Paired Associative Stimulation Induces Change in Presynaptic Inhibition of Ia Terminals in Wrist Flexors in Humans

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Persistent changes in synaptic efficacy have been proposed as a mechanism underlying memory and learning, as well as the cortical plasticity related to the acquisition and recovery of sensorimotor function. Several noninvasive techniques have been developed that are aimed at inducing changes in cortical organization and excitability that outlast the duration of the intervention (Ziemann et al. 2008). Among these techniques, paired associative stimulation (PAS), introduced by Stefan et al. (2000), has been extensively studied. Based on Hebb’s law of coincident summation (Hebb 1949), PAS consists of repetitive pairing of a peripheral nerve and a cortical stimulation, achieved by transcranial magnetic stimulation (TMS). It results in long-lasting after-effects on corticospinal (CS) excitability (Stefan et al. 2000, 2002; Weise et al. 2006; Wolters et al. 2003; Ziemann 2004). When the stimuli are timed to produce an approximately synchronous activation of cortical networks, the amplitude of motor-evoked potentials (MEPs) is enhanced.

This PAS-induced plasticity is referred to as long-term potentiation (LTP)-like plasticity as it shares several features with LTP processes described in vivo and in vitro at the cellular level in animal experiments (Cooke and Bliss 2006). PAS-induced changes in CS excitability in humans are monitored by measuring EMG amplitude of the MEPs before and after PAS intervention. Because MEPs represent the sum of processes occurring at the cortical, subcortical, and spinal levels (Rossini et al. 1994; Rothwell 1991), the exact site where PAS-induced plasticity manifest remains unclear and a current matter of debate (Ziemann et al. 2008).

A cortical effect of “excitatory” PAS (LTP-like effect) has been favored because both F-waves and motor-evoked potential (CMEP) evoked by electric/magnetic stimulation of the cervicomedullary junction are not affected by PAS targeting the abductor pollicis brevis (APB) (Meunier et al. 2007; Stefan et al. 2000; Wolters et al. 2003) and tibialis anterior (TA) (Mrachacz-Kersting et al. 2007) muscles. Similarly, TA H reflex recorded at rest (Mrachacz-Kersting et al. 2007) or under contraction (Roy et al. 2007) is not altered by PAS. Recently, direct evidence from recordings of corticospinal descending volley evoked by single pulse TMS over the motor cortex in patients who had an electrode implanted in the cervical epidural space showed that PAS specifically affected the amplitude of later descending I waves, consistent with a cortical origin of PAS-induced after-effects (Di Lazzaro et al. 2009, 2010).

However, in contrast with these studies, a concomitant PAS-induced increase of the MEP and H reflex has been reported in flexor carpi radialis (FCR) and APB muscles (Meunier et al. 2007) suggestive of changes in both cortical and spinal excitability.

CMEP, F waves, and H reflex rely on different principles (Pierrot-Deseilligny and Burke 2005; Taylor and Gandevia 2003), and this may account for the discrepancy observed in studies comparing two different techniques, e.g., H reflex and F waves (Carson et al. 2004; Meunier et al. 2007) or H reflex and CMEP (Hortobagyi et al. 2003). Actually, among these methods, only the H reflex is mediated by Ia afferents and therefore up- or downregulation of mechanisms controlling Ia...
afferent discharge may affect H reflex amplitude without concomitant change of CMEP or F waves amplitude.

Two mechanisms controlling Ia afferent discharge have been described thus far in both animals and humans: 1) presynaptic inhibition of Ia terminals that modulates the synaptic effectiveness of Ia afferents before they reach the target neurons through axo-axonic synapses (Franck and Fuortes 1957; Pierrot-Deseilligny and Burke 2005) and 2) postactivation depression, a phenomenon reflecting the frequency-related depression of the H reflex discharge (Hultborn et al. 1996), that plays a role in modification of synaptic transmission and is likely to involve changes in readily releasable transmitter at the Ia–MN synapse (Lev-Tov and Pinco 1992).

