The relationship between anaerobic lactate threshold and plasma catecholamines during incremental exercise in hereditary spastic paraplegia

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Accepted for publication: January 24th, 2003

Summary

Lower limb muscle chronic hyperactivity in hereditary spastic paraplegia (HSP) is the consequence of motor corticospinal tract involvement, which in turn has been hypothesized to be of mitochondrial origin. In order to assess skeletal muscle aerobic metabolism and sympathetic response during exercise in 10 HSP patients, we evaluated their blood lactate and catecholamine levels during an incremental workload bicycle exercise.
Lactate, but not epinephrine or norepinephrine, levels were significantly higher in the HSP patients than in control subjects, in both resting conditions and during exercise. In the patients, the anaerobic lactate threshold was reached prematurely (at 50% of the predicted normal maximal power output) when compared to normal controls. This finding was not related to any specific muscle morphology or histochemical activity.
Although other factors, including chronic spasticity and muscle deconditioning, have to be considered in the interpretation of our data, our results suggest the possible involvement of a mitochondrial mechanism, independently of sympathetic system overactivation, in exercising skeletal muscle of HSP patients.

KEY WORDS: catecholamines, exercise test, hereditary spastic paraparesis, lactate, mitochondria.

Introduction

The term hereditary spastic paraplegia (HSP) refers to a group of familial neurodegenerative disorders characterized by progressive lower limb spasticity. These disorders are classified both genetically, according to their pattern of inheritance, and clinically, with pure and complicated forms being recognized (1,2). Sporadic cases of spastic paraplegia can be included in the HSP group, provided other possible causes of pyramidal system involvement have been ruled out.
The fact that two of the eight HSP genes cloned to date encode mitochondrial proteins (namely Hsp60 in SPG13 and paraplegin in SPG7) argues for the involvement of aberrant mitochondrial processes in HSP. In fact, muscle biopsies in a subset of patients with paraplegin mutations have shown typical signs of mitochondrial involvement, suggesting the presence of impaired oxidative phosphorylation in these cases (3,4). However, contrasting results, arguing for (5) and against (6) this hypothesis, have been reported in not fully genetically characterized series of both familial and sporadic HSP patients. Furthermore, with the recent identification of new genes involved in different forms of HSP, evidence is accumulating to link long axon neurodegeneration with defective trafficking dynamics. Such defective trafficking dynamics would prevent essential, metabolic cargoes – including mitochondria – from reaching their correct cellular destination, which in turn would result in inadequate fuelling of axonal transportation (7).
In skeletal muscle, in the presence of aerobic metabolism involvement, deficient mitochondrial ATP supply to the contractile apparatus occurs at low exercise levels. The anaerobic lactate threshold (LT) is the critical point marking the transition from aerobic to anaerobic exercise metabolism, a transition that occurs with the participation of the sympathetic system, which stimulates anaerobic lactogenic glycogenolytic and glycolytic muscle pathways (8).
The aim of this study was to assess the LT as an index of aerobic skeletal muscle metabolism and to relate it to sympathetic system activation in 10 HSP patients during an incremental workload exercise.

Materials and methods

The study population was selected, from a group of 25 HSP patients followed in our clinic, according to the following criteria:
– Mild degree of neurological impairment: the patient had to be self-sufficient, i.e., scoring 2 on each Activi-

ties of Daily Living item (9), and theoretically able to perform the test exercise proposed, i.e., he had to have MRC (10) and Ashworth modified (9,11) scores of 4 and 2, respectively.
– Absence of cardiac and respiratory involvement, as assessed by ECG and cardiac ultrasound scan, chest X-ray and spirometric testing.
– Absence of joint or bone deformities.
– Body weight never above 20% of theoretical anthropometric value.
Ten patients (eight men and two women, median age±SE, 48.9±4.2, range 32-72 years) fulfilled the inclusion criteria. HSP was diagnosed both clinically and neurophysiologically according to accepted criteria (12) after excluding other possible causes of the spastic paraplegia. The main clinical characteristics, pattern of inheritance, and molecular studies in this group of patients are reported in Table I.
As controls, we enrolled a group of six untrained healthy volunteers, 3 male and 3 female, mean age±SE, 34.2±12.5 years.

