Involvement of spinal recurrent inhibition in spasticity
Further insight into the regulation of Renshaw cell activity

R. Mazzocchio and A. Rossi

Summary
Changes in the excitability of the soleus H-reflex arc were studied after oral administration of L-acetylcarnitine, a cholinomimetic substance, in eight healthy control subjects and 23 spastic patients presenting with slowly progressive paraparesis (n = 10), a cord lesion (n = 9) and a cerebral lesion (n = 4). Changes in the amount of recurrent inhibition of soleus motor neurons at rest were also estimated in order to assess the level of activity of Renshaw cells before and after L-acetylcarnitine administration. Recurrent inhibition elicited by a conditioning reflex discharge (H1) was assessed by a subsequent test reflex (H'). Four patients lacked an H' reflex. In ~50% of the remaining patients, recurrent inhibition was normal, while in the other half there was evidence of reduced or absent inhibitory activity at rest. Pooling the data relative to the effect of L-acetylcarnitine on the H-reflex in relation to the strength of recurrent inhibition disclosed that the ratio of peak-to-peak amplitude values of the maximum H reflex to maximum M wave responses (Hmax:Mmax) was reduced in all the cases in which the recurrent inhibition at rest was normal, while such a reduction was never observed in the patients in whom recurrent inhibition was found to be decreased at rest. In the former cases, the size of the H' reflex evoked by the same conditioning H1 discharge was further depressed after L-acetylcarnitine, pointing to a potentiating effect of the drug on Renshaw cells; in the latter cases no such effect was observed. A significant decrease in the mean Hmax:Mmax ratio after L-acetylcarnitine intake was also seen in the healthy control subjects. Possible changes in the amount of presynaptic inhibition on Ia terminals on soleus motor neurons after L-acetylcarnitine were ruled out. It is proposed that the differential effect of the drug on the H-reflex excitability is directly related to the level of Renshaw cell activity, a reduction of which probably follows a lesion interrupting reticulo-spinal pathways with tonic facilitatory influences on Renshaw cells. These findings support the hypothesis that Renshaw cell excitability is set via cortico-reticulo-spinal systems.

Keywords: recurrent inhibition; spasticity; L-acetylcarnitine; motor control; human

Introduction
Spasticity is generally defined as a motor disorder characterized by a velocity-dependent increase in tonic and phasic stretch reflexes (see Young, 1994). There are two sets of reasons to dispense with this restrictive definition. The first is that spastic features cannot be the same irrespective of the underlying cause and duration of the spastic syndrome. The evidence is that (i) most of the spinal pathways which could underly the stretch reflex exaggeration in spastic hemiparesis are normal (for a review, see Pierrot-Deseilligny, 1990); (ii) though a dysfunction of synaptic transmission at Ia terminals (Mailis and Ashby, 1990; Nielsen and Hultborn, 1993), presynaptic inhibition of Ia fibres (Mailis and Ashby, 1990; Faist et al., 1994), reciprocal Ia inhibition (Ashby and Wiens, 1989; Boorman et al., 1991; Crone et al., 1994) and recurrent inhibition (Katz and Pierrot-Deseilligny, 1982; Mazzocchio and Rossi, 1989a) has been observed in spastic paraparesis, no correlation between the amount of each of these dysfunctions and the severity of spasticity has been found; (iii) later changes in the mechanical properties of the muscle itself and in its connective tissue have been shown to be in part responsible for the increased stiffness of spastic limbs (see Sinkjaer and Magnussen, 1994; Given et al., 1995).

The second reason to dispense with the restrictive definition is that the deteriorated compliance of the spastic limb cannot be seen only as a linear sum of dysfunction in individual muscles (cf. Haggard et al., 1994). Indeed, an altered pattern
of muscle coactivational relationships may be important in determining an abnormal joint stiffness during natural movements (see Dewald et al., 1995).

Among the many spinal systems, the Renshaw recurrent inhibitory pathway may contribute to different motor synergies, given that its activity is not restricted to homonymous motor neurons but it is widely distributed throughout the limb via heteronymous projections (Meunier et al., 1990; Katz et al., 1993; Meunier et al., 1994). It is definitively clear that recurrent inhibition in the spinal cord has turned out to be a complicated system transcending the scheme of a simple negative feedback loop (see Windhorst, 1996).

During natural movements, the descending command provides a variable regulation of Renshaw-cell activity depending on the motor task (see Pierrot-Deseilligny et al., 1983; Mazzocchio et al., 1994; Nielsen and Pierrot-Deseilligny, 1996). This may contribute to determination of adequate synergies between muscles acting on the same or different joints (Meunier et al., 1990; Meunier et al., 1994; Rossi et al., 1995).

Although homonymous recurrent inhibition at rest may, or may not, be reduced (Mazzocchio and Rossi, 1989a), all spastics lack in task-dependent modulation of Renshaw cell activity during movement (Katz and Pierrot-Deseilligny, 1982). This may be the consequence of a lesion interrupting the corticomotor pathways conveying the intrinsic coordinates for the execution of the movement to Renshaw cells. Indeed, short-latency cortical influences on Renshaw cell activity have been demonstrated in humans (Mazzocchio et al., 1994). The loss of Renshaw cell adaptability to the motor task may make muscle synergies unflexible.