In this study, we hypothesized that PAS-induced increase in spinal excitability, as assessed by the H reflex (Meunier et al. 2007), is caused by an increase in the efficacy of the afferent volley through a decrease of the activity of those mechanisms (i.e., decreased presynaptic Ia inhibition and/or postactivation depression). To address this question, we first confirmed that PAS-induced changes in spinal excitability, as assessed by the FCR H reflex recruitment curve. To elucidate the mechanism responsible for such a change, we explored, using the same FC MR H reflex recruitment curve. To address this question, we first confirmed that PAS-induced changes in spinal excitability, as assessed by the FCR H reflex recruitment curve. To elucidate the mechanism responsible for such a change, we explored, using the same subjects, whether presynaptic Ia inhibition and postactivation depression are modulated by PAS.

METHODS

Subjects

Experiments were performed on 12 healthy volunteers (6 women, 6 men) 19 to 59 yr of age [mean, 34.5 ± 11.23 (SE) yr]. All subjects gave written informed consent, and the protocol was approved by the NINDS Institutional Review Board. All subjects, but one, were right-handed according to the Oldfield handedness inventory (Oldfield 1971). Subjects were comfortably seated in a reclining chair with the shoulder in slight abduction (60°), the elbow semi-flexed (110°), and the forearm pronated and supported by the arm of the chair. Responses were recorded on the dominant side of the subjects, i.e., right forearm for right-handed subjects.

EMG recording

Surface EMG was recorded from FCR muscle using pairs of surface Ag-AgCl disposable electrodes (Medtronic, Skovlunde, Denmark). EMG signals were amplified and filtered with a band-pass of 10 Hz to 1 kHz (Nicolet Viking Electromyography, Skovlunde, Denmark). EMG signals were digitized at 5 kHz sampling frequency using a data acquisition system built with the Labview graphical programming language (Kaelin-Lang and Cohen 2000) and stored on a personal computer for off-line analysis. EMG activity was continuously monitored during the experiment with a visual feedback to ensure complete relaxation of the examined muscle. All EMG signals were expressed as a percentage of the maximal motor response (M_max), which was measured in each experiment.

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) was performed at 0.2 Hz using a Magstim 200 mono-pulse unit (The Magstim Company, Dyfed, UK) connected to a figure-of-eight shaped coil (7 cm inner diameter for each half) positioned on the scalp over the contralateral hand area of the motor cortex. The coil was held tangentially to the skull with the handle pointing backward and laterally at a 45° angle to the sagittal plane. The optimal stimulus site was determined as the site where TMS at a slightly suprathreshold intensity consistently produced the largest MEPs. This site was marked with a pen on the cap worn by the subject to assure a constant placement of the coil throughout the session.

Resting motor threshold (rMT) was defined as the nearest 1% stimulator output as the lowest stimulus intensity that elicited ≥ 5 MEPs > 50 μV of 10 consecutive trials (Rossini et al. 1994).

Peripheral nerve stimulation

Peripheral nerves were stimulated using constant-current square-wave pulse of 1 ms duration (DS7A, Digitimer) delivered through bipolar surface electrodes (1.5 cm diam, half-balls 2 cm apart).

PAS intervention

Paired associative stimulation consisted of 240 pairs (0.2 Hz over 20 min; Russmann et al. 2009) of electrical stimulation to the ipsilateral median nerve followed by a single TMS pulse to the contralateral M1 at the optimal site for eliciting MEPs in FCR muscle on the dominant side. The interstimulus interval between the peripheral and cortical activation was set at 20 ms. This intervention is referred to as PAS20 in the following. The median nerve was stimulated at the elbow through bipolar electrodes (cathode proximal) at an intensity of 1.1 × motor threshold (MT). TMS intensity was adjusted at 1.2 × rMT.

Because subject’s attentional focus has been shown to be an important factor influencing PAS effectiveness (Stefan et al. 2004), all the subjects were instructed to direct their attention on the examined forearm and to report the number of pairs of pulses delivered at the end of the intervention.