Exercise protocol

The exercise protocol was approved by our institution's committee on human experimentation. All the subjects gave their informed consent to the study, once its purposes and procedures had been explained to them.
An electrically braked pedal-rate bicycle ergometer (Bik, Elettronica Trentina, Italy) was used so that the subjects could perform a series of 3-min exercise bouts starting at a minimum pedaling rate of 60-70 revolutions per minute and increasing the workload after 2-min rest intervals. This duration was chosen so that steady-state blood lactate levels could be obtained (13). Each patient's predicted normal maximal power output ($pnPO_{max}$) was provisionally defined on the basis of his/her sex, age, weight and height. The exercise started with an initial bout at 10% of the $pnPO_{max}$. Then, through successive 10% $pnPO_{max}$ increments, the work level was raised to the highest at which cycling could be maintained for 3 minutes. Expressed in Watt, this level, which corresponded to at least 60% of $pnPO_{max}$, was taken as the actual or real maximum power output (rPO_{max}). Our choice of protocol was based on the assumption that exercise will be mainly aerobic at the beginning of the test, before becoming progressively anaerobic as the power output increases, due mainly to the slow and fast motor unit recruitment sequence.
In addition to cardiac and respiratory function indices, blood lactate (in samples drawn from an antecubital vein) was assessed spectrophotometrically on an ERIS Analyzer 6170 (Eppendorf Geratebau, Hamburg, Germany) under basal conditions, during exercise, and during each inter-bout interval.
Free epinephrine (E) and norepinephrine (NE) levels were determined using a fully automated, high-performance liquid chromatography analyzer (HLC 725) developed by Tosoh (Tosoh Co., Tokyo, Japan) and distributed by Eurogenetics (Eurogenetics, Tessenderlo, Belgium). The following HLC columns were used: TSK pre-column CAI, 7.5 × 75 mm; TSK pre-column CA2, 4 × 60 mm; Catecholpak cation exchange gel column, 6 × 150 mm. The laboratory normal range for catecholamines was < 80 pg/ml, and < 500 pg/ml for E and NE.
To determine the LT in each patient, we calculated the exercise power output (work level) at which the slope of the best-fit lactate curve began to rise exponentially.

Muscle biopsy

In four of the 10 patients (patients no.s 1, 2, 3 and 4, Table I), a quadriceps muscle biopsy was performed and histochemically stained for haematoxylin-eosine,

Table I - Clinical features* of 10 HSP patients.

Patient no.	Age/Sex	Signs
1	32/F	a, b, nystagmus, pes cavus, dysarthria, adiadocokinesia
2	32/M	nystagmus, adiadocokinesia, impaired vibration sense
3	45/M	a, b, upper limb deep tendon hyperreflexia
4	64/M	a, dysarthria, impaired vibration sense
5	51/M	a, pes cavus, upper limb deep tendon hyperreflexia
6	53/M	a, b
7	72/M	a, b, upper limb deep tendon hyperreflexia
8	41/M	a, b
9	58/F	a, dysarthria, upper limb deep tendon hyperreflexia
10	40/M	dysarthria

* In addition to weakness, spasticity and deep tendon hyperreflexia in lower limbs. a=extensor plantar responses; b=ankle and/or knee clonus

Gomori's modified trichrome, ATPase pH 4.6 and 9.4, nicotinamide dehydrogenase tetrazolium reductase (NADH-TR), succinate dehydrogenase (SDH), and cytochrome c oxidase (COX). Serial muscle sections (8 µm) were studied for fibre-type characterization (at least 500-600 fibres per patient). Morphometry was performed using an automatic micrometric optical method at a magnification of 250x. The percentages of type 1 and 2 fibres, type 1 and 2 fibre diameters, and atrophy and hypertrophy factors were calculated (14). Also, the percentages of fibres with altered COX stain, altered SDH stain, or both were determined through automated imaging analysis (Matlab, Mathworks).

