in increasing order. In this plot, the larger the reduction in percentage threshold, the stronger the PAD (see "Methods"). Values obtained from the same terminal during spinal block are indicated by the corresponding data points (white circles). It is to be noted

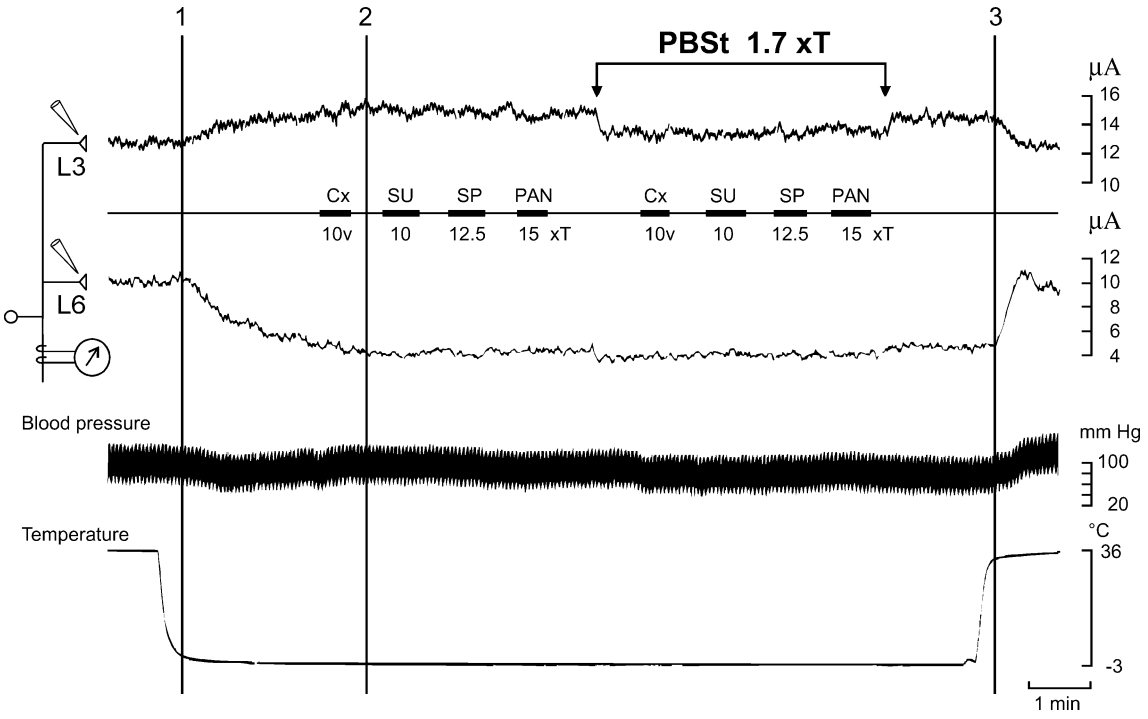

Fig. 2 Effects of cold block spinalization on the intraspinal threshold of a pair of L3 and L6 collaterals of the same afferent. Stimulating current pulses were applied alternatively through the L3 and L6 micropipettes once per second while recording the antidromic responses of a single GS fiber conducting at 63.1 m/s and with a peripheral threshold of 1.05×*T*. The intensity of the intraspinal current pulses was automatically adjusted in order to produce antidromic responses with a constant probability. The current was integrated and recorded as a continuous trace. *Traces from above downwards* are intraspinal threshold of L3 and L6 collaterals, blood pressure and temperature of cord surface below the cooling device. Stimulus intensities for SU, SP and PAN are indicated. Further explanations are given in the text

that in most (34/42) L3 collaterals the PBSt-induced PAD was only slightly affected by spinalization, regardless of the magnitude of the PAD elicited before the spinal block. In contrast, in 17/42 L6 collaterals, spinalization reduced the PAD produced by PBSt stimulation (Fig. 5B).

To compare the effects of spinalization on PAD in one collateral relative to the other, we subtracted the percentage threshold changes produced by the PBSt stimulus in the L3 collateral from the changes produced in the corresponding L6 collateral. The differences

