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Effects of chronic growth hormone treatment in aged rats on the biophysical and pharmacological properties of skeletal muscle chloride channels

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1 The effects of a 4-month daily treatment with recombinant human growth hormone (GH) (150 μg kg^{-1}) to aged rats were evaluated on the passive and active membrane electrical properties of extensor digitorum longus (EDL) muscle fibres *in vitro* by means of a two intracellular microelectrode technique.

2 Chronic GH treatment completely restored the diameter and the membrane capacitance of aged EDL muscle fibres and significantly lowered the membrane resistance towards the adult value. There was also an increase of the threshold current, a shortening of the latency and an increase of the amplitude of the action potential and a significant amelioration of the membrane firing capability.

3 The effects were almost fully attributable to a significant 50% increase of resting conductance to chloride ions (G_{Cl}), although an observed restoration of potassium conductance and a possible effect on voltage-activated sodium channels could contribute to the effects.

4 EDL muscle fibres of untreated aged rats showed a different pharmacological response to 2-(p-chlorophenoxy) propionic acid (CPP) enantiomers from that seen in adult rats; the **S**-(−) isomer was less potent in blocking G_{Cl} and the **R**-(+) isomer always increased G_{Cl} instead of producing the typical biphasic effect observed in adult fibres (an increase of G_{Cl} at 1–10 μM and a decrease at higher concentrations). The 4-month-GH-treated aged rats showed a pharmacological sensitivity to CPP enantiomers similar to that of adults.

5 The *in vitro* application of insulin-like growth factor I (IGF-I), the peripheral mediator of GH, produced a significant and irreversible increase of G_{Cl} of EDL muscle of untreated aged rats, an effect not observed in adults. This effect was completely inhibited by preincubation with 0.5 μM okadaic acid, suggesting that the IGF-I receptor transduction pathway can act on the phosphorylation state of the chloride channel through a serine-threonine protein phosphatase.

6 The results show that the skeletal muscle chloride channel is a target of the impairment of GH/IGF-I axis occurring in aged subjects. The acute and chronic effects observed on G_{Cl} of aged muscle fibres suggest that the GH/IGF-I stimuli act through a modulation of channel phosphorylation state and through the synthesis of 'adult'-like type chloride channels.

Keywords: Ageing; skeletal muscle; growth hormone; insulin-like growth factor-I; chloride channels; phosphorylation

Introduction

A decline of muscle function, i.e. a loss of muscle mass and a reduced strength of contraction, occurs with advanced age in both man and animals and is the result of multifactorial mechanisms involving both nervous and hormonal stimuli, environmental influences and changes of muscular apparatus itself (Sonntag *et al*., 1985; Carmeli & Reznick, 1994). Growth hormone (GH) secretion is severely reduced by ageing, with a consequent decrease of serum insulin-like growth factor-I (IGF-I), which mediates most of the peripheral actions of GH (Florini *et al*., 1991; Cocchi, 1992; Cohick & Clemmons, 1993; Ho & Hoffman, 1993). Rudman *et al*. (1990) observed that a 6-month treatment with GH to humans over 60 years old was able to restore IGF-I levels and lean body mass. These results led to the proposal that pharmacological interventions able to increase GH levels and/or secretion may be beneficial for the elderly (Rudman *et al*., 1990; Ho & Hoffman, 1993). Administration of high doses of GH to old rats restores the reduced protein synthesis occurring in skeletal muscle (Sonntag *et al*., 1985; Ullman *et al*., 1990). Furthermore, we found that a 6–8 week treatment with a therapeutic dose of GH (150 μg kg^{-1}) (Rudman *et al*., 1990; Beshyan *et al*., 1995) to aged female rats, known to be highly susceptible to the age-dependent decrease of GH secretion (Jansson *et al*., 1985), counteracted the alteration in membrane electrical properties, including the specific reduction of resting membrane conductance to chloride ions (G_{Cl}), occurring with ageing (De Luca *et al*., 1992a; 1994a). A large G_{Cl} is a characteristic of fast-twitch muscle fibres and works to control membrane excitability and muscle contractility (Lehmann-Horn & Rüdel, 1996). Thus, the extensor digitorum longus (EDL) muscles of GH-treated aged rats have significantly higher values (by 30%) of G_{Cl} compared to untreated aged ones (De Luca *et al*., 1994a). In support of the link between the decrease of GH secretion and the impairment of muscle G_{Cl} during ageing, we concomitantly observed that the GH treatment had little effect on adult rats, although it was slightly more potent in female compared to male rats (De Luca *et al*., 1994a).