However, the evidence of a decreased recurrent inhibition at rest in some spastic patients (Mazzocchio and Rossi, 1989b; Mazzocchio et al., 1990) is suggestive of a more complex organization of the descending control on these interneurons. In the present study, we investigated the possibility that the level of excitability of Renshaw cells is maintained within an appropriate operative range, by descending tonic facilitatory influences. The hypothesis is put forward that, regardless of the location of the lesion, the possible consequences of the dysfunction of the Renshaw system in spasticity may be the inability of the nervous system to determine the optimal set of relationships between muscles and limb segments necessary to accomplish the movement.

A preliminary account of these findings has been presented (Mazzocchio and Rossi, 1992).

Patients
Ten patients had a clinical picture of a slowly progressive spastic paraparesis. Four patients had a cerebral lesion: ischaemic infarction in two; anteriovenous malformation in one; and infantile encephalopathy in one. Nine patients had a spinal cord lesion: cervical spondylosis in four; intradural spinal tumour in two; and traumatic myelopathy in three. At the time of the investigation the duration of the illness varied from 2 months to 27 years.

Clinical assessment of spasticity comprised Achilles tendon jerks, resistance to passive ankle dorsiflexion, the amount and duration of ankle clonus, and examination of gait. An increase in calf muscle tone was graded according to the Ashworth scale (Ashworth, 1964): 0 = normal tone; 1 = slightly increased tone giving a catch on abrupt passive stretch of the triceps surae muscle; 2 = increased tone; 3 = severely increased tone; and 4 = passive movement of the ankle joint hardly possible. Achilles tendon reflexes were graded as 0 = diminished or absent; 1 = normal; 2 = increased; 3 = ankle clonus (that is, four or more reflex contractions produced by tendon percussion or abrupt ankle dorsiflexion). All patients had signs of spasticity in the lower limbs. All had hyperactive Achilles tendon reflexes and all but three subjects had ankle clonus. Spasticity was more conspicuous than weakness in most of the subjects. Some clinical characteristics are reported in Table 1. At the time of investigation, none of the patients had received medical treatment known to influence spasticity. The clinical assessment of spasticity and the neurophysiological testing were performed independently by different investigators.

General experimental arrangement
The subjects were comfortably seated and the leg to be examined was clamped with the knee semi-flexed and the ankle in plantar flexion (110°). In most of the patients both legs were examined. Surface electrodes were used for both stimulation and recording. The soleus H-reflex was obtained by stimulating the posterior tibial nerve at the popliteal fossa with rectangular pulses of 1-ms duration, every 10 s. The same unipolar electrode provided the conditioning and test stimuli. The reflex responses were recorded by two nonpolarizable disc electrodes placed 2 cm apart over the distal third of the soleus muscle. The reflex amplitude was computer analysed (peak-to-peak) and expressed as a percentage of the maximum muscle potential.

Method of estimating recurrent inhibition
The homonymous recurrent inhibition of soleus motor neurons was evaluated by the method originally developed by Bussel and Pierrot-Deseilligny (1977). Renshaw interneurons were activated by a conditioning reflex response (H1) of the ‘corresponding’ soleus α-motor neurons elicited by an electrical conditioning stimulus (S1), and the resulting
Recurrent inhibition in spasticity

Table 1  Clinical and neurophysiological features of patients

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<th>Tonus (Ashworth)</th>
<th>H' max % of M max</th>
<th>H' min % of M max</th>
<th>H max (mV) before/after L-acetylcarnitine</th>
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*Lesion: 1 = progressive spastic paraparesis; 2 = cerebral; 3 = cord. †Same patient, both legs tested (see text).

inhibitory effect was evaluated by the test reflex response (H') elicited by a subsequent supramaximal stimulus of the same nerve. If the conditioning-test interval is appropriate, the H1 reflex discharge collides with the antidromic motor volley caused by the supramaximal test stimulation. This collision opens the way for an orthodromic reflex response to the supramaximal stimulation, the test H' reflex. Since H' can only pass along the motor axons in which previous collision has taken place, only motor neurons which have already fired in the first conditioning discharge have their excitability assessed by the following test H' volley. It follows that, not only recurrent inhibition but also, motor neuronal after-hyperpolarization is expected to affect the size of the test H' reflex (for further details see Mazzocchio et al., 1994). By exploiting the cholinomimetic properties of L-acetylcarnitine (Nicetile®), a substance which can be safely administered in humans (Schieppati et al., 1989), it has been possible to demonstrate that recurrent inhibition is the only factor responsible for the increasing depression of the test H' reflex with increasing conditioning H1 reflexes (Mazzocchio, Rossi, 1989b). This was not possible in four patients in whom the H' reflex was either absent or not large enough (<5% of the maximum-amplitude motor response, M max) to allow reliable measurements. Secondly, series of test H' reflexes of maximum (H' max) and minimum (H' min) size were collected using conditioning H1 reflexes of appropriate amplitudes. H' reflexes were considered of minimum amplitude when their size was decreased to the smallest possible by conditioning H1 reflexes of maximum size.