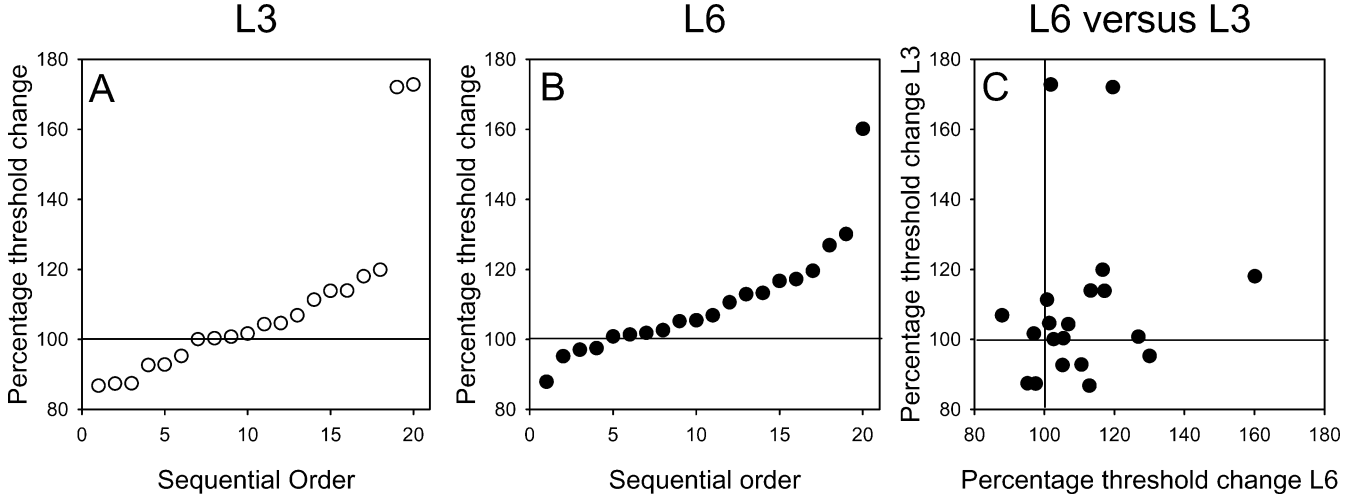

Fig. 3A–C Cold block spinalization changes the resting threshold of segmental and ascending collaterals of group I afferents. **A, B** Effects of cold block spinalization on the resting threshold of the L3 and L6 collaterals. Changes in resting threshold are expressed as percentage relative to control and were plotted in increasing order and displayed sequentially, as indicated. Values smaller than 100% indicate reduction and values above 100% indicate increases of resting threshold. **C** Percentage changes in resting threshold produced by spinalization in the L6 collateral versus changes produced in the L3 collaterals of the same afferent fiber. Further explanations are given in the text

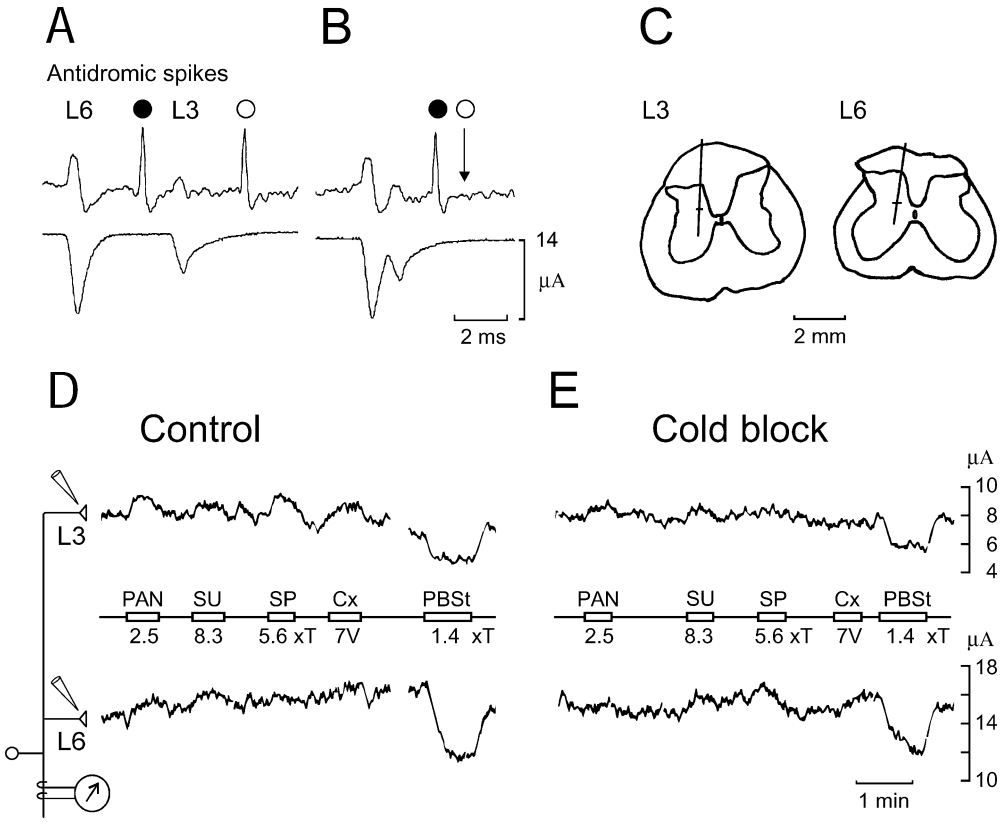

Fig. 4A–E Effects of spinalization on phasic PAD and PAH produced in the segmental and ascending collaterals of a single afferent. **A, B** Test for refractoriness between antidromic spikes elicited by L6 and L3 stimulation. *Upper traces* Antidromic responses (marked by *open and closed circles*). *Lower traces* Stimulating current. Note that at short time intervals between the L6 and the L3 pulses, the latter produces no antidromic responses. **C** Histological reconstructions of the micropipette tracks. **D** Changes in intraspinal threshold of the L3 and L6 collaterals produced by stimulation of muscle and cutaneous nerves and of the motor cortex. **E** Same as **C**, but during spinal block at T12. This fiber had a peripheral threshold of $1.33\times T$ and a conduction velocity of 73.1 m/s, derived from the latencies of the antidromic responses produced by L6 stimulation