The effects of GH on sarcolemmal electrical properties are due either to a direct action on GH receptors (Mathews *et al*., 1989; Florini *et al*., 1991) or to a stimulation of hepatic and tissue synthesis of IGF-I; this latter then acts on skeletal muscle in both endocrine and autocrine/paracrine manner (Turner *et al*., 1988; Cohick & Clemmons, 1993; Gosteli-Peter *et al*., 1994). Both GH and IGF-I stimulate muscle protein synthesis (Sonntag *et al*., 1985; Cohick & Clemmons, 1993); thus both could increase the number of ion channels and consequently G_{Cl}. Additionally, the decrease of G_{Cl} occurring during ageing may involve an alteration of the intracellular pathways controlling the phosphorylation state of the channel

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(De Luca *et al*., 1994b). Furthermore, the results of studies with the enantiomers of the chloride channel ligand 2-(*p*-chlorophenoxy) propionic acid (CPP), led us to hypothesize that isoforms of chloride channels with different pharmacological properties appear during ageing. In adult rat muscle, the **S**-(−)-CPP is a potent chloride channel blocker, whereas the **R**-(+) isomer produces a typical biphasic effect, increasing G_{Cl} in the range of 1–10 μM and decreasing it at higher concentrations (De Luca *et al*., 1992b). Aged rats have a decreased sensitivity to the blocking effects of **S**-(−)-CPP isomer and lack the biphasic response to **R**-(+) isomer which only increases G_{Cl} (De Luca *et al*., 1992a). In the present study we have evaluated the membrane electrical parameters of skeletal muscle of aged rats by extending the duration of the GH treatment to 4 months. To clarify the molecular mechanism of the GH treatment on G_{Cl} of aged rat skeletal muscle, we have performed an *in vitro* pharmacological characterization with the enantiomers of CPP. To evaluate the tissue sensitivity of IGF-I during ageing, we have tested the effect of acute *in vitro* application of the somatomedin on muscle G_{Cl} of untreated aged and adult rats.

Methods

Chronic GH treatment

Adult (6–9 months old) and aged (20–22 months old) female rats of the Wistar Kyoto strain (Charles River, Calco, Italy) were used for all the experiments. As previously shown (De Luca *et al*., 1994a), female animals are highly susceptible to the impairment of GH secretion with ageing (Jansson *et al*., 1985). Different groups of rats, each one of at least 10 animals, were used: GH treated aged rats, receiving 150 μg kg^{-1} of biosynthetic human GH (Genotropin, sterile powder-Kabi Vitrum, Stockholm, Sweden) s.c. 6 days a week for 4 months; untreated aged rats and untreated adult rats, receiving 0.1 ml distilled H_2O s.c. daily for the whole period of treatment. The aged animals were 20–22 months old at the beginning of the treatment period and were in good health, with no impairment of hind limb movements or locomotor activity (De Luca *et al*., 1992a; 1994a). Before the treatment was started, the plasma levels of insulin-like growth factor-I (IGF-I) were measured in randomly selected aged and adult rats (see below) to verify the state of age-related impairment of the GH axis described in the aged subjects. The health conditions of the aged rats were monitored during the entire treatment period. No differences were observed between treated and untreated groups. The animals were generally in good health. In both groups, 3 out of 10 aged rats died before the end of the treatment period of unknown sudden causes. Those in the untreated group died within the first month of the trial whereas those of the GH treated group died during the third and fourth month. One aged rat of the untreated group developed a skin tumour and was therefore discarded from the study. The mean weight of the aged rats finishing the trial period, evaluated at the beginning and end of the treatment, was not significantly different between the two experimental groups.

Electrophysiological recordings

The electrophysiological experiments were made *in vitro* at the end of the treatment period. The extensor digitorum longus (EDL) muscles were removed under urethane anaesthesia (1.2 g kg^{-1}, i.p.) from both untreated adult and aged rats, and GH treated aged rats. Soon after the removal of the muscles, the rats, still anaesthetized, were killed by i.p. injection of a urethane overdose. The muscles were placed in a muscle bath at 30°C for the electrophysiological recordings and superfused with normal and chloride-free physiological solutions.

