Enhancement of recurrent inhibition by intravenous administration of L-acetylcarnitine in spastic patients

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Abstract

The recurrent inhibition of the soleus alpha-motoneurons at rest, evaluated by a specially designed method of paired H reflexes, was estimated in 10 patients with spastic paraparesis. In three of these patients, Renshaw cell activity produced inhibition of the corresponding alpha-motor neurons comparable to that obtained in normal subjects, while the inhibition was reduced in five and absent in two. The effects of intravenously administered L-acetylcarnitine on the activity of Renshaw cells were studied in these patients. In all patients except the two with no evidence of recurrent inhibition, L-acetylcarnitine was found to significantly increase the amount of recurrent inhibition. Its effect became evident at about 30 minutes, reached a maximum around 50 minutes and vanished about 70 minutes from the onset of administration. A significant correlation was found between Renshaw cell activity and the ability of the drug to increase it. L-acetylcarnitine appeared to act specifically by enhancing recurrent inhibition, since no variation in the excitability of the monosynaptic reflex arc was observed.

Recurrent inhibition, first described by Renshaw,¹ is mediated by axon collaterals of motor neurons (MNs) projecting to Renshaw cell (RC) inhibitory interneurons, which in turn project back to alpha-²⁻⁴ and gamma-⁵ MNs, Ia inhibitory interneurons⁶ and other RCs.⁷ One of the suggested roles for RC is as a variable gain regulator of MN output from descending fibre inputs.⁸

This possible role in motor control has been particularly researched in normal⁹ and spastic subjects.¹⁰⁻¹² From the latter studies it can be inferred that supraspinal control of RCs was lacking during voluntary contraction, while recurrent inhibition at rest, if it changed at all, was decreased only in a limited number of patients.

Although current evidence does not support the idea that exaggeration of passively induced stretch reflexes in spastic patients may be related to a decrement in RC activity, it is reasonable to hypothesise that strengthening of the inhibitory activity of RCs could contribute to reducing the hyperactive stretch reflexes in these patients.

Preliminary results¹³ have demonstrated that the intravenous administration of L-acetylcarnitine (L-AC), a well-known cholinomimetic drug¹⁴ is capable of increasing the recurrent inhibition of soleus MNs in normal subjects. It was therefore of particular interest to study the effects of this drug on recurrent inhibition in spastic subjects.

Materials and methods

Experiments were performed on 10 patients (eight males and two females, aged 13-58 years) who gave their informed consent after agreement from the local Ethical Committee. They were selected from a population of 17 subjects that participated in a recent investigation of the activity of soleus-coupled RCs in spasticity of spinal origin.¹² Of the original 17 patients, four were excluded from this study because they were unwilling to enter an acute pharmacological trial and three because of protocol noncompliance.

The new population presented with spastic paraparesis, its duration ranging from two to 25 years. The clinical picture was very similar in all patients, differing only in its severity: a degree of paresis and increased tendon jerk reflexes and tone were present in both lower limbs. One patient (Case 1 in the table) had a complete post-traumatic transection of the cord at low thoracic level; another (Case 2) had a compressive myelopathy from intervertebral disc protrusion at C5-C6; the remaining eight patients suffered from hereditary spastic paraparesis of Strumpell-Lorrain (Cases 3-10): the diagnosis was based on inheritance, lack of either electromyographic alterations of the peripheral nervous system or changes in brain computerised tomography (CT) and/or magnetic resonance imaging (MRI) scans. (See table.)

Method of estimating recurrent inhibition

The subjects were seated on an examination chair, the knee and ankle joint at 110 and 90 deg respectively. The electromyographic activity of the soleus was recorded through surface electrodes set 2 cm apart, placed posteriorly on the lower third of the leg. The soleus H reflex was obtained by stimulating the tibial nerve at the popliteal fossa with rectangular pulses of 1 ms duration. The same unipolar electrode provided the conditioning and test stimuli.