obtained before the spinal block were ranked in increasing order from negative to positive (Fig. 5C, filled circles). Negative values would indicate that the PAD was larger in the L6 than in the L3 collateral, while positive values would indicate the opposite, that is, a larger PAD in L3 than in L6 collaterals. In 22/42 fibers spinalization produced changes below 5% in the relative magnitude of the PAD, while in 17/42 fibers the differences were shifted upwards and even reversed in sign during spinalization (Fig. 5C, open circles). That is, the PAD elicited in the L6 collaterals was reduced by spinalization more than the PAD produced in the corresponding L3 collaterals.

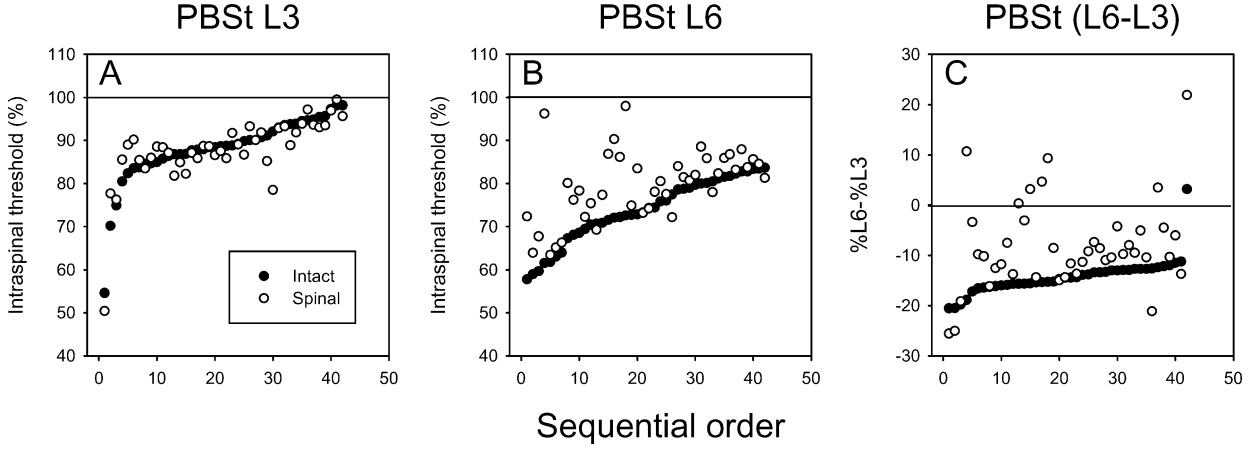

Fig. 5A–C Summary of effects of spinalization on PAD produced by PBSt stimulation in pairs of segmental and ascending collaterals. **A, B** *Ordinates* Percentage threshold changes produced in L3 and L6 collaterals by stimulation of the PBSt nerve with strengths maximal for group I afferents. Data points were ranked in ascending order according to the magnitude of the threshold changes produced before the spinal block and displayed sequentially (*filled circles*). Data obtained from the corresponding fibers during spinal block are shown by *open circles*. **C** Differences between the percentage threshold changes produced in the L6 collaterals and the L3 collaterals. As in **A** and **B**, data points obtained with the intact neuroaxis are also ranked in increasing order (*filled circles*). Data obtained during spinalization are indicated with *open circles*. Further explanations are given in the text

Effects of spinalization on PAD and PAH produced by stimulation of cutaneous and joint afferents

We have shown previously that stimulation of cutaneous and joint nerves can produce either PAD or PAH of group I muscle afferents (Rudomin et al. 2004), and also that in many fibers spinalization increases the cutaneous-induced inhibition of the PAD (Quevedo et al. 1993). The data depicted in Fig. 4D, E provide one example where cold block spinalization reduced the PAH produced in the L3 collaterals by stimulation of the PAN, SU and SP nerves (see also Fig. 7E, F). In other fibers reversible spinalization increased the PAH (see below).