The membrane electrical properties were obtained with the two-intracellular microelectrode technique in which a hyperpolarizing square current pulse is passed through one electrode and the membrane voltage response is monitored at two distances from the current electrode (Bryant, 1969; Bryant & Conte Camerino, 1991; De Luca *et al*., 1992b; 1994a). According to the theory of an infinite linear cable, the experimental cable parameters measured were the space constant λ (logarithmic decay of the electrotonic potential with distance from the current electrode), the fibre input resistance, Rin (steady-state electrotonic potential divided by the current intensity) and the time constant, τ (84% rise time of the electrotonic potential). The fibre diameter (d_{calc}) has been calculated from λ and Rin, while a constant value of 125Ω* cm was assumed for the myoplasmic resistivity on the basis of previous histological determinations (Bryant, 1969). From the above parameters, the membrane resistance (*R*m) and the total membrane capacitance, *C*m, that includes both surface (*C*s) and T-tubular (*C*t) membrane capacitance sources (Bryant, 1969; Bryant & Conte Camerino, 1991; De Luca *et al*., 1994a), were calculated. The current pulse generation, acquisition of the voltage records and calculation of the fibre constants were done under computer control, as described elsewhere (Bryant & Conte Camerino, 1991). The component resting membrane conductances to chloride and potassium ions were calculated from *R*m values in normal and chloride-free solutions (De Luca *et al*., 1992a; 1994a). For each fibre, the total membrane conductance *G*m, was considered to be 1/*R*m in the normal physiological solution. The potassium conductance, G_K, was 1/*R*m in the chloride-free physiological medium. The mean chloride conductance, G_{Cl}, was calculated as the mean *G*m minus the mean G_K. The excitability characteristics were determined by recording the intracellular membrane response to square-wave constant current pulses. In each fibre the membrane potential was set by a steady holding current to −80 mV before the depolarizing pulses were passed (De Luca *et al*., 1994a).

Solutions and drugs

The normal physiological solution had the following composition (in mM): NaCl 148, KCl 4.5, $CaCl_2$ 2.0, $MgCl_2$ 1.0, $NaHCO_3$ 12, NaH_2PO_4 0.44 and glucose 5.55. The chloride-free solution was made by equimolar substitution of methylsulphate salts for NaCl and KCl and nitrate salts for $CaCl_2$ and $MgCl_2$. Stock solutions of the **S**-(−) and **R**-(+) enantiomers of 2-(p-chlorophenoxy) propionic acid (CPP) were prepared in 1% aqueous sodium bicarbonate solution and the final concentrations to be tested *in vitro* were obtained by further dilution in normal and chloride-free physiological solution (De Luca *et al*., 1992a,b). On each preparation no more than two concentrations were applied to the muscle and each concentration was incubated for at least 45 min before recording to allow the steady-state of drug effect to be reached. Insulin-like growth factor I (IGF-I; human, recombinant, Sigma Immuno chemicals) was reconstituted in a stock solution of 10 μg in 100 μl of 0.1 M acetic acid. The final concentrations of IGF-I to be tested *in vitro* were obtained with further dilution in normal and chloride-free physiological solutions. Stock solutions of okadaic acid (Sigma, St. Louis, U.S.A.) were prepared in dimethylsulphoxide (DMSO) and added in μl amounts to the bath solutions, as needed. A 0.5% DMSO solution, much higher than the maximum DMSO concentration used, was without effects on the parameters studied. All solutions were gassed with 95% O_2 and 5% CO_2 (pH = 7.2–7.4).

IGF-I radioimmunoassay

Blood samples were collected from the caudal vein of randomly selected aged and adult rats before the beginning of the treatment by scalpel incision of the ventral side of the tail under local anaesthesia (xylocaine spray 2%; Astra Chemicals). The samples were placed in centrifuge tubes rinsed with EDTA (150 mM) and centrifuged at 600 × *g* for 10 min. The plasma was then separated and stored at −20°C until used for the IGF-I assay.

Plasma IGF-I concentrations were evaluated after acid – ethanol extraction (87.5% ethanol and 12.5% 2 M HCl), according to the method described by Daughaday *et al*. (1980). Samples were assayed by heterologous radioimmunoassay with IGF-I antibody (provided by NIADDK) and iodinated antigen (Amersham Int. Co., Amersham Buck, U.K.). The sensitivity of the assay was 0.1 ng ml^{-1}.

Statistics

The data are expressed as mean ± s.e.mean. The estimates of s.e.mean of G_{Cl} values were obtained from the variance and degrees of freedom of G_m and G_K, by assuming no covariance, by standard methods (Green & Margerison, 1978). The estimates of s.e.mean and of the number of values (n) of normalized G_{Cl} were obtained as described by Green & Margerison (1978). The logistic function used to non-linear least square fit the data of the concentration-response curves of **S**-(−)-CPP in the various experimental conditions was the following: Effect = $-100/1+(K/[\mathbf{S}\text{-}(-)]^n)$; where Effect = % change of G_{Cl}; −100 maximal % block of G_{Cl}, $K = EC_{50}$ of **S**-(−); n = logistic slope factor; [**S**-(−)] = molar concentration of **S**-(−)-CPP (De Luca *et al*., 1992a). The goodness of fit was calculated from the minimum χ^2 values for each fit and the number of degrees of freedom and was considered acceptable when larger than 0.001 (Press *et al*., 1986). Statistical comparison between experimental groups was performed by analysis of variance; comparison between individual means was made by unpaired Student's t test (Tallarida & Murray, 1987).