Recurrent inhibition was evaluated by the method originally developed by Bussel and Pierrot-Deseilligny¹⁵ and extensively described

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Received 14 April 1989
and in revised form
31 August 1989.
Accepted 18 September 1989

Table

Case	Sex	Age	Diagnosis	Duration of disease in years	Clinical spasticity	H/M ratio	Pattern of H' variations	L-AC maximal effect		
								H' reflex %	p	Time in mins
1	M	57	ST	10	++++	0.9	Plateau-like	82	<0.05	35-45
2	M	56	CC	21	+++	0.9	Bell-shaped	88	<0.01	35-45
3	M	43	SL	25	+++	0.9	Plateau-like	81	<0.025	25-35
4	M	54	SL	8	++	0.8	Bell-shaped	33	<0.001	45-55
5	M	46	SL	21	+	0.9	Bell-shaped	71	<0.005	45-55
6	M	58	SL	18	++	0.9	Plateau-like	79	<0.001	45-55
7	F	42	SL	24	+++	0.8	Plateau-like	61	<0.001	55-65
8	F	19	SL	9	++	0.8	Plateau-like	71	<0.01	55-65
9	M	13	SL	10	+++	0.9	Straight line	93	NS	25-35
10	M	26	SL	8	+++	0.9	Straight line	95	NS	15-25

Diagnosis: ST = Spinal Transection; CC = Cord Compression; SL = Strumpell-Lorain Disease. L-AC Maximal Effect: H' reflex amplitude expressed as a % of H' before L-AC administration; Time from onset of administration.

in a previous paper.¹⁶ Briefly, RCs were activated by a conditioning reflex response (H1) of the "corresponding" alpha-MNs of the soleus elicited by an electrical conditioning stimulus (S1), and the resulting inhibitory effect was evaluated by the test reflex response (H') elicited by a successive supramaximal stimulus (SM) of the same nerve, 10 ms after the conditioning stimulus. The collision of the H1 reflex with the antidromic volley following the supramaximal stimulation prevents the appearance of the H1 reflex response and opens the way for an orthodromic monosynaptic reflex response (H') to the same supramaximal test stimulus.

The stimulation protocol was as follows: a) the conditioning stimulus S1 was given alone to check the size of the H1 conditioning reflex; b) the stimuli S1 + SM followed 10 s later. The sequences a and b were repeated with randomly varied intensities of the S1 conditioning stimulus to study the effects of different amplitudes of the H1 conditioning discharge on the H' test reflex. In this way, the H'/H1 curve was obtained by plotting the amplitude of the H' reflexes as a function of the amplitude of the H1 reflexes. All values were expressed as a percentage of the maximal motor response (Mmax).

Method of estimating the effect of L-AC on recurrent inhibition

All patients had been free of drugs for two months before participating in the study. To test the effects of L-AC on the H' reflex, the intensity of S1 conditioning stimulus was adjusted to have an H1 conditioning reflex about 80% of its maximal amplitude. The H' reflexes, evoked in this way, were then acquired at a rate of 6-8 per minute throughout the experimental session.

L-Acetylcarnitine (Nicetile®) was obtained from Sigma-Tau (Pomezia, Italy) 2000 mg of the drug were dissolved in a volume of 100 ml of 0.9% saline solution. At the beginning of the experiments, two iv infusion sets were prepared, connected to the patient via a two-way tap. The first bottle contained saline, the second the solution of L-AC. A continuous iv infusion of saline was started after setting the appropriate stimulation parameters. L-AC was then administered for 11-17 minutes (mean 14.5) after which saline infusion was resumed. It is important to stress that, although the order

of treatment (saline, L-AC, saline) was constant, the patients could not realise when L-AC replaced saline and vice versa. Before, during and after L-AC administration, H1 and H' reflexes were randomly evoked.