Figure 6 summarizes the effects of cold block spinalization on the PAD and PAH induced by stimulation of cutaneous and joint afferents on 42 pairs of L3 and L6 collaterals of single afferents. As in Fig. 5A, B, we have expressed the effects produced in each collateral as percentage change relative to baseline threshold. Values above 100% would indicate threshold increase (that is, PAH) and values below 100% would indicate PAD (see Rudomin et al. 2004). In each graph the data points obtained before the spinal block are ranked in ascending order (filled circles). Values obtained from the same terminal during spinal block are indicated by the corresponding data points (white circles). We found that, in contrast to the effects produced by stimulation of the PBSt nerve, the cutaneous and joint nerves increased the threshold in about half of the L3 and L6 collaterals before the spinal block (filled circles in Fig. 6A, B, D, E, G, H). During spinal block the PAD and PAH were either increased or decreased in both collaterals (see below).

The heterogeneity of the effects produced during spinalization is more clearly illustrated in Fig. 6C, F, I.

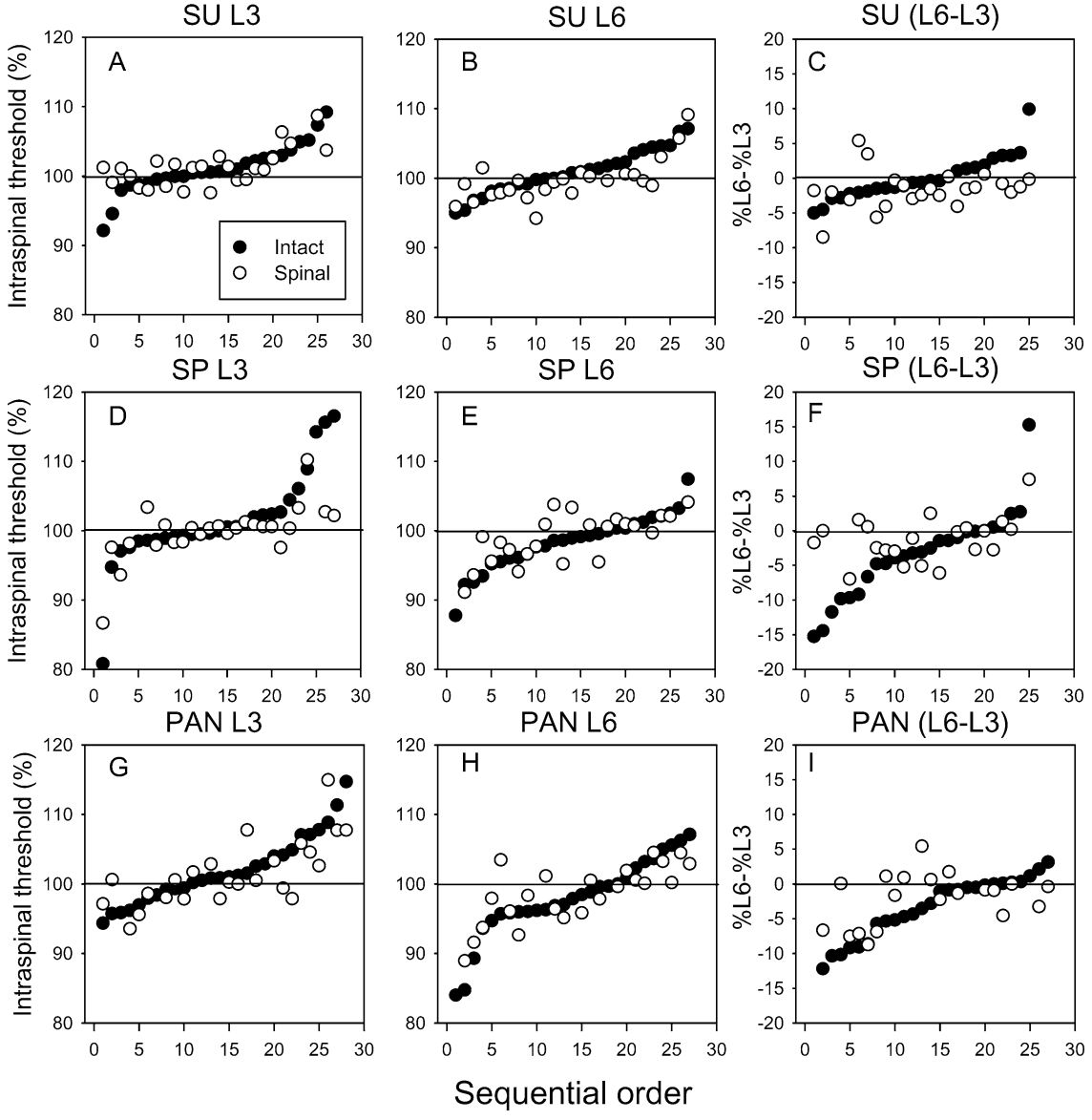

Fig. 6A–I Effects of spinalization of PAD and PAH produced by stimulation of cutaneous and joint nerves. Same format as in Fig. 5. Further explanations are given in the text

As in Fig. 5C, these graphs show the effects produced by a given input in the L6 collateral minus the effects produced in the L3 collateral of the same fiber. Spinalization also affected the magnitude of the PAD and of the PAH produced in one collateral relative to the other collateral. Although there was no clear trend in the effects produced by the spinal block, this finding indicates that the interneuronal pathways that mediate actions of cutaneous and joint afferents on muscle afferents are subjected to descending modulation, even under barbiturate anesthesia, and that this modulation has dual effects: it may increase PAD in some collaterals and reduce PAD in other collaterals of the same afferent (see "Discussion").