Results

Effects of 4 months treatment with GH on passive cable properties, ionic conductance and excitability characteristics of extensor digitorum longus muscle fibres of aged rats

Passive cable properties In agreement with our previous studies (De Luca *et al*., 1992a; 1994a), the passive cable properties of extensor digitorum longus (EDL) muscle fibres were affected by the ageing process in that the fibre diameter (d_{calc}) tended to decrease although this did not reach statistical significance and the membrane resistance (Rm) and the total capacitance (Cm) increased significantly (Figure 1). These parameters were significantly less changed in the EDL muscles of the aged rats in the GH treated group. In particular, the d_{calc} of EDL muscle fibres of GH-treated aged rats was even larger than that of adult rats and was also significantly different with respect to the d_{calc} of age-matched untreated animals (Figure 1a). In the GH-treated group, the value of Cm decreased significantly by 50% with respect to those of age-matched untreated rats so that it was only slightly but not significantly higher than the value of adult control rats (Figure 1c). In GH-treated aged rats there was also a partial but significant restoration of Rm that was significantly lower (by 34%) than that of age-matched untreated rats, albeit still significantly higher with respect to the adult value (Figure 1b).

Component ionic conductances The lower value of Rm in the aged group treated with GH was due to a significant (50%) increase of G_{Cl} with respect to age-matched untreated rats (Table 1). In addition, the high value of G_K observed in muscles of untreated aged rats, was not found in the GH treated group where G_K was completely restored to the adult value (Table 1), as has previously been observed after 6–8 weeks of treatment (De Luca *et al*., 1994a).

Excitability characteristics In parallel with the significant restoration of G_{Cl}, the 4-month treatment with GH improved the excitability characteristics of aged muscle fibres related to this parameter (Table 2). The threshold current to elicit the first action potential, a parameter significantly reduced in the untreated aged rats, was instead maintained close to the adult value by the GH treatment. The prolongation of the latency of the action potential observed in untreated aged rats was not found in GH treated aged rats that showed a latency value close to the adult one. Also the other excitability parameters affected the ageing process but not strictly related to G_{Cl} were ameliorated by the GH treatment (Table 2). In fact the am-