Statistical analysis

Goodness-of-fit models, in terms of minimal square residuals, were utilized to fit all the lactate curves. After a Kolmogorov-Smirnov test had confirmed that the data did not present a Gaussian distribution, the Mann-Whitney and, when necessary, the Kruskal Wallis non-parametric tests were used in order to estimate differences between patients and controls; 0.5% was taken as the level of significance.

Results

Resting

The mean resting value of lactate in the HSP patients was 2.53±0.22 mmol/L, higher than in the normal subjects (1.44±1.14 mmol/L, $p<0.01$). The mean resting value of E was significantly higher ($p<0.05$) in the patients than in the normal control subjects (96.5±24.2 vs 42.7±4.1 pg/ml), whereas the NE mean level did not differ significantly between the two groups (430.7±96.4 vs 368.0±60.2 pg/ml).

Exercise

In the HSP patients, the rPO_{max} ranged from 60 to 70% of the $pnPO_{max}$. Mean lactate values, normalized to resting values, started to increase from the fourth step and were significantly higher in the HSP patients than in the controls ($p<0.05$) from the sixth step of the exercise (60% of $pnPO_{max}$) onwards (Fig. 1).
As expected, the slope of the lactate curve in the control subjects started to increase exponentially at 70% of $pnPO_{max}$. On the contrary, in the HSP patients the LT was reached at an exercise level of 50% of $pnPO_{max}$ (5th step of the exercise protocol) (Fig. 1). A progressive increase of lactate, up to 350% of the resting values (peak lactate), was further observed at 70% of $pnPO_{max}$ (271% in controls).
The exercise-induced increase in plasma catecholamine levels was higher in the HSP patients than in the controls, although the difference was not significant (normalized peak E 410±130.5% vs 210±25.3%; peak NE 377±149.6% vs 250±23.1% (Fig. 2). A significant positive correlation was found between lactate and both E ($p<0.05$) and NE ($p<0.01$) levels during exercise in both patients and controls.

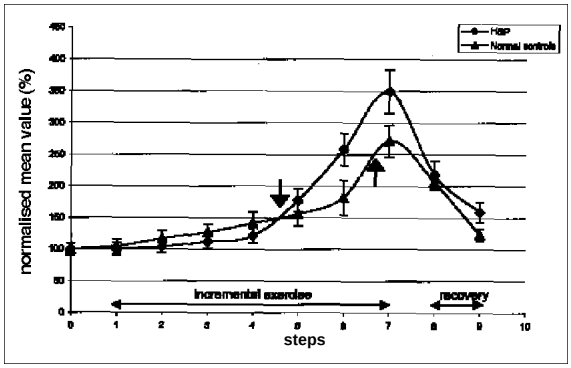

Figure 1 - Lactate curve in HSP patients and normal controls (normalised mean values and standard deviations). Arrows indicate LT in the two groups.

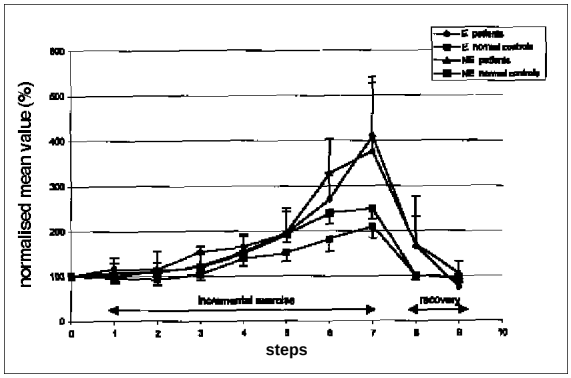

Figure 2 - E and NE curves in HSP patients and normal controls (normalised mean values and standard deviations).

Recovery

Unlike the controls, the HSP patients did not show a return to basal lactate values 30 min after exercise. On the contrary, both E and NE returned to baseline levels in both patients and controls.