Effects of spinalization on the cutaneous-induced inhibition of PAD

We have shown previously that spinalization changes the magnitude of the inhibition that cutaneous and joint afferents produce on the PAD of group I muscle afferents ending in the L6 segment (Quevedo et al. 1993). We have now compared the effects of spinalization on pairs of segmental (L6) and ascending (L3) collaterals of individual group I muscle afferents (see also Lomelí et al. 1998).

Figure 7 provides a representative example of the differential control exerted by supraspinal structures on the inhibition of PAD in the L3 and L6 collaterals. Before the spinal block, graded stimulation of the PBSt nerve reduced the threshold of both the L3 and L6 collaterals by about

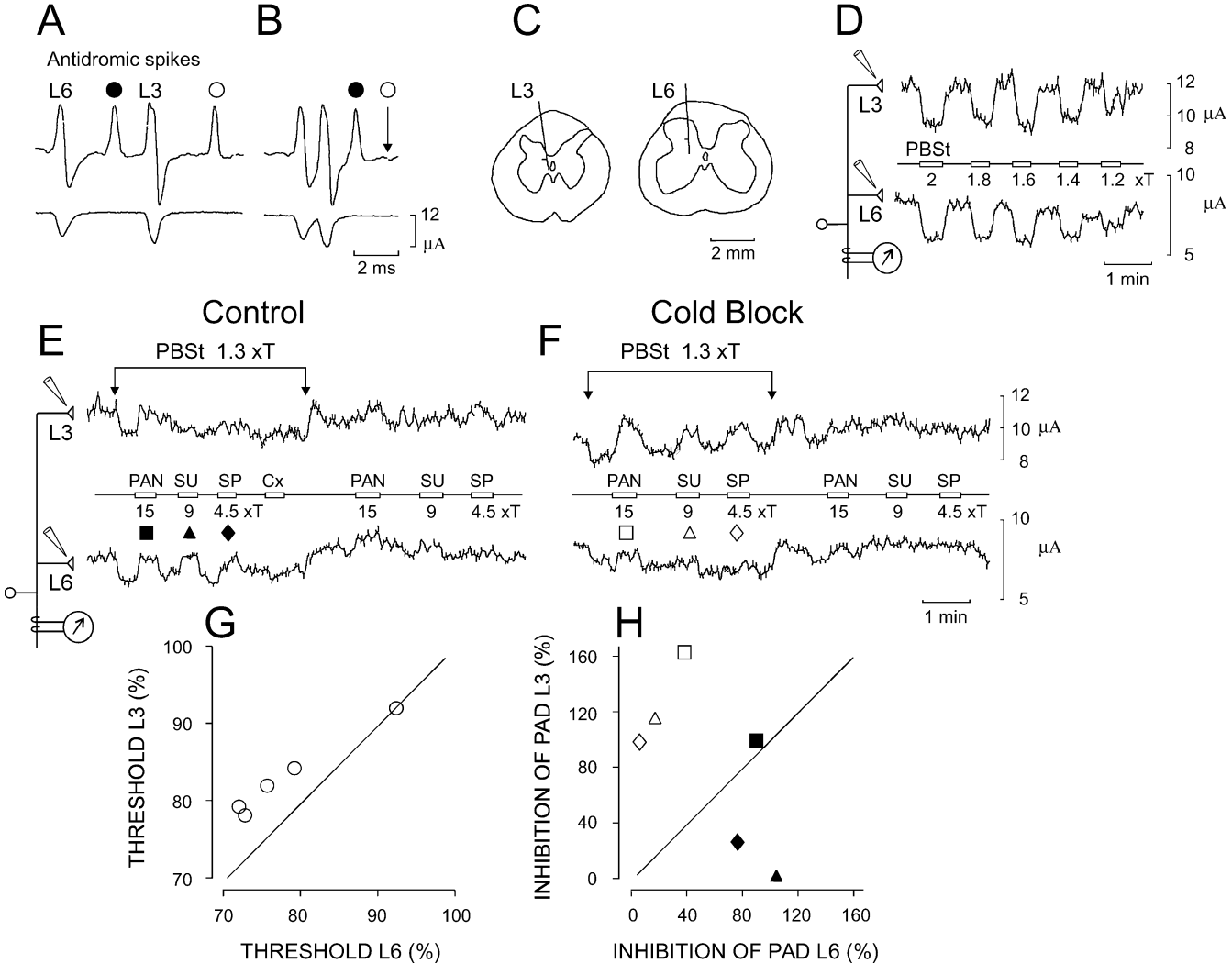

Fig. 7A–H Effects of reversible spinalization on the PAD patterns of L6 and L3 collaterals of a single afferent fiber. **A, B** Test for refractoriness between antidromic spikes elicited by L6 and L3 stimulation. **C** Histological reconstructions of the micropipette tracks. **D** Effects of graded stimulation of the PBSt nerve at the indicated strengths. **E** Effects produced by stimulation of PAN, SU and SP on the PBSt-induced PAD and on the resting threshold. **F** Same as **E**, but during spinal block at T12. **G** Percentage threshold changes produced by PBSt stimulation in the L6 collateral versus changes produced in the L3 collateral before spinal block. **H** Percentage inhibition of PBSt-induced PAD in the L3 versus inhibition in the L6 collateral. *Filled symbols* before, *open symbols* during, spinal block, as indicated. This fiber had a peripheral threshold of 1.07×*T* and a conduction velocity of 84 m/s, derived from latencies of antidromic responses produced by L6 stimulation. Part of the records displayed in **E** and **F** have been published in a preliminary communication (Lomelí et al. 1998) and are reproduced with permission

the same extent (Fig. 7D, G) and conditioning stimulation of the SU and SP nerves preferentially inhibited the PBSt-PAD generated in the L6 collateral (Fig. 7E). During spinal cold block the situation was reversed: stimulation of the SU and SP nerves now produced a stronger inhibition of PAD in the L3 than in the L6 collateral. In contrast, the effects of spinalization on the relative magnitude of the inhibition produced in both collaterals by PAN stimulation were not so evident as for the inhibition produced by SU and SP stimulation (Fig. 7F, H).