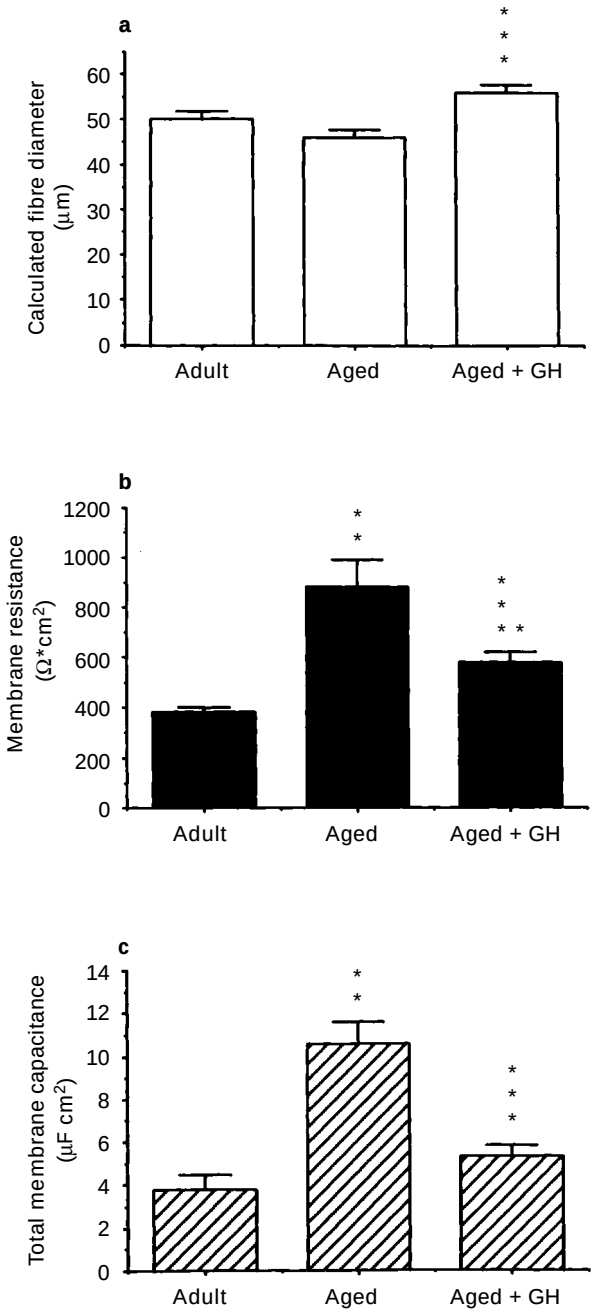

Figure 1 (a) Calculated diameter (d_{calc}), (b) membrane resistance (Rm) and (c) membrane capacitance (Cm) of extensor digitorum longus muscle fibres of 6–9 month-old rats (Adult), 24–26 month-old rats (Aged) and 24–26 month-old rats that in the preceeding 4 months received a daily treatment with 150 $\mu g\ kg^{-1}$ recombinant human growth hormone (Aged + GH). Each column is the mean ± s.e.mean of 26–73 fibres. Statistical evaluation by analysis of variance as follows: $^{**}P<0.005$ between adult and aged rats (dF = 1/92; $F = 12.8$ for Rm and $F = 15.9$ for Cm). $^{***}P<0.005$ between aged and GH-treated aged rats (dF = 1/139; $F = 110.9$ for d_{calc}; $F = -52.4$ for Rm; $F = 21.9$ for Cm). $^{*}P<0.025$ between adult and GH-treated aged rats (dF = 1/97; $F = 7.05$. No significant differences were found for d_{calc} and Cm with $F = 1.72$ and $F = 2.82$, respectively).

Table 1 Effect of a 4-month daily treatment with growth hormone to aged rats on resting ionic conductances of extensor digitorum longus muscle fibres

Experimental conditions	n	G*m* (μS cm^{-2})	n′	G_{Cl} (μS cm^{-2})	G_K (μS cm^{-2})
Adult	26	2808 ± 136	33	2492 ± 93	316 ± 31
Aged	68	1720 ± 100	53	1258 ± 97	462 ± 30
Aged + GH	73	2292 ± 124	61	1949 ± 122	343 ± 24

The columns from left to right are as follows: experimental conditions; the electrophysiological recordings were performed *in vitro* on extensor digitorum longus muscles removed from adult, aged and GH-treated aged (Aged + GH) rats, as detailed in the Methods. *n*: number of fibres sampled for the total membrane conductance (*G*m) and *n*′: number of fibres sampled for chloride (G_{Cl}) and potassium (G_K) conductances. Statistical comparison between experimental groups by analysis of variance as follows: Untreated aged vs adult rats: $P<0.005$ for G_m ($F=34.8$, dF = 1/92), G_{Cl} ($F=57.09$) and G_K ($F=10.15$, dF = 1/84); GH treated aged vs age-matched untreated rats: $P<0.005$ for *G*m($F=-96.3$; dF = 1/139), G_{Cl} ($F=25.04$) and G_K ($F=9.42$; dF = 1/112); GH treated aged vs adult rats: $P<0.025$ for *G*m ($F=5.32$; dF = 1/97) and G_{Cl} ($F=5.61$, dF = 1/92). No significant differences for G_K ($F=0.471$) were found.

Table 2 Effect of a 4-month daily treatment with growth hormone to aged rats on resting membrane potential and excitability characteristics of extensor digitorum longus muscle fibres

Experimental conditions	n	*RMP* (mV)	n′	*Ith* (nA)	*AP* (mV)	*Lat* (ms)	*N spikes*
Adult	28	−74.7 ± 1.4	10	110 ± 9	88.5 ± 2.3	6.5 ± 0.5	5.0 ± 0.2
Aged	68	−73.8 ± 0.8	14	48 ± 13	70.7 ± 2.8	13 ± 2.3	1.4 ± 0.2
Aged + GH	73	−73.5 ± 0.9	23	107 ± 18	80.5 ± 3.2	7.7 ± 1.3	3.1 ± 0.6