Organization of the experiments

The stimulus protocol was as follows: (i) a single conditioning stimulus was delivered so that the H1 reflex response could be obtained; (ii) a single test stimulus supramaximal for α-axons was given so as to obtain a maximum M wave; (iii) the conditioning and test stimuli were combined to produce the H' reflex (conditioning–test interval usually being 10 ms). The relationship between conditioning H1 reflexes of increasing amplitude and the resulting test H' reflexes was first studied to estimate the strength of recurrent inhibition and hence the activity of the Renshaw cell at rest (see Figs 1A and 2A; Mazzocchio et al., 1994). By exploiting the cholinomimetic properties of L-acetylcarnitine (Nicetile®), a substance which can be safely administered in humans (Schieppati et al., 1989), it has been possible to demonstrate that recurrent inhibition is the only and Rossi, 1989b). This was not possible in four patients in whom the H' reflex was either absent or not large enough (<5% of the maximum-amplitude motor response, M max) to allow reliable measurements. Secondly, series of test H' reflexes of maximum (H' max) and minimum (H' min) size were collected using conditioning H1 reflexes of appropriate amplitudes. H' reflexes were considered of minimum amplitude when their size was decreased to the smallest possible by conditioning H1 reflexes of maximum size.
Fig. 1 Estimation of recurrent inhibition at rest in Group 1 patients. (A) Pattern of variations of the soleus H’ test reflex after a conditioning H1 discharge in a representative subject (Case 5). The amplitude of the H’ reflex is plotted against that of the H1 reflex, both expressed as a percentage of the maximum motor response (M_max). The H’_max corresponds to the maximum size of the H’ reflex, while the H’_min corresponds to the smallest H’ amplitude obtained after a conditioning H1 reflex of maximum size. The identity line represents the theoretical curve which would be obtained if H’ equaled H1. Each point is the mean of five measurements. (B) EMG responses from the soleus muscle obtained by electrical stimulation of the posterior tibial nerve at the popliteal fossa in the same subject. Upper traces: isolated conditioning posterior tibial nerve stimuli of different intensity producing (left) medium- and (right) maximum-sized H1 reflexes; lower traces: combined conditioning and test stimuli at 10 ms intervals producing (after the M_max) H’ reflexes of (left) maximum and (right) minimum size. Each record shows five superimposed traces. (C) Comparing the mean size (+SE) of the H’_max and the H’_min (n = 11). The difference between the two reflexes was significant (P < 0.001).

amplitude (preceded, if present, by an M-wave of negligible size). Thirdly, peak-to-peak amplitude values of the maximum H-reflex (H_max) and maximum M wave (M_max) were measured to calculate the H_max:M_max ratio. Electrode positions, and the voltage and current which elicited the best H and M responses were noted. All subjects, both patients and controls were then given a twice-daily oral dose of L-acetylcarnitine (30 mg/kg/day). Each subject served as his/her own control. Subjects were tested on four different occasions, at 5, 10, 15 and 30 days after the start of the daily drug administration.

In 10 subjects (seven patients and three healthy controls), presynaptic inhibition of Ia fibres was also assessed before (control conditions) and after L-acetylcarnitine administration, by two independent methods. (i) A short-lasting vibration (10 ms: three shocks at 3-ms intervals) was applied to the skin above the tendon of the tibialis anterior muscle by a vibrator (Bruel and Kjaer model 4809) driven by monophasic rectangular pulses of 2 ms duration. The strength of vibration was graded using a power amplifier. The time course of the effect of the tendon tap on the soleus H reflex was obtained in eight subjects. When using an interval of 40–60 ms between the vibratory stimulus and the H-reflex, the depression of the H-reflex is thought to reflect the amount of presynaptic inhibition of the Ia fibres mediating the H-reflex (Hultborn et al., 1987). A conditioning–test interval of 60 ms between the conditioning tap and the H-reflex was used before (control) and after L-acetylcarnitine oral administration. Although the same amplitude of tap was used in the two conditions, we also checked that the conditioning stimulus produced a small facilitation (of similar size) of the H-reflex at conditioning–test intervals of 20 ms but no significant effect on the size of the H-reflex at conditioning–test intervals of 500 ms. The lack of inhibition at these long interstimulus intervals suggests
that activation of homonymous Ia afferents (due to spread of vibration to the soleus muscle), which could lead to post-activation depression of the soleus H-reflex (Hultborn et al., 1996), minimally contributes to the inhibition observed at conditioning–test intervals of 60 ms. (ii) The soleus H-reflex facilitation produced by femoral nerve stimulation was measured. The femoral nerve was stimulated by a monopolar ball electrode placed in the femoral triangle just lateral to the femoral artery. The indifferent electrode was placed on the posterior aspect of the thigh. As it was critical to have a constant conditioning stimulus under the two different conditions, the stimulus intensity was adjusted to be supramaximal (more than four times the motor threshold).