The effects of spinalization on the cutaneous-induced differential modulation of the PBSt-induced PAD on the whole sample of examined fibers are summarized in Fig. 8. The data points were ranked according to the magnitude of the percentage changes produced by the conditioning volleys before the spinal block. Positive values indicate inhibition of PAD and negative values facilitation of PAD. In confirmation of a previous report (Quevedo et al. 1993), we found that in many L6 collaterals (and also in L3 collaterals), cold block spinalization increased the magnitude of the inhibition exerted on the PBSt-induced PAD. However, in other collaterals spinalization reduced, and even abolished, the inhibition of PAD.

To compare the effects of spinalization on modulation of PAD in one collateral relative to modulation exerted on the other collateral, for each fiber we subtracted from the percentage threshold changes produced in the L6 collaterals the changes produced in the L3 collaterals (Fig. 8C, F). The differences obtained before the spinal block were also ranked in increasing order (filled circles). We found no consistent patterns in the effects of spinalization. In some fibers the differences in the cutaneous-induced changes of the PAD were shifted towards positivity during spinalization (that is, the inhibition became stronger in the L6 than in the L3 collaterals), while in other fibers they were shifted towards negativity relative to the effects observed before the spinal block (the inhibition of PAD became stronger in L3 than in the L6 collaterals). It thus seems that suppression of supraspinal influences disrupted the relative balance of the excitatory and inhibitory inputs affecting the PAD observed in the L3 and L6 collaterals of individual fibers.

Discussion

Tonic modulation of PAD

Previous work has indicated that in the anesthetized cat, cold block spinalization can modify the phasic PAD of single group I afferents ending in the L6 segment, as well