Experimental design

Volunteers were invited to participate in three experiments (Table 1), conducted on 3 separate days, with an interval of ≥ 3 days between two consecutive sessions. For each experiment, measurements were done at baseline (T0) and between 15 and 45 min after the end of PAS intervention (T1). All experiments were performed at rest.

Experiment 1: Recruitment curves of the FCR H reflex and MEP

The H reflex in the FCR muscle was elicited by stimulating the median nerve in the antecubital fossa, ~ 2 cm above the elbow. To...
create the recruitment curve of the H reflex, 10 responses were recorded and averaged at each of a range of intensities at 0.2 Hz. Intensity of stimulation started just below the threshold intensity for evoking the H reflex to best fit a sigmoid function and was increased by steps of 5–10% of MT (threshold intensity for evoking a M wave) until reaching the plateau value (Hmax). To rule out the possibility that electrodes moved or changed their conduction properties during the experiment, M waves were recorded simultaneously and compared before and after the intervention. If M waves were mismatched between the pre- and post-intervention, the corresponding file was discarded from analysis.

To ensure the efficacy of PAS20 to modulate MEP amplitude, MEP recruitment curves were also determined by collecting 10 MEPs at five different intensities ranging from 1.1 to 1.5 × rMT before and after the intervention.

Experiment 2: Presynaptic inhibition of FCR Ia terminals

Stimulating group I afferents in the nerve supplying antagonist muscles evokes three successive inhibitory phases of the agonist motoneuron (MN) pool as tested by the H reflex technique (Berardelli et al. 1987). The first phase has been attributed to disynaptic Ia reciprocal inhibition. The second phase (D1 inhibition) has been attributed to presynaptic inhibition exerted on the Ia afferent volley of the test reflex, whereas the third phase (D2 inhibition) is of uncertain origin, but may also be caused, at least partly, by presynaptic inhibition (Berardelli et al. 1987; Mizuno et al. 1971; see DISCUSSION). Conditioning stimuli (1 ms duration) were applied through bipolar electrodes to the radial nerve in the spiral groove. The intensity of stimulation was set at 0.95 MT (Berardelli et al. 1987; Lamy et al. 2009). Conditioning test intervals of 10, 13, 15, 20, 25, and 30 ms were studied at random. Moreover, in six subjects, we studied interstimulus intervals (ISIs) of 50, 75, 100, 200, and 500 ms. ISIs from 10 to 30 ms reflect D1 inhibition, whereas ISIs from 50 to 500 ms reflect D2 inhibition. Twenty unconditioned reflexes and 20 conditioned reflexes were collected at each ISI.

In addition, 10 MEPs were collected at 1.2 × rMT (MEPs strongly increased at this intensity in most of our subjects in experiment 1) before and after the intervention to ensure the efficacy of PAS20.

Experiment 3: Postactivation depression

Similarly to previous studies (Crone and Nielsen 1989; Lamy et al. 2005), postactivation depression at the homonymous Ia fiber-FCR MN synapse was assessed by comparing the effects of the following different stimulus rates on FCR H reflex amplitude: 0.1, 0.125, 0.166, 0.25, 0.5, and 1 Hz. At the beginning of the session, the intensity of the stimulation was adjusted so that the amplitude of the FCR H reflex at 0.1 Hz (postactivation depression is absent or very minimal at this frequency) was Hmax/2 (i.e., Hmax/2 at 0.1 Hz ranged from 5 to 28% of Mmax). After the PAS20, we carefully adjusted the stimulus intensity so that the amplitude of the H reflex at 0.1 Hz was very much the same as that before the intervention. In addition, it was verified that the amplitude of the reflex at 0.1 Hz remained constant throughout the session. The sequence of stimulus rates randomly varied. Twenty reflexes (after discarding of the 1st one) were recorded for each frequency (0.1–1 Hz).

To ensure the efficacy of PAS20 to modulate MEP amplitude, 10 MEPs were collected at 1.2 × rMT before and after the intervention.