A time course of the effect of the femoral nerve stimulation on the soleus H-reflex was obtained before and after l-acetylcarnitine administration in one normal subject and in one patient with spastic hemiplegia. The earliest conditioning–test interval at which it was possible to elic hemononymous Ia facilitation of the test reflex was used as the first of a series of consecutives intervals where there was a facilitation lasting >1 ms, and where the maximum value of the facilitation was statistically significant. It has been shown that this facilitation is mediated through a monosynaptic Ia pathway, and that during its first 0.5 ms, this facilitation is not yet contaminated by any non-monosynaptic effect (Hultborn et al., 1987). The size of the unconditioned control H-reflex was adjusted to be between 15 and 25% of M-wave, H-reflex, H1, and H2, respectively. The maximum size of the H-reflex obtained with the same experimental session. Care was taken to ensure that stimulation and recording conditions before and after drug intake were as similar as possible; in particular, it was verified that maximum M-waves were of approximately equal amplitude and shape in both situations.

Data analysis
The data were stored on a computer for later statistical analysis. The means and standard deviations of maximum M-wave, H-reflex, H1max, H1min, and H2max:H1max ratio were determined. The statistical significance of differences in these measurements before and after l-acetylcarnitine oral administration was evaluated by Student’s paired t test.

Results
The electrophysiological data for each patient is shown in Table 1 together with some clinical findings. The average Hmax:Max ratio (80 ± 2.3%) was higher than the normal control values (P < 0.001) but not significantly different within the spastic population. Nevertheless, two groups of patients could be distinguished judging from the amount of recurrent inhibition at rest (Table 1): Group 1 (Patients 1–11) showing a decrease of the H’ reflex with increasing H1 reflexes, and Group 2 (Patients 12–20) showing no depression of the H’ reflex. In a further group (Group 3, Patients 21–24) the H’ reflex could not be evoked. Although no conclusion can be drawn on the amount of recurrent inhibition, the absence of the H’ reflex might be suggestive of an increased recurrent inhibition at rest (Katz and Pierrot-Deseilligny, 1982).

Figure 1 summarizes the behaviour of the H’ reflex in Group 1 patients. A representative example of the full pattern of test H’ reflex changes after increasing H1 conditioning reflexes is illustrated in Fig. 1A (Case 5). The size of the H’ reflex increased in parallel with the H1 reflex up to a certain value (H1max) beyond which, as the H1 continued to increase, there was a gradual decrease in H’ reflex amplitude. This pattern was very similar to the H’ variations observed in a normal subject but for the fact that, due to the hyperexcitability of the monosynaptic reflex arc, maximum depression of H’ reflex amplitudes (H’min) were obtained with significantly larger H1 reflex amplitudes. An example of the H’max and H’min reflexes produced by H1 amplitudes of ~40% and 80% of Mmax respectively, are shown in Fig. 1B (also Case 5). Figure 1C illustrates the mean and standard errors of the H’max and H’min reflexes for Group 1 patients. The difference in the size of the two reflexes was statistically significant (t = 13.5; P < 0.001). This group was considered as having normal recurrent inhibition at rest.

Figure 2 summarizes the behaviour of the H’ reflex in Group 2 patients. A representative example of the full pattern of variations of the H’ reflex is shown in Fig. 2A (Case 19). Having reached its maximum size, the H’ reflex showed no sign of successive reduction as H1 continued to increase. Figure 2B shows that the size of the H’ reflex obtained with the largest H1 reflexes (80% of Mmax) was very similar to the maximum H’ size obtained with much lower H1 amplitudes (25% of Mmax in this case). Figure 2C shows that there was virtually no difference in the mean size of the two reflexes when pooling the data for Group 2 patients. This group was considered to have a reduced amount of recurrent inhibition at rest (see also Mazzocchio and Rossi, 1989a; Mazzocchio et al., 1990).

Finally, in one patient, both patterns of H’ variation were present. For this reason, data from the two legs were separated into Groups 1 and 2 accordingly (11 and 18 in Table 1).

Effects of chronic administration of l-acetylcarnitine on recurrent inhibition
After oral intake of l-acetylcarnitine (30 mg/kg daily), the size of the H’ reflex was affected differently in Group 1 and 2 patients (Fig. 3). A representative example (Case 6) of the full pattern of H’ variations before (open circles) and after (closed circles) l-acetylcarnitine is shown in Fig. 3A for Group 1 patients. As previously reported (Mazzocchio and Rossi, 1992), the decay phase of the H’ reflex could be obtained with smaller H1 amplitudes after drug intake; in
addition, the same conditioning H1 discharge produced a much greater depression of the H’ size within its decay phase. This is further shown for five patients (Cases 1, 2, 6, 7, and 8) in Fig. 3B where the average values of the H’ reflex elicited by a conditioning H1 reflex equal to 55% of M_max (corresponding to the average reduction in H1 maximum size) are compared before (open columns) and after (filled columns) L-acetylcarnitine intake. The difference was statistically significant \((P < 0.002, t = 7.512)\). Figure 3C (Case 14) and Fig. 3D (Cases 12, 13, 14, 15, and 17) show that the drug did not produce the same effects in Group 2 patients. There was no significant change in the size of the H’ reflex produced by the same conditioning discharge under the two conditions.