The amplitude of H reflexes was automatically measured (peak to peak) by computer. The H1 and H' reflexes occurring within every 10 min period from the start of the experiment were then averaged, and the results obtained during and after L-AC plotted as a function of the time elapsed from the onset of administration, in correspondence with the centre of each time-interval. For example, the mean value at 50 minutes comprises those reflexes occurring from 45 to 55 minutes. The values of the H' reflexes recorded during and after L-AC administration were expressed as a percentage of the values of the H' reflexes evoked before L-AC administration, which were considered as controls. The same criterion was used for the H1 reflexes. The changes in the H' and H1 amplitude induced by L-AC administration were therefore evaluated by comparison with the pre-infusion H' and H1 values. The mean and standard deviation (SD) were computed for all the single reflexes evoked in a given time interval, and compared by the two-tailed Student's *t* test both within and between patients.

The H'/H1 curve and the effect of L-AC on H' amplitude were studied only in one normal subject in this investigation. The results are entirely consistent with those we have already reported.¹³

Results

Figure 1A illustrates the typical pattern of variations in the H' test reflex amplitude as the H1 conditioning reflex amplitude was increased by augmenting the strength of the S1 stimulus in a normal subject. At low H1 reflex amplitude, the H' reflex closely followed H1 up to a specific value (H' max) beyond which, as the H1 continued to increase, there was a gradual decrease in the H' reflex amplitude. The effects of the administration of L-AC on the amplitude of the H' (fig 1B) and H1 (fig 1C) reflexes are also illustrated in the same figure. These effects were obtained by S1 stimulation yielding an H1 of about 80% of its maximum amplitude. In agreement with our preliminary findings,¹³ a

Figure 1 Pattern of variations of the H' test reflex after the H1 conditioning discharge and the effect of L-AC on the H' and H1 reflexes in a normal subject. In A, the amplitude of the H' reflex is plotted against that of the H1 reflex (both expressed as a percentage of Mmax). Each point is the mean of 5 measurements. The time-course of the H' (B) and H1 (C) reflexes, expressed as a percentage of the H' and H1 reflexes respectively evoked before L-AC administration, is plotted as a function of the time from the onset of L-AC administration. Each point is the mean of 15 measurements ± 1 SD. Significant points are indicated by * ($P < 0.05$).

decrease in the amplitude of H' became evident at about 30 minutes, and was greatest around 50 minutes from the onset of L-AC administration. The effect was over between 70 and 80 minutes when the amplitude of H' attained the values observed before administering the drug (control values). On the contrary, no significant variation in the amplitude of H1 at the same time intervals was present.

Three representative examples of that observed in spastic patients are illustrated in figs 2, 3 and 4. The pattern reported in fig 2A was observed in three patients (Cases 2, 4 and 5 in the table). In the example illustrated (Case 4), the H' reflex, having reached its maximum amplitude was only weakly depressed by further augmentation of H1. In all the three patients of this group, the H' max ranged between 10 and 15% of Mmax. L-AC induced in the same case (fig 2B), as in the other two, a significant decrease in the amplitude of H' which was apparent at about 30 minutes, greatest around 50 minutes and vanished about 80 minutes from the onset of administration. The mean value of greatest H' inhibition,

observed within 35–55 minutes from the start of L-AC administration, was $36 \pm 28\%$ of the control values in these patients (table). No effect of the drug on the H1 conditioning reflex was evident in the same patient (fig 2C) or in the others.

Figure 3A illustrates a representative example of the pattern observed in five patients (Cases 1, 3, 6, 7 and 8). The H' reflex reached a plateau and showed no sign of successive reduction as H1 continued to increase. In these patients, the H' max ranged between 35 and 45% of Mmax. The effects of L-AC on H' (fig 3B) and H1 (fig 3C) reflexes in one patient (Case 6) are shown. The mean value of the greatest inhibitory effect induced by L-AC administration on H', within 35–55 minutes, was $25 \pm 9\%$ in the five patients of this group.

Figure 4 illustrates the results in two patients (Cases 9 and 10). In fig 4A (Case 9), the amplitude of H' continuously increased when H1 was increased and showed no sign of diminishing. The administration of L-AC produced no significant effect on the amplitudes of H' (fig 4B) and H1 (fig 4C) reflexes at any time interval

Figure 2 Pattern of variations of the H' test reflex (A) and the effect of L-AC on the H' (B) and H1 (C) reflexes in Case 4. A, B and C as in Fig 1.