Data analysis and statistics

Measurements were made on the peak-to-peak amplitudes of EMG responses (±SD) and expressed as a percentage of Mmax. For the D1 and D2 inhibitions, the size of the conditioned responses was expressed as a percentage of the unconditioned responses recorded in the same block of trials.

The following three-parameter sigmoid function was used to fit the ascending limb of each individual FCR H reflex recruitment curve (Klimstra and Zehr 2008)

\[
H \text{ reflex (S)} = \frac{H_{\text{max}}}{1 + e^{m(S_0 - S)}}
\]

This equation has three parameters that have been determined for statistical analysis: 1) the maximal value of the H reflex (Hmax, 2) the S50 (stimulus needed to obtain 50% of the maximal response Hmax), and 3) the slope m. In addition, we determined the stimulus intensity necessary to elicit a threshold response (Hthreshold) by quantifying as the x-intercept (SD; linear regression) of the tangent to the function at the point of maximal slope (i.e., S50) (Carroll et al. 2002; Landby-Jensen and Nielsen 2008). Calculation of the area under each curve (AUC) was used to analyze recruitment curves of both M waves and MEP because their full curves have not been collected. AUC of the H reflex recruitment curve was also determined.

A one-way repeated-measures ANOVA was used to test the effects of PAS20 on each parameter of the recruitment curves of the FCR H reflex (Hthreshold, Hmax, S50, and slope m), on AUC M waves and on AUC MEP.

To test the effects of PAS20 intervention on radial-induced depression of the FCR H reflex (D1 and D2 inhibitions) and on postactivation depression, two-way repeated-measures ANOVA were used. The factors tested are explained in more detail in RESULTS. Conditional on a significant F-value, post hoc paired t-test were used to explore the strength of main effects.

To plot individual results, we determined the overall modulation by PAS20 on the radial-induced depression of the FCR H reflex, M waves, and MEP. Figures 1A and 1B show the modulation by PAS20 as a function of the conditioning stimulus rate. The ordinate shows the change of the radial-induced depression in the H reflex, M waves, and MEP.

To further study PAS20-induced change of spinal excitability, the three parameters of the H reflex sigmoid function and Hthreshold as well as M wave amplitudes and MEP size (see METHODS) were determined before (open box plots) and after
There was a tendency for a correlation between increased MEP amplitude (mean AUC: 2.09 ± 1.34 (T0) vs. 1.95 ± 1.43 (T1), P = 0.17; Fig. 2E), whereas FCR MEP amplitude was significantly increased by PAS intervention [mean AUC: 1.75 ± 1.18 (T0) vs. 2.49 ± 1.4 (T1), P < .003; Fig. 2F]. There was a tendency for a correlation between increased MEP and increased H reflex (r = 0.57; P = 0.05).

These modulations occur without concomitant change of the M wave amplitude [mean AUC: 2.09 ± 1.34 (T0) vs. 1.95 ± 1.43 (T1), P = 0.17; Fig. 2E], whereas FCR MEP amplitude was significantly increased by PAS intervention [mean AUC: 1.75 ± 1.18 (T0) vs. 2.49 ± 1.4 (T1), P < .003; Fig. 2F]. There was a tendency for a correlation between increased MEP and increased H reflex (r = 0.57; P = 0.05).

Figure 3 shows the modeling of the FCR H reflex recruitment curves before (continuous line) and after (dashed line) PAS20 for the whole population (12 healthy volunteers). These curves were obtained by fitting averaged values of individual H\textsubscript{threshold}, slope parameter m, S\textsubscript{50}, and H\textsubscript{max} values to a sigmoid function. The most striking effects were a significant increase of both the slope parameter m and the plateau value (H\textsubscript{max}).

**Experiment 2: Presynaptic inhibition of FCR Ia terminals**

In Fig. 4, the H reflex depression induced by radial nerve activation was assessed, at various ISIs, before and after PAS. Because PAS20 affected H reflex amplitude (experiment 1), we carefully adjusted the unconditioned H reflex size to the same proportion of M\textsubscript{max} before (T0) and after (T1) the intervention. In this respect, there was no change in size of the unconditioned H reflex before and after PAS20 intervention [mean size: 12.26% M\textsubscript{max} ± 3.81 (T0) vs. 12.7% M\textsubscript{max} ± 4.39 (T1), P = 0.57]. PAS20 increased the amplitude of 10 MEPs at 1.2 × rMT (P < 0.001).