Effects of chronic administration of L-acetylcarnitine on the \(H_{\text{max}} : M_{\text{max}}\) ratio

Figures 4 and 5 show the values of \(H_{\text{max}}\), \(M_{\text{max}}\) and the \(H_{\text{max}} : M_{\text{max}}\) ratio before and after L-acetylcarnitine oral administration for all the patients. The data from Group 3, in whom recurrent inhibition could not be estimated, was pooled according to the drug effect on the H reflex. A representative example of the time course of L-acetylcarnitine effects on the \(H_{\text{max}}\) and \(M_{\text{max}}\) in Group 1 patients is shown in Fig. 4A (same case as in Fig. 1A). While there was no significant variation of the maximum M wave during the 30 days of treatment, a significant drop in the size of the maximum H-reflex was observed throughout the period of treatment, starting approximately on the fifth day. Figure 4B shows maximum M and H waves before and at the peak of drug effect (10 days later in this case). Figures 4C and 4D show the mean and standard errors of the maximum M wave and the H reflex, and the \(H_{\text{max}} : M_{\text{max}}\) ratio, respectively, before and after L-acetylcarnitine intake for Group 1. The differences between the mean H-reflexes and mean \(H_{\text{max}} : M_{\text{max}}\) ratios before and after L-acetylcarnitine were highly significant \((t = 5 \text{ and } t = 12.2, \text{ respectively}; P < 0.001)\).

Figure 5A shows a representative example of the time course of L-acetylcarnitine effects on the \(H_{\text{max}}\) and \(M_{\text{max}}\) of Group 2 patients (same case as in Fig. 2A). There was no reduction in the size of the \(H_{\text{max}}\) in Group 2 patients on any of the days selected for the test, which was different from the other group of patients. In fact, there was an increase in the size of the \(H_{\text{max}}\) compared with its control.
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Fig. 3 Effect of L-acetylcarnitine on recurrent inhibition. (A) Pattern of variations of the H’ reflex before (open circles) and after (closed circles) the oral intake of L-acetylcarnitine in a subject representative of Group 1 patients (Case 6). Each point is the mean of five measurements. (B) Average size (±SE) of the H’ reflex evoked by a conditioning H1 discharge corresponding to an average size of 55% of M_max before (open columns) and after (filled columns) L-acetylcarnitine in five patients from Group 1 (Cases 1, 2, 6, 7 and 8). The difference was statistically significant (P < 0.002). C and D are as A and B but represent Group 2 patients (Cases 12, 13, 14, 15 and 17). Note the lack of effect of the drug on the H’ size in C (Case 14). In D, the slight difference between the average H’ sizes before and after L-acetylcarnitine is not significant (P = 0.35).

value (Fig. 5B). This tendency was confirmed by a consistent increment (P = 0.051) in the mean values of the maximum H reflex, without a parallel M_max change (Fig. 5C) and, to a lesser extent, in the H_max : M_max ratio (Fig. 5D) after L-acetylcarnitine, when pooling all data for Group 2.

**Effect of L-acetylcarnitine on the H_max : M_max ratio of normal subjects**

The effect of L-acetylcarnitine on maximum H reflexes was also tested in a group of normal subjects (Table 2). Despite a notable individual variability, the mean H_max : M_max ratio and the mean amplitude of the absolute H reflex was significantly reduced after L-acetylcarnitine administration (t = 3.297 and t = 2.88, respectively; P < 0.05). Larger H_max : M_max ratios appeared more likely to show a significant decrease in their values after drug intake. Such an effect may perhaps go undetected, if the gain of the reflex arc is too low, as in the case of the subjects with very low H_max : M_max ratio.

**Effect of L-acetylcarnitine on presynaptic inhibition of Ia fibres**

Possible changes in the amount of presynaptic inhibition of soleus Ia terminals due to L-acetylcarnitine administration were assessed by studying the effect of a conditioning vibratory stimulus applied to the tibialis anterior tendon on the soleus H-reflex in six patients, representing the three groups (Cases 5 and 9; 19 and 21; and 23 and 24) and in two healthy subjects (Subjects 2 and 5). Such a conditioning stimulus normally evokes an inhibition of the H-reflex, starting at a conditioning–test interval of 40 ms and lasting ~300 ms (Fig. 6A, open circles; Case 5 as in Figs 1A and 4A). A similar time course was found in three other cases (9, 21, and 23). In the remaining two cases (19 and 24), a
Fig. 4 Effect of L-acetylcarnitine on the excitability of the H-reflex arc in Group 2. A, B, C and D as in Fig. 3. The case illustrated in A and B refers to the same subject as in Fig. 2A and B (Case 19). Note the lack of depression in the size of the H-reflex (A and B). In fact, the average size of the H-reflex (C) and the H$_{\text{max}}$ : M$_{\text{max}}$ ratio (D) showed small but insignificant increases (n = 11, including Cases 23 and 24).