Figure 3 Pattern of variations of the H' test reflex (A) and the effect of L-AC on the H' (B) and H1 (C) reflexes in Case 6. A, B and C as in Fig 1.

Figure 4 Pattern of variations of the H' test reflex (A) and the effect of L-AC on the H' (B) and H1 (C) reflexes in Case 9. A, B and C as in Fig 1.

in the range explored.

The general time course of the effects of L-AC, the grand mean variation in the amplitudes of H' (fig 5A) and H1 (fig 5B) reflexes after the administration of L-AC, from all the patients except Cases 9 and 10, where there was no evidence of recurrent inhibition, is shown in fig 5. The value of the greatest inhibition in H' amplitude, the time interval at which it occurred and the relative Student's *t* values for each patient are reported in the table, along

Figure 5 Time-course (grand mean) of the effect of L-AC on H' (A) and H1 (B) reflexes obtained by averaging all the values from the eight patients who exhibited a sizable recurrent inhibition in control conditions. Significant points are indicated by * ($P < 0.05$).

Figure 6 Relationship between the amplitude of H' test reflex expressed as a percentage of H1 conditioning reflex (abscissa) and the percentage decrement of H' test reflex produced by L-AC (ordinate) in all the patients. The equation of the straight line is $y = 0.57x - 5$, $r = 0.79$ ($P < 0.01$).

Figure 7 Pattern of H' variations following administration of L-AC (A) and saline solution (B) administration (Case 5). A significant point is marked by * ($P < 0.005$).

with the H/M ratios and the various patterns of H' variations.

To decide whether the changes in H' reflex produced by L-AC were related to the control H' amplitude (that is, before L-AC administration), we plotted the maximal amounts of inhibition induced by L-AC versus the H' amplitude measured before administering the drug for each single patient. Figure 6 shows that there is a clear-cut and significant regression between the two variables. Assuming that the ratio H'/H1 in part reflects the amount of recurrent inhibition, it becomes apparent that the ability of the drug to decrease the H' amplitude is significantly dependent on the level of RC activity before L-AC administration in each subject.

Control experiments

Saline solution alone was administered in three patients (Cases 5, 7 and 6 see table). In fig 7, the effects of L-AC (fig 7A) and of saline (fig 7B) on H' amplitude in Case 5 are compared. In no case did saline produce significant changes in the amplitude of the H' reflexes.

The possibility was also considered that the lack of effect of L-AC on the H1 conditioning reflex could, in part, depend on its being of greater amplitude than H'. It is in fact known that the susceptibility to conditioning events of H reflexes is partly dependent on their size.¹⁷ Therefore, the effect of L-AC on unconditioned H reflexes, of amplitude equal to the H' control values, was tested in one patient (Case 6). No significant effect of the drug on unconditioned H reflexes of such amplitude was observed.

Adverse reactions

None of the patients complained of any adverse reactions during or after L-AC administration. A burning sensation at the injection site was reported in some cases.

Discussion

Our findings clearly show that the iv administration of L-AC induces a powerful enhancement of recurrent inhibition of the soleus MNs in most spastic subjects, as measured by the decrease in H' test reflex.