At ISIs ranging from 10 to 30 ms FCR H reflex inhibition reflects D1 inhibition (Fig. 4B; 12 subjects), whereas at ISIs ranging from 50 to 500 ms FCR H reflex inhibition reflects D2
inhibition (Fig. 4C; 6 subjects). Because the mechanisms account-able for D1 and D2 inhibitions might be different (see DISCUSSION), a two-way repeated-measures ANOVA was conducted separately on the data from each phase with TIME (T0 vs. T1) and ISI (D1 inhibition: 10, 13, 15, 20, 25, and 30 ms; D2 inhibition: 50, 75, 100, 200, and 500 ms) as main factors. For both D1 and D2 inhibitions, there was a significant main effect of TIME (D1 inhibition: $F_{11.005} = 10.53; P < 0.002$; D2 inhibition: $F_{9.005} = 7.75; P < 0.05$), but no effect of ISI and no TIME × ISI interaction. Post hoc paired $t$-test indicated that this finding was because PAS 20 intervention reduced the amount of both D1 and D2 inhibition.

Importantly, individual results (Fig. 4D) showed that when PAS induced a decrease in D1 inhibition, there was a concomitant facilitation of the H reflex (subjects 1–10 in Fig. 4D), whereas when PAS 20 intervention was not able to modulate D1 inhibition (subjects 11 and 12 on Fig. 4D), the H reflex amplitude was not modulated. There was a tendency for a correlation between decreased D1 inhibition and enhanced H reflex amplitude but it did not reach statistical significance ($r = 0.52; P = 0.08$). Decreased D2 inhibition was not correlated with increased H reflex amplitude ($r = 0.6; P = 0.24$).

Experiment 3: Postactivation depression

In Fig. 5, frequency-related depression of FCR H reflex was assessed before and after PAS. Here again, because PAS 20 affects spinal excitability (experiment 1), FCR H reflex size evoked at 0.1 Hz was carefully adjusted to the same proportion of $M_{\text{max}}$ before (T0) and after (T1) the intervention [15.71% $M_{\text{max}}$ ± 3.32 (T0) vs. 15.96% $M_{\text{max}}$ ± 3.21 (T1), $P = 0.82$]. One subject (subject 3) was removed from the analysis, because the amplitude of the H reflex evoked at 0.1 Hz was not matched between T0 and T1. Here again, PAS 20 was effective in modulating the amplitude of 10 MEPs at 1.2$r_{\text{MT}}$ ($P < 0.02$).

To explore the effect of PAS 20 onto postactivation depression, we computed a two-way repeated-measure ANOVA with...
factors TIME (T0 vs. T1) and FREQUENCY (0.1; 0.125; 0.166; 0.25; 0.5; 1 Hz). ANOVA showed a significant effect of FREQUENCY ($F = 3.38; P < 0.007$) because of the depressive effect of increasing the frequency on H reflex amplitude, i.e., the phenomenon of postactivation depression by itself. However, there was no effect of TIME ($F = 0.11; P = 0.74$) and no TIME $\times$ FREQUENCY interaction ($F = 0.02; P = 0.99$) pointing out that postactivation depression was unchanged by PAS$_{20}$.

**DISCUSSION**

This series of experiments has confirmed that PAS, targeting the motor representation of the FCR muscle, significantly affects spinal segmental excitability, as assessed by H reflex technique. Change in the recruitment curve of the H reflex was accompanied by a significant decrease in the long-latency depression of the FCR H reflex (both D1 and D2 inhibitions), whereas postactivation depression was unchanged by the intervention. Together, these observations suggest that enhancement of segmental excitation by PAS relies on a selective effect of PAS on interneurons controlling presynaptic inhibition of Ia terminals.