Fig. 5 Effect of L-acetylcarnitine on the excitability of the H-reflex arc in Group 2. A, B, C and D as in Fig. 3. The case illustrated in A and B refers to the same subject as in Fig. 2A and B (Case 19). Note the lack of depression in the size of the H-reflex (A and B). In fact, the average size of the H-reflex (C) and the H$_{\text{max}}$ : M$_{\text{max}}$ ratio (D) showed small but insignificant increases (n = 11, including Cases 23 and 24).

method based on the estimation of the amount of heteronymous facilitation from quadriceps to soleus (see Patients and methods). The time course of the effect of supramaximal conditioning stimulation of the femoral nerve on the soleus H-reflex (open circles) is shown for a control subject (Subject 1) and for an hemiplegic patient (Case 6, the same as in Fig. 3A) in Fig. 6C and D, respectively. It is seen that the conditioning stimulation evoked a clear-cut facilitation starting at a conditioning–test interval of ~7.5 ms (the negative conditioning–test interval designates that the conditioning stimulus followed the test stimulus). This facilitation reached a maximum within 0.6 ms after its onset. This fast rise time is compatible with its monosynaptic origin; therefore, changes in the amount of this facilitation can be ascribed to variation in ongoing presynaptic inhibition of heteronymous Ia afferents on the soleus motor neurons (see Hultborn et al., 1987). After L-acetylcarnitine administration (closed circles in Fig. 6C and D), which produced a consistent reduction of the H$_{\text{max}}$ : M$_{\text{max}}$ ratio in both subjects (see Table 1 and 2), there was no significant change in the amount of this early heteronymous facilitation. Thus, the decrease in excitability of the soleus H-reflex after L-acetylcarnitine is caused by a postsynaptic effect on the motor neurons.
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Table 2  Neurophysiological data from control subjects

<table>
<thead>
<tr>
<th>Control subject</th>
<th>Age (years)</th>
<th>H&lt;sub&gt;max&lt;/sub&gt; (mV) before/after l-acetylcarnitine</th>
<th>M&lt;sub&gt;max&lt;/sub&gt; (mV) before/after l-acetylcarnitine</th>
<th>H&lt;sub&gt;max&lt;/sub&gt; : M&lt;sub&gt;max&lt;/sub&gt; ratio (%) before/after l-acetylcarnitine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>12.8/7.9</td>
<td>20.4/19.5</td>
<td>63/40</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>12.4/11.2</td>
<td>20.6/20.4</td>
<td>61/55</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>9.12/6.5</td>
<td>15.6/15.6</td>
<td>58/42</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>5.61/3.8</td>
<td>10.8/10.5</td>
<td>52/36</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>2.7/2.4</td>
<td>12.2/13.7</td>
<td>22/17.5</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>5.0/4.93</td>
<td>22.3/22.3</td>
<td>22/22</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>3.46/3.37</td>
<td>22.5/22.4</td>
<td>15/15</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>12.4/9.9</td>
<td>19.0/19.0</td>
<td>65/52</td>
</tr>
<tr>
<td>Mean (SE)</td>
<td>34.5</td>
<td>7.94/6.25</td>
<td>17.7/17.7</td>
<td>45/35</td>
</tr>
</tbody>
</table>

Excluding the patients in whom recurrent inhibition could not be estimated (Group 3), it was possible to inhibit soleus α-motor neurons recurrently in ~50% of our spastic population (Group 1), under resting conditions by means of a maximum conditioning reflex discharge, while in the remaining patients (Group 2), this inhibition could not be obtained, the H<sup>9</sup> reflex size showing no significant change despite the increasing conditioning discharge. This has been explained by an increased 'surplus excitation' protecting the α-motor neurons from inhibition (see Katz and Pierrot-Deseilligny, 1982). However, if the hyperexcitability of the reflex arc, as inferred from the high H<sub>max</sub> : M<sub>max</sub> ratio, opposed the manifestation of Renshaw inhibitory effects, no difference in the amount of recurrent inhibition at rest should be observed, given that both groups of spastics shared a similarly high H<sub>max</sub> : M<sub>max</sub> ratio. Moreover, assuming that the slightly larger size of the absolute H<sup>9</sup> reflex in Group 2 may be due to a reduction of motor neuron after-hyperpolarization, this should favour the appearance of recurrent inhibition (see Hultborn et al., 1979; Mazzocchio and Rossi, 1989). On the other hand, the absence of a decay phase in H<sup>9</sup> size with increasing conditioning reflex discharges (as observed in Group 2), has also been reported to occur in motor nuclei in which no evidence of recurrent inhibition was found (Rossi and Mazzocchio, 1991, 1992; Katz et al., 1993). On the basis of these findings, we conclude that the lack of H<sup>9</sup> changes, observed in a part of our spastic population, may be taken to indicate a reduced amount of recurrent inhibition at rest (see also Mazzocchio and Rossi, 1989a; Mazzocchio et al., 1990).