The columns from left to right are as follows: Experimental conditions; the electrophysiological recordings were performed *in vitro* on extensor digitorum longus muscles removed from adult, aged and GH-treated aged (Aged + GH) rats, as detailed in the Methods. *n*: number of fibres sampled for the resting membrane potential (RMP) and *n*′: number of fibres sampled for threshold current (Ith), amplitude (AP) and latency (Lat) of the action potential, and membrane firing capability (N spikes). Statistical comparison between experimental groups by analysis of variance as follows: Untreated aged vs adult rats: $P<0.005$ for Ith ($F=13.1$, dF = 1/22), AP ($F=21.3$) and N spikes ($F=51.1$); $P<0.025$ for Lat ($F=6.23$). GH (treated) aged vs age-matched (untreated) rats: $P<0.05$ for Ith ($F=5.53$; dF = 1/35), AP ($F=4.48$) and Lat ($F=5.57$); $P<0.025$ for N spikes ($F=6.60$). No significant differences were observed between adult and GH-treated aged rats or between the three groups for the RMP values.

plitude of the action potential was significantly decreased in aged muscles, but significantly restored after the GH treatment. The untreated aged muscles showed a serious impairment in the firing capability of the membrane, as few fibres were able to generate more than one action potential. A partial but significant restoration of the firing capability towards the adult value was observed in the GH-treated aged muscles. The resting membrane potential values (RMP) were not significantly different between the three experimental groups.

Pharmacological characterization of skeletal muscle chloride channels in GH-treated aged rats

As shown previously (De Luca *et al*., 1992a,b), **S**-(−)-CPP produced a concentration-dependent block of G_{Cl} in adult EDL muscle with an EC_{50} of 14.6 μM, but it was less effective in untreated aged rats where it decreased G_{Cl} with an EC_{50} of 64.8 μM. On the other hand, in the aged rats treated with GH, in parallel with the significant amelioration of G_{Cl}, we found a chloride channel sensitivity to the **S**-(−)-CPP similar to that of adult, the calculated EC_{50} being 21.6 μM (Figure 2). Similar results were obtained with the **R**-(+)-isomer of CPP. This compound produced the typical biphasic effect on G_{Cl} of adult muscle fibres; indeed at the concentration of 3 μM it produced an increase of G_{Cl}, whereas a higher concentration such as 500 μM, produced a decrease of this parameter (Figure 3). As shown previously (De Luca *et al*., 1992a), the biphasic effect was missing in the untreated aged rats (Figure 3). In contrast, the typical biphasic effect observed in the adult rats, was again detectable in the aged rats treated with GH (Figure 3).

Effects of in vitro *application of IGF-I on chloride conductance of adult and aged skeletal muscle fibres*

Plasma levels of IGF-I determined by RIA were significantly lower in aged rats than in adult ones. The amount of free IGF-I (determined at the beginning of the treatment) was 336.3 ± 21 ng ml^{-1} in aged ($n=10$) and 694.4 ± 43 ng ml^{-1} ($n=5$) in adult rats ($P<0.001$ by unpaired Student's *t* test). *In vitro* application of IGF-I (3.3 nM) on EDL muscle of adult rats did not produce significant changes of either G_{Cl} (Figure 4) or G_K (data not shown). However, in untreated aged rat muscles, IGF-I significantly increased G_{Cl} by 70% up to a value overlapping that of normal adults. This effect was specific for G_{Cl}; IGF-I was without effect on G_K of untreated aged rats, this parameter being 447 ± 42 μS cm^{-2} ($n=14$) and 492 ± 59 μS cm^{-2} ($n=18$) before and after the application of IGF-I, respectively. Also no effects were observed on d_{calc} and *C*m upon *in vitro* application of IGF-I to aged muscle. The effect of IGF-I appeared after just 10 min of incubation and the increased G_{Cl} remained even after the removal of the peptide from the bathing solution (Figure 4). To evaluate if IGF-I could increase G_{Cl} by acting on the phosphorylation state of the channel, we tested its effects in the presence of the phosphatase-inhibitor okadaic acid (0.5 μM). The *in vitro* application of okadaic acid on muscle of untreated aged rats produced a significant 20% decrease of G_{Cl} and this pre-incubation completely antagonized the effect of IGF-I on G_{Cl} (Figure 4).