Muscle biopsies

In the 4 biopsied patients we found a slight increase in size variability and in hypertrophy factors in type 1 fibres. A prevalence of type 1 fibres was present in case 3 (Table II, see over). While the presence of muscle fibres with COX hyporeactivity was rarely observed, the percentage of SDH hyperreactivity varied between 2% and 23% among type 1 and between 2% and 18% among type 2 fibres. No correlation was found between lactate levels and percentage of type 1 fibres or hypertrophy.

Discussion

One of the most reliable markers of *in vivo* functional mitochondrial impairment is excessive muscle lactate production during submaximal exercise (15). We stud-

Table II - Muscle biopsy in 4 HSP patients.

Patient no.	Type1 fibres %	Type1 fibres Atrophy/ hypertrophy factors	Type1 fibres with COX hyporeactivity no. (%)	Type1 fibres with SDH hyperreactivity no. (%)	Type2 fibres %	Type2 fibres Atrophy/ hypertrophy factors	Type2 fibres with COX hyporeactivity no. (%)	Type2 fibres with SDH hyperreactivity no. (%)
1	33	0/320	0	19 (13)	67	0/18	0	6 (2)
2	35	62/268	5 (3)	34 (18)	65	0/9	2 (1)	34 (18)
3	46	130/250	0	2 (2)	54	30/0	0	0
4	33	65/402	0	38 (23)	67	15/18	0	7 (2)

ied venous lactate kinetics during incremental exercise by assessing the anaerobic lactate threshold, i.e., the critical point at which the transition from aerobic to anaerobic exercise occurs. Studies in normal subjects undergoing an incremental exercise workload (16) have drawn attention to the role of sympathetic system activation as a factor involved in determining muscle lactate concentrations (17). It is not clear whether this role relates to a co-activation of skeletal muscle and the neuroendocrine system by a "central command" or whether it depends upon III/IV fibre-afferent sympathetic reflex activation by muscle/extracellular pH changes (18). During exercise, E stimulates β2-mediated muscle glycogenolysis (19) and hepatic neoglucogenesis (8), resulting in sustained blood glucose levels and the induction of muscle lactate production. As far as NE is concerned, its role during exercise is related to its β3-receptor mediated systemic lipolysis (20) and to the redistribution of blood away from organs and tissues that normally remove lactate (8).

Consistent with our preliminary observations (21), we found abnormal muscle aerobic metabolism during exercise in the HSP patients, as indicated by the accumulation of lactate starting from 40% of the $pnPO_{max}$. This increment can be attributed to additional recruitment of glycogenolytic fibres at low levels of muscle contraction during incremental exercise (22). Furthermore LT was achieved at a workload of 50% of the $pnPO_{max}$, an advance when compared to the control group. This phenomenon, however, was not found to relate to a disproportionate amount of any fibre type or to deficient OXPHOS as measured by COX and SDH stain in muscle. In the interpretation of our data other factors should be taken into account, particularly the deconditioning effect of disuse (23) and the consequences of chronic spasticity on structural and functional properties of type 1 oxidative fibres (24).

Although basal E was higher in the HSP patients, their NE and E peak increments during exercise were comparable to those of the controls, which rules out a major role for sympathetic activation in fostering muscle lactate overproduction in HSP. It is possible, however, that the absence of any significant difference between the patients and the controls in exercise-induced NE and E levels may relate to the small size of the control group. An exercise-related increase of lactate without a concomitant increase in catecholamine levels has previously been demonstrated in patients affected by mitochondrial myopathies performing an incremental intermittent exercise (25). This reinforces the hypothesis that lactate increase in HSP patients during incremental exercise is due to a defect of oxidative metabolism at skeletal muscle level. The full significance of this defect, and its role in the cascade of events leading to muscular damage in HSP remains to be determined.

Acknowledgments

The financial support of the Italian Health Ministry, Telethon - Italy (project n. 1179c) and the DNA/Tissue Bank for Neuromuscular Disorders, Muscle Disease Center, Department of Neurosciences of the University of Pisa, is gratefully acknowledged.

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