After a daily regimen of 30 mg/kg of l-acetylcarnitine, the absolute mean H-reflex amplitude in Group 1 patients and in the control group was significantly less than that obtained before treatment. Given no significant differences in absolute M-wave amplitudes within each group, these changes cannot be due to a decrease in neuromuscular transmission but could, presumably, be due to a reduction of spinal cord excitability. This may occur at a presynaptic

Discussion

This study provides definite evidence that not all spastics exhibit a normal recurrent inhibition at rest (cf. Pierrot-Deseilligny and Mazieres, 1985; Pierrot-Deseilligny, 1990; Young, 1994).

Fig. 6  Effect of l-acetylcarnitine on presynaptic inhibition of Ia terminals on soleus motor neurons. (A) Time-course of the depression of the H-reflex following a tap applied to the tibialis anterior tendon in a patient representative of Group 1 (open circles, Case 5 as in Figs 1A and 4A) and in a patient representative of Group 2 (closed circles, Case 19 as in Figs 2A and 5A). The ordinate represents the size (±SE) of the conditioned reflex as a percentage of the control reflex size and the abscissa represents the conditioning–test interval in ms. (B) The average size of the depression of the H-reflex evoked by a tibialis anterior tendon tap at a conditioning–test interval of 60 ms before (open column) and after (filled column) l-acetylcarnitine administration in four patients (Cases 5, 9, 21 and 23) shows no significant change. (C) Facilitation of the soleus H-reflex evoked by a supramaximal stimulation of the femoral nerve in a healthy subject (Subject 1) before (open circles) and after (closed circles) l-acetylcarnitine administration. The negative intervals in the abscissa indicate that the conditioning stimulation was applied after the test stimulus. The dashed line represents the size of the unconditioned reflex. (D) The same as in C but in a spastic patient (Case 6).
level via an increased inhibition of soleus Ia terminals or through postsynaptic mechanisms. Using two independent electrophysiological methods, no evidence was found, in either spastics or controls, for an increase in presynaptic inhibition after L-acetylcarnitine. This is in line with the observation this drug does not affect GABAergic transmission in the animal (Tempesta et al., 1985). Thus, the decrease in the excitability of the soleus H-reflex is caused by a postsynaptic effect on the motor neurons. In view of the evidence that L-acetylcarnitine has no effect on Ib inhibition, while it is able to potentiate the recurrent inhibition elicited via motor neuron reflex discharge (Mazzocchio and Rossi, 1989b), the decrease in motor neuron excitability may be ascribed to an increase in Renshaw cell activity. Since restriction in the size of the motor neuronal pool has occurred in the absence of motor activity, it follows that L-acetylcarnitine is, by itself, able to activate the recurrent inhibitory pathway. In the cat, Renshaw cells can be spontaneously active (Beneke et al., 1974; Walmsley and Tracey, 1981) and an increase in their discharge rate can be obtained by the systemic administration of cholinomimetic agonists (see Ryall, 1983).

The apparently contradiction between the acute (Mazzocchio and Rossi, 1989b; Schieppati et al., 1989; Mazzocchio et al., 1990; Schieppati et al., 1991; Rossi et al., 1995) and chronic (Mazzocchio and Rossi, 1992; present results) effects of L-acetylcarnitine on the H reflex (no significant variation in the former, significant decrease in the latter) deserves some comment. Under acute conditions, the drug-induced potentiation of Renshaw-cell activity produces no measurable variation of motor neuron excitability, as tested by the H-reflex technique (Mazzocchio and Rossi, 1989b); under chronic conditions, there is probably a long-term potentiation of Renshaw cell activity capable of producing a lasting reduction in the number of motor neurons recruited by a reflex discharge.

In the patients with a reduced recurrent inhibition at rest, i.e. in those who showed no H'-reflex depression with increasing conditioning reflex discharges (Group 2), there was no reduction in the $H_{\text{max}} : M_{\text{max}}$ ratio after L-acetylcarnitine administration. This was unlikely to be due to a reduced efficacy of the drug or non-compliance with the prescribed therapy, since it was observed in a large number of subjects. Moreover, in the same subject (see Table 1), it was possible to observe a significant decrease in the $H_{\text{max}} : M_{\text{max}}$ ratio after L-acetylcarnitine intake on the side in which recurrent inhibition was normal, while no significant change in the $H_{\text{max}} : M_{\text{max}}$ ratio was seen on the other side, in which recurrent inhibition was lacking. We think this may be interpreted as being due to the loss of descending tonic facilitatory influences on Renshaw cells. Indeed, the absence of the H' reflex decay phase (cf. Fig. 2A) has never been observed under conditions in which a physiological reduction of Renshaw cell activity occurs (see fig. 3 in Pierrat-Deseiligny et al., 1983; fig. 6B in Mazzocchio et al., 1994).