Discussion

The present results show that the efficacy of GH treatment on skeletal muscle function of aged rats, as measured by membrane electrical parameters, is maintained and even enhanced by prolongation of the time of treatment. *R*m, *C*m and G_{Cl}, as well as the excitability characteristics related to chloride and sodium channel function, were significantly more ameliorated after 4 months of treatment than after 6–8 weeks (De Luca *et al*., 1994a). A time-dependent effect of GH therapy on the recovery of muscle strength has been observed in GH-deficient adult humans (Beshyan *et al*., 1995) and Rudman *et al*. (1990) found neither GH resistance nor tachyphylaxis upon hormone-

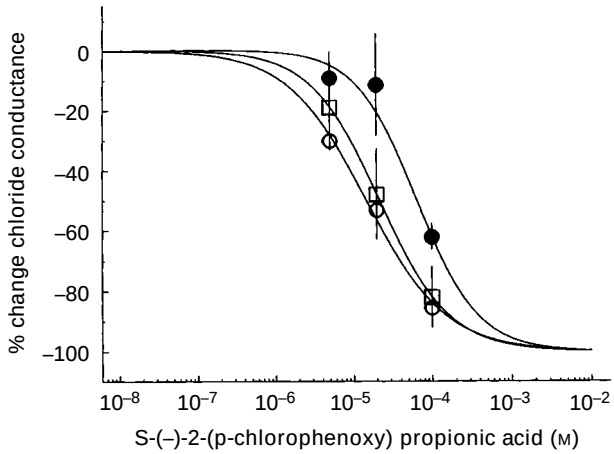

Figure 2 Effects of **S**-(−) enantiomer of 2-(p-chlorophenoxy) propionic acid (CPP) on membrane chloride conductance (G_{Cl}) of extensor digitorum longus muscle fibres of adult (○), untreated aged (●) and GH-treated aged rats (□). The mean values of G_{Cl} obtained at each concentration of **S**-(−)-CPP (from 15–33 fibres from 3–4 preparations for each group of rats) have been normalized to the group-related mean values of G_{Cl} (from 31–80 fibres) recorded in the absence of drug. Thus each point is the normalized % change of G_{Cl} in the presence of drug (from 21–64 normalized values); vertical lines show s.e.mean. The fit of the data to the logistic equation described in Methods led to the calculation of the fitting parameters K (EC_{50}) and n (slope of the concentration-response curve) as follows: $K = 14.6$ μM and n = 0.86 for adult (goodness-of-fit = 0.2), $K = 64.8$ μM and n = 1.13 for aged (goodness-of-fit = 0.47) and $K = 21.6$ μM and n = 0.99 for aged GH-treated (goodness-of-fit = 0.99).

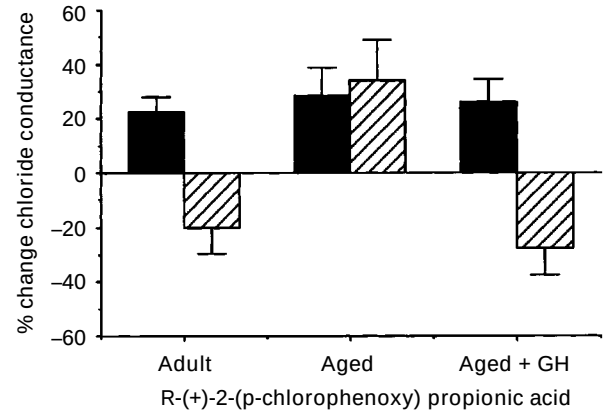

Figure 3 Effects of 3 μM (solid columns) and 500 μM (cross-hatched columns) **R**-(+)-2-(p-chlorophenoxy) propionic acid (CPP) on membrane chloride conductance (G_{Cl}) of extensor digitorum longus muscle fibres of adult, aged and GH-treated aged rats. The mean values of G_{Cl} obtained at each concentration of **R**-(+)-CPP (18–31 fibres from 3 preparations for each group of rats) have been normalized to the group-related mean values of G_{Cl} (from 32–49 fibres) recorded in the absence of drug. Thus each point is the normalized % change of $G_{Cl} \pm$ s.e. in the presence of drug (from 27–78 normalized values).