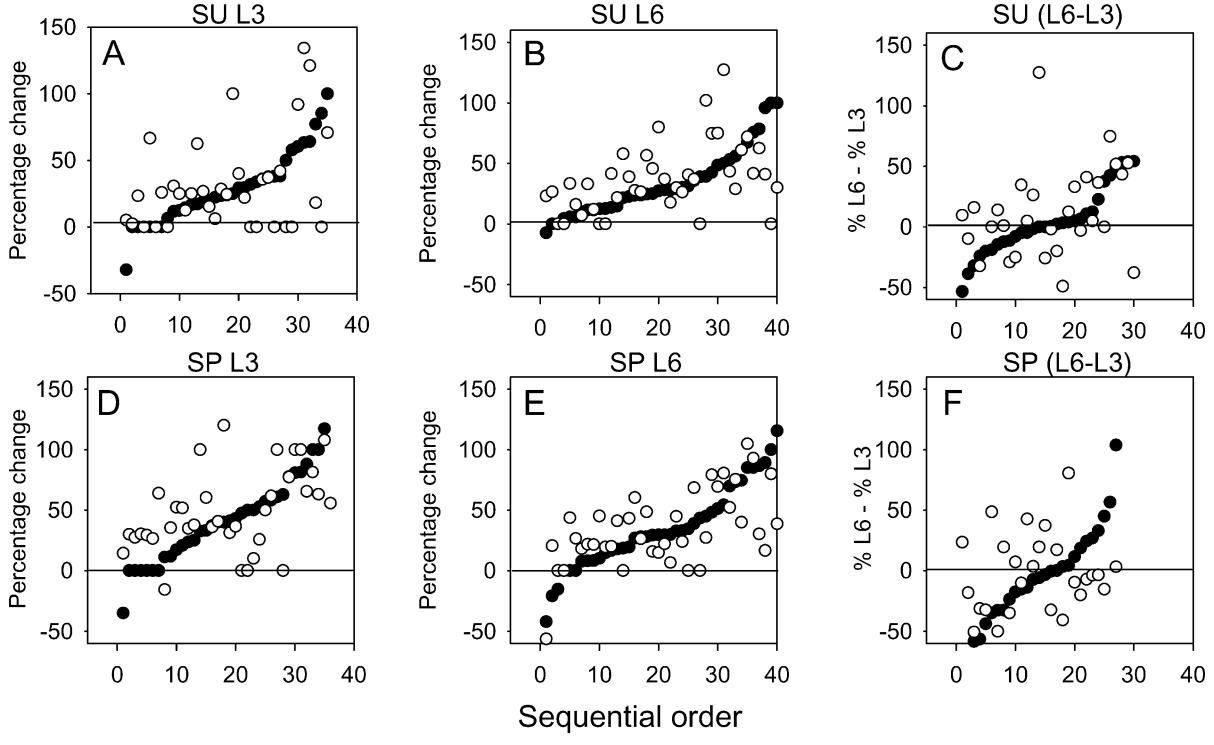

Fig. 8A–F Spinalization changes the magnitude of facilitation and inhibition of the PBSt-induced PAD in pairs of L6 and L3 collaterals. **A, B, D, E** *Ordinates* show percentage changes of the PBSt-induced PAD in the L3 and L6 collaterals following conditioning stimulation of the SU and SP nerves. Data points obtained from individual collaterals before spinal block are ranked in ascending order and displayed sequentially (*filled circles*). Intraspinal threshold changes obtained in the corresponding collaterals during spinal block are shown by *open circles*. Positive values indicate inhibition of PAD and negative values facilitation of PAD. **C, F** Differences between the percentage threshold changes produced in the L6 collaterals and the L3 collaterals of the same afferent. *Ordinates* show data obtained before the spinal block, ranked in increasing order (*filled circles*). *Open circles* Data obtained from corresponding fibers during spinal block. Negative values indicate that inhibition of PAD in the L3 collateral was larger than inhibition in the L6 collateral. Further explanations are given in the text

as the inhibitory actions of cutaneous and joint afferents on the phasic PAD. This has been taken as evidence for the existence of a tonic descending modulation of transmission in pathways mediating PAD of group I afferents (Quevedo et al. 1993). The present observations further support this view and show in addition that spinalization may either increase or decrease the resting threshold of pairs of collaterals of the same afferent fiber. The increase in resting threshold during spinal block may be ascribed to a decrease of the background PAD, and the reduction in resting threshold to decreased inhibitory influences acting on the pathways mediating PAD (Lomelí et al. 1998; see also Burke and Rudomin 1977; Rudomin and Schmidt 1999).

Although differences in the magnitude of the tonic PAD affecting L6 and L3 collaterals of the same afferent could lead to a differential spatial distribution of the tonic PAD, the present set of data reveals no trend suggesting that these differences are related to the segmental level of projection of the collaterals (see Lomelí et al. 1997).

At present, the sources of the descending tonic modulation of PAD of group I afferents have not been fully identified (see Rudomin and Schmidt 1999). In the anesthetized cat, there is a system of spontaneously active dorsal horn neurons in laminae III–VI that are longitudinally and bilaterally distributed along several spinal segments (García et al. 2004). These neurons respond with mono- and oligosynaptic latencies to the activation of low threshold cutaneous afferents and affect impulse transmission in several reflex pathways, including those that mediate PAD and presynaptic inhibition of cutaneous and muscle afferents (Manjarrez et al. 2000). Their activity is most likely controlled by descending influences (Sandkuhler and Eblen-Zajjur 1994) and may thus become an important source of the tonic modulation of PAD. However, it is clear that there could be other sources of tonic PAD as well, among them the interneurons in Lissauer's tract (Lidierth and Wall 1998).

Changes in phasic PAD

Studies dealing with tonic descending influences exerted on the pathways mediating PAD have been concerned with changes of the DRPs produced by stimulation of cutaneous and of muscle afferents (Brink et al. 1984). To our knowledge there is only one study dealing with effects of spinalization on individual group I muscle afferents (Quevedo et al. 1993). This study has shown that the inhibitory actions that cutaneous and joint nerves exert on the PAD of group I terminals ending in segment L6 are subjected to a tonic descending modulation. However, it provides no information on how this modulation affects collaterals ending at more rostral levels.