In the cat, conditioning stimulation of the nucleus raphe magnus increases recurrent inhibition and Renshaw-cell activity, apparently via non-serotonergic descending pathways (Wada et al., 1989); on the other hand, recurrent inhibition is under a tonic inhibitory influence of a supraspinal system involving both serotonin (Sastry and Sinclair, 1976) and noradrenaline (Fung et al., 1987). L-acetylcarnitine has been reported to have a facilitatory effect on the spontaneous activity of medullary-pontine reticular formation neurons and their responses to acetylcholine and serotonin (Tempesta et al., 1985). Cholinergic and monoaminergic transmission could interact at the medullary-pontine reticular formation (see Pompeiano, 1995), from where the majority of the intraspinal fibres then contact interneurons located in laminae VII, VIII and IX (see Peterson, 1984).

The disruption of the resulting modulatory effect at the spinal level could account for the reduced level of activity of Renshaw cells, and for the possible prevalence of serotonergic influences on spinal cord circuitry. Serotonin is known to increase tonic motor activity mainly through a depression of motor neuron after-hyperpolarization (see Jacobs and Fornal, 1993). This might explain the additional increase in the excitability of the reflex arc in the patients with reduced recurrent inhibition at rest, after L-acetylcarnitine administration (see Fig. 5C).

Pathophysiological considerations

Decreased recurrent inhibition does not appear to be typical of a particular disease process, although it tends to be more often associated with progressive spastic paraparesis (see also Mazzocchio and Rossi, 1989a; Mazzocchio et al., 1990). This condition is somehow different from other processes causing spasticity for the presence of marked leg stiffness with relatively little loss of muscle power and unsteadiness of gait mainly due to impoverished movements in spastic limbs (see Bruyn and Scheltens, 1991). Most interestingly, the pathological evidence is of an involvement of the dorsal reticulo-spinal tract in the thoracolumbar cord, with a degree of sparing of the corticospinal tract (see Chou, 1992).

In patients tested after L-acetylcarnitine administration, a reduction of hyperexcitable H reflexes was accompanied by some clinical improvement, most notably in the reduction of flexor spasms and clonus. The response to passive movement was generally unaltered. This may depend on concomitant changes in the mechanical properties of ankle muscles usually associated with long-standing lesions (see Hufschmidt and Mauritz, 1985; Pierrat-Deseilligny, 1990). The presence of such changes is perhaps responsible for the difficulty in identifying major clinical differences in patients with normal or reduced recurrent inhibition at rest. Nevertheless, in the young paraplegic patient who was studied 2 months after injury, there was a clear-cut clinical difference between the two legs, one already showing an increased resistance to stretch with intense clonus and flexor spasms, the other showing no such signs and being still rather flaccid. Interestingly, the former was associated with reduced
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hemiparetic patients (Dewald et al., 1995; Levin, 1996). A lesion interrupting the reticulo-spinal projections with a facilitatory influence on Renshaw cells would lower the level of excitability of these interneurons making them less sensitive to any supraspinal input (with the same consequences as above) but also to the recurrent motor neuronal drive. This may have, in theory, complex consequences. (i) Transmission through the pathways mediating reciprocal Ia inhibition of the antagonists may be increased. This has been observed in patients with spinal cord lesions (Ashby and Wiens, 1989; Boorman et al., 1991). (ii) A reduction in the duration of motor neuron after-hyperpolarization (Hultborn and Kiehn, 1992) or in post-activation depression (Davies and Murphey, 1994) might be secondary to a loss of tonic Renshaw inhibition on $\alpha$-motor neurons. Indeed, there is evidence for a decrease in post-activation depression in spastics (Mailis and Ashby, 1990; Nielsen and Hultborn, 1993). (iii) Disinhibition of gamma motor neurons may produce an increase in spindle discharges. Although this has not been generally observed (Hagbartha et al., 1973), it would be worth specifically readdressing the issue in spastics in whom recurrent inhibition is definitely reduced (see above) or Ia transmission to motor neurons could be enhanced compared with normals (Mailis and Ashby, 1990; Faist et al., 1994). (iv) Disinhibition of ventral spinocerebellar tract cells activated monosynaptically by Ia input (see Windhorst, 1996) may produce an excess of information on muscle dynamics to supraspinal structures responsible for the control of posture and locomotion. Thus, lesions of the reticulo-spinal pathways have the potential of altering the excitability of Renshaw interneurons. The resulting loss of input specificity on one side and of output gain control on the other may contribute to the development of extreme limb stiffness causing a significant reduction in the passive and active range of motion, as seen in patients with spastic paraparesis.

In conclusion, the excitability of Renshaw interneurons is probably maintained within an operative range by reticulo-spinal systems which allow the cortical command to set or adapt the level of activity of Renshaw cells according to the motor task.

**Acknowledgements**

We wish to thank all the volunteers and patients who participated in this study, and Drs M. Giacchi and C. Scarpini who helped with some aspects of this work. L-acetylcarnitine was kindly supplied by Sigma-Tau Co., Pomezia (Italy). This work was financed, in part (60%), by grants from the Italian MURST.

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Received January 23, 1997. Accepted February 18, 1997