From the findings of Katz and Pierrot-Deseilligny¹⁰ and Katz,¹¹ and from our previously reported data,¹² it appears that spastic patients exhibit three different patterns of behaviour of the H' test reflex in relation to the amplitude of H1 conditioning reflexes: (a) a "bell-shaped" pattern, similar to that observed in normal subjects, in which the H' amplitude, having reached its maximum, gradually decreases as the H1 amplitude is progressively augmented. A pattern of this kind indicates a normal or even increased RC activity at rest, in view of the monosynaptic reflex arc hyperexcitability of these patients.¹⁰ In our previous study,¹² this pattern was observed in nine of 17 patients (53% of cases); (b) a "plateau-like" pattern, in which the H' amplitude having attained its maximum remains unchanged in spite of the progressive increase in H1 amplitude. This pattern was observed in six of 17 patients (35% of cases). The fact that there is no evidence of a decay phase of the H' test reflex in these patients could be related to a reduced RC activity, the H' reflex thus being primarily exposed to the action of the after-hyperpolarisation (AHP) that MNs are undergoing after the conditioning discharge.^{11,12} This behaviour, however, could also be explained by the hyperexcitability of the monosynaptic reflex arc;¹⁰ (c) a "straight line" pattern (observed in two of 17 patients, that is, 12% of cases) in which the H' amplitude increases in parallel with the H1 amplitude showing no sign of decreasing. An "extended" susceptibility of alpha-MNs to Ia excitatory effects, coupled with a complete loss of recurrent inhibition, could make the Ia test volley effective enough to overcome the AHP to which MNs are subject after the conditioning discharge, thus reactivating them all. Although it is not possible to know to what extent each of these factors contributes to such observed behaviour, we can conclude, at least for spastic patients with "plateau-like" and "straight line" patterns, that RC inhibitory activity is unable to restrain the "corresponding" MNs which are in a hyperactive state.

In a preliminary study performed in normal subjects,¹³ we showed that the iv administration of L-AC remarkably enhances recurrent inhibition of soleus MNs. In the present paper, a similar effect has also been demonstrated in spastic subjects. In the patients exhibiting both a "bell-shaped" and a "plateau-like" pattern (80% of the present cases), L-AC produced a significant decrease in the amplitude of the H' reflex. On administering 2000 mg of the drug in about 15 minutes, the effect was evident at about 30 minutes, maximum around 50 minutes and vanished between 60 and 70 minutes from the start of administration.

In the two patients with a "straight line" pattern, in whom there was no evidence of recurrent inhibition (that is, the H' reflex continuously increased with increasing H1), L-AC was unable to produce any significant effect on the H' amplitude.

In all patients, the administration of L-AC did not significantly modify the amplitude of the H1 conditioning reflex. Since its amplitude

was usually much greater than that of H', it could be argued that this might determine a lower susceptibility of H1 to the drug with respect to H'. This, however, can be ruled out since no effect was observed when L-AC was tested on unconditioned H reflexes, the amplitude of which was equal to that of the H' test reflex. These observations exclude the possibility that L-AC influences MN excitability, neuromuscular transmission and presynaptic inhibition on Ia afferent fibres. Thus, we can hypothesise that an increase in recurrent inhibition is the main factor responsible for the depression of the H' amplitude produced by L-AC. This conclusion is strengthened by the significant correlation between the amount of H' inhibition produced by the drug and the level of RC activity before L-AC administration. The fact that the lower the recurrent inhibition, the less the drug is effective, indirectly supports the hypothesis that spastic subjects with a "straight line" pattern of H' variations lack recurrent inhibition.

A further observation is prompted by the fact that L-AC is capable of enhancing recurrent inhibition also in patients with a "bell-shaped" pattern, in whom RC activity is supposed to be increased.¹⁰ This indicates that there is still margin for additional increases in RC activity even in these cases. The possibility of strengthening such inhibitory activity may have some weight in resetting the balance between excitatory and inhibitory inputs to spinal MNs in spastic patients.

It must be stressed that the patients selected for this study represent a fairly homogeneous model of spasticity in that the spinal cord was the principal site of disease and the nature of spasticity was similar among the subjects. Since spasticity can arise from a multitude of lesions and its characteristics depend heavily on the precise localisation and combination of lesions, our results might not be generalised.

It is worth emphasising that the effects of L-AC on recurrent inhibition were also observed in a patient with complete transection of the cord at low thoracic level. This suggests that the drug exerts its action directly at spinal level.

Our conclusion that the effects of L-AC on the H' test reflex result from an increase in RC activity is coherent with the demonstration that these interneurons have both nicotinic and muscarinic excitatory receptors¹⁸ which may be activated by systemic administration of blood-brain barrier-penetrating cholinergic agonists.¹⁹

Dr R Mazzocchio received a grant from Sigma-Tau, Italy. This work was partially supported by a grant from Regione Toscana and the Italian MPI.

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