Variations in the level of tonic PAD could well contribute to the variability of the effects of spinalization on the phasic PAD and on the inhibition of PAD that follows stimulation of cutaneous and joint nerves. This variability can be considered as the expression of the high degree of differentiation in the control of transmission along the pathways mediating PAD (see Rudomin and Schmidt 1999). The balance of the information flowing through the L3 and L6 collaterals in preparations with intact neuraxis would be determined, to some extent, by descending influences which are eliminated by spinalization. The observed differences in the PAD and in the inhibition of PAD in pairs of collaterals of individual fibers require in addition that PAD has a local character and that the PAD produced in both collaterals (L3 and L6 in this case), as well as the inhibition of PAD, are mediated by separate sets of interneurons (see Jankowska and Padel 1984; Harrison and Jankowska 1984).

It thus seems reasonable to conclude, in agreement with our previous proposal (Rudomin et al. 2004), that the PAD patterns of individual collaterals are the expression of combinations of excitatory and inhibitory inputs of peripheral and descending origin acting at that particular moment on the populations of spinal interneurons mediating PAD (see also Jankowska 1992). This would allow the intraspinal arborizations of the afferent fibers to behave as dynamic substrates capable of addressing information to selected groups of spinal neurons.

Some functional considerations

The results depicted in Fig. 7 provide an interesting example of the switching of the inhibition of PAD from one to the other collateral during spinalization. Before the spinal block, stimulation of the SU and SP nerves inhibited mostly the PBSt-PAD produced in the L6 collaterals. During spinalization, the inhibition of the PAD became more apparent in the L3 collaterals. In other words, in this fiber the direction of information flow (segmental or ascending) at the time of the activation of cutaneous afferents is set by descending influences. Most likely this has some bearing on what occurs during the onset of voluntary contraction in humans, where there is a reduction of the tonic presynaptic inhibition of Ia afferents from the muscles to be contracted and increased presynaptic inhibition of afferents from other muscles (Hultborn et al. 1987). This differential control of PAD may also underlie the reduction in background presynaptic inhibition of Ia fibers that follows activation of skin afferents (Iles 1996; Aimonetti et al. 1999). In this case, activation of skin receptors may signal the end of a pre-programmed action such as stepping, or the unexpected finding of an obstacle during walking (Prochazka et al. 1978; Fossberg 1979). Under those circumstances the reduction of presynaptic inhibition would allow the information generated from the muscle receptors to flow again and play a relevant role in the re-programming of the required compensatory reactions (Ménard et al. 2002; Côte and Gossard 2003).

In this regard it is quite interesting to note that the inhibition of PAD that follows activation of skin and joint receptors seems to be more affected by spinalization than the PAD itself. That is, the descending control that is

disclosed by spinalization appears to affect more the pathways that produce inhibition of PAD than those mediating PAD (see also Quevedo et al. 1993). This fits well with all of the available information pertaining to the descending control of the effectiveness of cutaneous and joint afferents compared with the actions of group I muscle afferents (Quevedo et al. 1993) and underscores the role of cutaneous and joint afferents in the integration of a variety of motor behaviors (LaBella et al. 1992; Seki et al. 2003).

Concluding remarks

We have made a systematic exploration of the effects produced by reversible spinalization on the PAD and PAH of pairs of collaterals of single group I muscle afferents. The underlying hypothesis was that this procedure eliminates tonic supraspinal influences that affect transmission along the interneuronal pathways mediating PAD and inhibition of PAD, even in preparations under barbiturate anesthesia. The results obtained agree with this prediction. Unfortunately there was no clear-cut trend that could be easily interpreted in functional terms. This is not at all surprising because the data were collected from many animals, not necessarily all of them under the same set of conditions. In fact, as discussed in the preceding paper (Rudomin et al. 2004), it is clear that we are dealing here with a distributed system of interneurons whose output will strongly depend on the balance of excitation and inhibition of segmental and supraspinal influences received by the ensemble at a given moment. This view is supported by the finding that even in the barbiturate-anesthetized preparation there is a substantial amount of spontaneous activity of dorsal horn neurons that are the source of the variations of PAD of group I afferents and of monosynaptic reflexes (Manjarrez et al. 2000). In other words, what we have described here and in the preceding paper (Rudomin et al. 2004) are the basic features of the modulation of the synaptic effectiveness of different collaterals of the same afferent fiber. It is possible that the effects following reversible cooling would have been much larger in decerebrate unanesthetized preparations, but this needs to be investigated. Nevertheless, the recent observations of Seki et al. (2003) suggest that during the execution of a specific motor task in awake monkeys, the involved interneurons will exert more coherent actions on the synaptic effectiveness of segmental and ascending collaterals of the afferent fibers.

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