**Origin of D1 and D2 inhibitions**

Radial nerve stimulation induces a threefold inhibition of the FCR H reflex (Day et al. 1984): 1) an early inhibition peaking at 0 ms (disynaptic inhibition); 2) a second phase of inhibition for ISIs ranging from 5 to 40 ms (D1 inhibition); and 3) a third phase of inhibition lasting $\leq$500 ms (D2 inhibition). At ISIs ranging from $\sim$10 to 30 ms, Berardelli et al. (1987) showed that radial-induced depression of FCR H reflex was not paralleled by change of FCR MEPs amplitude evoked by TES. Because a postsynaptic effect at MNs level will depress both H reflex and MEP, it is thus likely that for intervals ranging from 10 to 30 ms, the conditioning radial volley acts at a premotorneuronal level.

**Does D2 inhibition also reflect presynaptic inhibition?**

The origin of D2 inhibition is still an open question. It has been proposed that D1 and D2 inhibitions are not distinct phenomena but that both reflect presynaptic inhibition of Ia afferents. They are eventually separated from each other by superimposed facilitation (occurring for ISIs 30–60 ms) involving cutaneous afferent activation (Berardelli et al. 1987). Another possibility would be that D2 inhibition involves long
loop inhibitory connections from radial nerve to brain stem or even transcortical connections that may return back to the spinal cord (Berardelli et al. 1987; Huang et al. 2004). It does not exclude that D2 might not be a single phenomenon over its whole duration. Data regarding the relationship between D2 inhibition and presynaptic inhibition is conflicting in the literature with data supporting the idea that D2 inhibition reflects presynaptic Ia inhibition (Perez et al. 2005) or, in contrast, not (Huang et al. 2004, 2009).

Obviously, our experimental design does not allow us to determine if D2 inhibition faithfully reflects presynaptic Ia inhibition. However, decreased D2 inhibition after PAS (even though to a lesser extent than D1 inhibition) suggests that spinal excitability is affected by PAS and supports our result on D1 inhibition. Finally, it has to be kept in mind that at ISIs ranging from 13 to 20 ms, radial nerve stimulation strongly inhibited the FCR MEPs when evoked by a TMS (Bertolasi et al. 1998; Lourenço et al. 2007). It is unclear why magnetically evoked FCR MEPs are suppressed by radial activation, but it must be stressed that the mode of activation of the corticospinal tracts differ between these techniques, TES activating the corticospinal tract below the pyramidal cell bodies, whereas TMS activates interneuronal pathways in the superficial layers of the motor cortex upstream to the pyramidal cell bodies. Whatever the mechanisms regarding the findings, if radial-induced depression of FCR H reflex had been postsynaptic in origin, both electrically and magnetically evoked MEPs would have been inhibited.

**Contribution of decreased presynaptic Ia inhibition to H reflex enhancement**

The H reflex recruitment curve reflects the recruitment of αMNs by Ia afferent inputs that proceeds in an orderly fashion from smallest [the more excitable, with the larger Ia excitatory postsynaptic potentials (EPSPs)] to largest (the less excitable,
with the smaller Ia EPSPs) MNs according to the size principle (Heckman and Binder 1993; Hennemann et al. 1965). Changes in H reflex recruitment curve, as observed after PAS in our study, may thus reflect changes occurring at pre- and/or postsynaptic level (Pierrot-Deseilligny and Mazevet 2000). We observed a significant decrease in the long-lasting depression of the H reflex (D1 inhibition) after PAS that was relatively, but not significantly \( (r = -0.52; P = 0.08), \) correlated to FCR H reflex enhancement, suggesting that decreased presynaptic inhibition of Ia terminals likely contributes, at least partly, to increased H reflex.