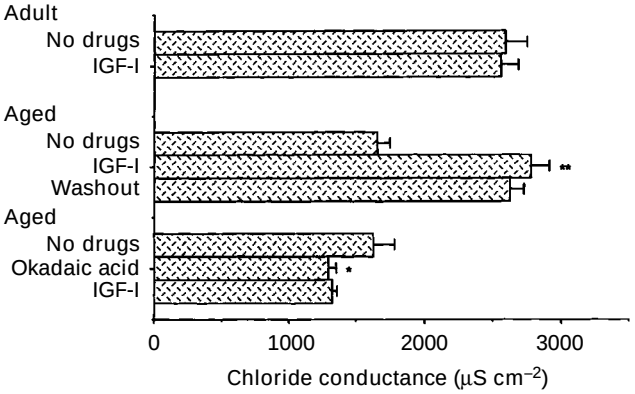

Figure 4 Effects of *in vitro* application of 3.3 nM insulin-like growth factor-I (IGF-I) on membrane chloride conductance (G_{Cl}) of extensor digitorum longus muscle fibres of adult and untreated aged rats. Each group of columns shows the following experimental conditions: G_{Cl} of adult rats in the absence and presence of IGF-I; G_{Cl} of aged rats in the absence and presence of IGF-I and after washout; G_{Cl} of aged rats in the absence and presence of 0.5 μM okadaic acid and effect of IGF-I application. Each column is the mean ± s.e.mean G_{Cl} of 10–44 fibres from 2–3 preparations for each experimental condition. Significantly different from age-matched G_{Cl} value in the absence of drug (by unpaired Student's t test) $^{**}P < 0.001$ and $^{*}P < 0.05$.

replacement therapy in aged subjects. These findings suggest an increased sensitivity to GH when the hormonal secretion is impaired (Cocchi, 1992; Ho & Hoffman, 1993). In support of this, we found that the plasma level of IGF-I, an indicator of GH secretion, was lower in untreated aged rats than in the adult group. Thus the use of doses of GH near to the physiological levels can be important for the long term efficacy of pharmacological treatments with the hormone.

We found that in the GH-treated rats, the amelioration of G_{Cl} was accompanied by a complete restoration of the pharmacological response to the enantiomers of CPP, specific muscle chloride channel ligands (De Luca *et al*., 1992a,b). The change in the pharmacological profile of the G_{Cl} of aged muscle fibres may be due to the presence of different isoforms of chloride channels (De Luca *et al*., 1992a). A likely hypothesis is that GH fully re-establishes the function of the chloride channels through an *ex-novo* synthesis of the adult isoform. This mechanism can be mediated by IGF-I whose expression in both skeletal muscle and liver is directly proportional to the duration of GH treatment (Turner *et al*., 1988; Gosteli-Peter *et al*., 1994). IGF-I exerts long-term effects on skeletal muscle properties through the synthesis of myogenin, a promoter of muscle specific protein genes (Florini *et al*., 1991; Cohick & Clemmons, 1993). The gene for ClC-1, the muscle chloride channel, belongs to this family and shows potential sequence motifs for binding MyoD and myogenin (Klocke *et al*., 1994). The hypothesis that IGF-I may contribute to the synthesis of ClC-1 channels is supported by observations in muscle of postnatally developing animals. During the first month of rat postnatal life, there is the highest density of muscle IGF-I receptors, and in parallel an increase of mRNA for ClC-1 and of G_{Cl} has been observed (Alexandrides *et al*., 1991; Steinmeyer *et al*., 1991; De Luca *et al*., 1992a). This process is accompanied by a gradual gain of the adult-like pharmacological responses of G_{Cl} to the enantiomers of CPP (De Luca *et al*., 1992b).

Furthermore, we found that *in vitro* application of IGF-I, at concentrations able to activate IGF-I receptor specifically (Florini *et al*., 1991; Cohick & Clemmons, 1993), produced a rapid increase of G_{Cl} in EDL muscle of untreated aged rats, but not in adult rats. IGF-I did not affect either G_K or other cable parameters, suggesting that these latter parameters require a long-term effect of GH/IGF-I stimuli on muscle metabolism and morphology. For example, we proposed that C_m increases upon ageing for a compensatory proliferation of the T-tubular system (De Luca *et al*., 1994a), a process that cannot be rapidly counteracted by the *in vitro* application of the somatomedin. Thus, chloride channels of skeletal muscle of aged animals are a specific target of IGF-I, and this is the first evidence of a channel function acutely modified by the somatomedin. The effect of IGF-I was washout-resistant; this rules out any direct action on the kinetic and/or the conductance of the channels. We previously proposed that an overactivity of a Ca^{2+}- and phospholipids-dependent protein kinase C (PKC) can contribute to the decrease of G_{Cl} in aged muscle by maintaining the

chloride channels in non- or low-conductive phosphorylated states (De Luca *et al*., 1994b). The acute effect of IGF-I on G_{Cl} was prevented by preincubation with okadaic acid, thus the biochemical cascade following the activation of the IGF-I receptor may involve an okadaic-acid-sensitive serine-threonine phosphatase able to counteract the PKC-induced phosphorylation of the channel (Cohen *et al*., 1990; Csermely *et al*., 1993). The activity of a phosphatase can be physiologically required to maintain the channel in a conductive state, as okadaic acid *per se* was able to decrease G_{Cl} further.

Further investigations are required to evaluate the role of the acute effect of IGF-I in the amelioration of G_{Cl} observed upon the chronic GH treatment.

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