Previous studies in animals have shown that changes in presynaptic inhibition of the synapses between sensory afferents and MNs are critical in the up-/downregulation of the reflex circuitry during conditioning protocols. For example, it has been shown that the habituation of the gill-withdrawal reflex in *Aplysia* is caused by a depression of synaptic transmission between sensory afferents and MNs mediated through changes in presynaptic inhibition (Kandel et al. 2000). Moreover, in both rats and monkeys, measure of presynaptic inhibition during classical operant conditioning showed that MN firing threshold is a critical mechanism underlying long-term adaptations in the magnitude of the H reflex (Wolpaw and Tennissen 2001), whereas short-term adaptations of the H reflex amplitude, as observed in our study, is likely associated with changes in presynaptic inhibition (Wolpaw 1997).

However, even though decreased presynaptic inhibition is likely one mechanism contributing to enhanced H reflex after PAS, it does not exclude that other mechanisms controlling the H reflex pathway may also be affected by the intervention (i.e., postsynaptic pathways, not tested in this study). Alternatively, it is also possible that PAS-induced changes of intrinsic properties of the MNs are responsible for the H reflex. Such changes have been shown in monkeys (Carp et al. 2001) and can involve a shift in MN firing threshold, change in AHP amplitudes, changes in synaptic contacts on MNs, or even a change in the firing threshold of the axon (Wolpaw and Carp 2006).

**Respective role of peripheral and TMS stimulation in PAS-induced after-effects**

It is conceivable that our observations are not caused by the pairing of peripheral and cortical stimulations but by the effects of each stimulation alone (i.e., the effect of repetitive TMS pulses alone or of repetitive peripheral nerve stimuli alone). However, several arguments in the literature suggest that repetitive low-frequency TMS (0.2 Hz in our experiments) by itself is not responsible for PAS-induced after-effects: 1) stimulation of the motor cortex at 0.1 Hz for 1 h did not change cortical excitability (Chen et al. 1997), 2) with stimulation at 1 Hz, a minimum of 750 TMS pulses are needed to modulate MEP amplitude from 0 to 15 min after the intervention, whereas 600 TMS stimuli at 1 Hz failed to modulate FCR H reflex (Touge et al. 2001); this is well above the total number of pulses we delivered in our experiment, and our recording post-PAS (T1) was done 15–45 min after the end of the intervention, and 3) PAS-induced plasticity is governed by the temporally asymmetric Hebbian rule: changes in MEP amplitude are conditionally dependent on temporal contingency between the TMS pulse and peripheral nerve stimulation. When both peripheral and cortical inputs are not temporally synchronized, PAS failed to modulate MEP amplitude (Machacz-Kersting et al. 2007; Stefan et al. 2000; Wolters et al. 2003), suggesting that repetitive cortical (or peripheral) inputs alone are not critical to induce PAS-induced after-effects.

Similarly, it is also unlikely that repetitive peripheral nerve stimulation by itself plays a role in PAS-induced change in spinal excitability: 1) it has been shown that H reflex amplitude typically decreases during the course of an experiment (Crone et al. 1999), whereas the opposite was found in our series of experiments, and 2) using a similar design to our experiment 1, Roche et al. (2009) recently explored the effect of 20 min of median nerve stimulation and anodal transcranial DC stimulation (i.e., the same duration as our PAS intervention) onto FCR H reflex recruitment curves; they reported no modification of FCR H reflex recruitment curves during or 0–20 min after the intervention. The total number of pulses they delivered to the median nerve was comparable to our paradigm, suggesting that repetitive peripheral nerve stimulation of median nerve does not affect spinal excitability.

We therefore favor the view that the repetitive coupling between peripheral and cortical stimulation is necessary to induce the enduring changes of presynaptic inhibition observed after PAS.

**Where does plasticity responsible for PAS-induced changes in presynaptic inhibition develop?**

It is not possible from our data to make any certain conclusions regarding the exact mechanism of the decreased presynaptic inhibition following PAS, but some possibilities may be discussed. A first possibility is that a synaptic associative plasticity develops at the spinal level in presynaptic network caused by repetitive coincident activation of interneurons by descending and peripheral sensory inputs. Presynaptic [primary afferent depolarization (PAD)] interneurons are good candidates for such coincident activation because they receive extensive projections from peripheral afferents (Ia, Ib, and cutaneous) and from various descending tracts (Rudomin and Schmidt 1999). A second possibility would be that PAS-induced plastic changes in the motor cortex affect durably the tonic pattern of discharge of cortical cells and then the descending tonic control exerted by the corticospinal pathways on the presynaptic spinal network. Indeed, in humans, presynaptic Ia inhibition on FCR MNs is decreased during a voluntary contraction of the FCR muscle (Aymard et al. 2001), which

**TABLE 2. Mean data of AUC**

<table>
<thead>
<tr>
<th></th>
<th>Before PAS (T0)</th>
<th>After PAS (T1)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H reflex</td>
<td>5.32 ± 4.03</td>
<td>8.69 ± 8.55</td>
<td>0.04</td>
</tr>
<tr>
<td>M wave</td>
<td>2.09 ± 1.34</td>
<td>1.95 ± 1.43</td>
<td>0.17</td>
</tr>
<tr>
<td>MEP</td>
<td>1.75 ± 1.18</td>
<td>2.49 ± 1.4</td>
<td>0.0003</td>
</tr>
<tr>
<td>D1 inhibition</td>
<td>771.79 ± 446.38</td>
<td>599.1 ± 447.98</td>
<td>0.0006</td>
</tr>
<tr>
<td>D2 inhibition</td>
<td>18497.7 ± 1837.4</td>
<td>15556.5 ± 11424.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Postactivation depression</td>
<td>93.61 ± 74.56</td>
<td>97.13 ± 81.82</td>
<td>0.48</td>
</tr>
</tbody>
</table>

AUCs are expressed in arbitrary units because they were computed from normalized curves (i.e., for FCR H reflex recruitment curve, ordinate was the amplitude of FCR H reflex, expressed in percent of \( M_{\text{max}} \), and abscissa was the stimulus intensity expressed in \( \times \text{MT} \)).
necessarily implies a tonic level of presynaptic inhibition of Ia terminals to FCR MNs in resting conditions. Such a tonic control has also been described in the cat with acute spinal transection after administration of DOPA (Anden et al. 1966). TMS applied over the motor representation of the FCR muscle induced increased presynaptic inhibition of FCR Ia terminals (Meunier and Pierrot-Deseilligny 1998) suggesting that the motor cortex facilitates PAD interneurons at the cervical level. Several mechanisms could contribute to the tonic level of presynaptic inhibition at rest including 1) a tonic inhibition from higher centers of the brain stem structures through which reticulospinal pathways maintain tonic inhibition of PAD interneurons, 2) a tonic inhibitory control of inhibitory interneurons transmitting cutaneous inhibition to PAD interneurons, and 3) a possible tonic vestibulospinal influence on PAD interneurons (see Pierrot-Deseilligny and Burke 2005 for details). Thus it is conceivable that PAS-induced cortical changes may affect the descending control onto presynaptic interneurons as PAD interneurons are powerfully controlled by suprasegmental structures. If so, both mechanisms and pathways supporting such changes have still to be identified.

Finally, an alternative explanation could rely on a possible interaction between presynaptic inhibition (though to be mediated by GABA) and postactivation depression that has been suggested from both physiological and pharmacological studies in animals. Indeed, in the cat spinal cord, it has been shown that 1) postactivation depression is altered by administration of benzodiazepines, and this effect is related to the prolongation of the primary afferent depolarization (Davies et al. 1985), and 2) repetitive activation of peripheral afferents may decrease the efficacy of presynaptic inhibition (Enriquez-Denton et al. 2002). On the whole, this suggests that changes in postactivation depression could result in changes in presynaptic Ia inhibition and vice versa. However, we can reasonably discard this possibility, because postactivation depression is unchanged after PAS.

In conclusion, we showed that PAS, targeting the motor representation of the FCR muscle, decreases presynaptic inhibition of Ia terminals and that this is concomitant with an increase of the H reflex. This result indicates that modulation of cortical circuits by PAS may impact spinal network excitability and highlights the fact that PAS after-effects should be considered in regard to spinal motor circuits in addition to cortical ones.

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