

Chronic Administration of Taurine to Aged Rats Improves the Electrical and Contractile Properties of Skeletal Muscle Fibers¹

SABATA PIERNO, ANNAMARIA DE LUCA, CLAUDIA CAMERINO, RYAN J. HUXTABLE and DIANA CONTE CAMERINO

Unit of Pharmacology, Department of Pharmacobiology (S.P., A.D.L., D.C.C.), Faculty of Pharmacy, University of Bari, Bari, Italy and Department of Pharmacology (C.C., R.J.H.), College of Medicine, University of Arizona, Tucson, Arizona

Accepted for publication May 8, 1998 This paper is available online at <http://www.jpvet.org>

ABSTRACT

A reduction of resting chloride conductance (GCl) and a decrease of the voltage threshold for contraction are observed during aging in rat skeletal muscle. The above alterations are also observed in muscle of adult rat after taurine depletion. As lower levels of taurine were found by others in aged rats compared to young rats, we tested the hypothesis that a depletion of taurine may contribute to the alteration of the electrical and contractile properties we found in skeletal muscle during aging. This was accomplished by evaluating the potential benefit of a pharmacological treatment with the amino acid. To this aim 25-mo-old Wistar rats were chronically treated (2–3 mo) with taurine (1 g/kg p.o. daily) and the effects of such a treatment were evaluated *in vitro* on the passive and active membrane electrical properties of extensor digitorum longus muscle fibers by means of current-clamp intracellular microelectrode technique. Excitation-contraction coupling was also evaluated by measuring the voltage threshold for contraction with the intracellular microelectrode "point" voltage clamp method. In parallel muscle and blood taurine contents were determined by high-performance liquid chromatography. Taurine supplementa-

tion significantly raised taurine content in muscle toward that found in adult rats. Supplementation also significantly increased GCl vs. the adult value, in parallel the excitability characteristics (threshold current and latency) related to this parameter were ameliorated. The increase of GCl induced by taurine was accompanied by a restoration of the pharmacological sensitivity to the R(+) enantiomer of 2-(p-chlorophenoxy) propionic acid, a specific chloride channel ligand. In parallel also the protein kinase C-mediated modulation of the channel was restored; in fact the potency of 4- β -phorbol 12,13-dibutyrate in reducing GCl was lower in taurine-treated muscles vs. untreated aged, being rather similar to that observed in adult. The treatment also improved the mechanical threshold for contraction of striated fibers which in aged rats is shifted toward more negative potentials, moving it toward the adult values. Our results suggest that the reduction of taurine content could play a role in the alteration of electrical and contractile properties observed during aging. These findings may indicate a potential application of taurine in ensuring normal muscle function in the elderly.

Taurine is a sulfonic amino acid ubiquitously and abundantly distributed in tissues of numerous animal species. The high concentrations parallel its involvement in many physiological processes such as osmoregulation, calcium mobilization and antioxidant action (Huxtable, 1992). Taurine is essential for normal development and proper function of the excitable tissues of mammals (Huxtable, 1992; Pion *et al.*, 1987; Sturman, 1993). As far as skeletal muscle is concerned, taurine plays a fundamental role in the electrical stabilization of cell membrane (Conte Camerino *et al.*, 1987). In fact *in vitro* application of taurine on skeletal muscle fibers reduces

fiber excitability by specifically increasing GCl (Conte Camerino *et al.*, 1987), the parameter that mostly contributes to the electrical stability of sarcolemma (Lehmann-Horn and Rüdél, 1995). We have proposed that this effect is mediated by the interaction with a low affinity site controlling chloride channels, because taurine analogs are less effective on GCl (Pierno *et al.*, 1994).

The physiological role of taurine in skeletal muscle has been more clearly demonstrated by the alterations of the electrical and contractile properties consequent to an experimentally produced taurine deficiency. Chronic administration of GES, a competitive inhibitor of taurine transporter (Huxtable, 1992), causes a fall in muscular taurine content and produces a marked decrease of GCl along with an increase of excitability of rat skeletal muscle fibers (De Luca *et*

Received for publication June 16, 1998.

¹This work has been supported by Grants CII*-CT 94-0037 from the CEE. C.C. has been partially supported by Taisho Pharmaceutical Co. (RH).

ABBREVIATIONS: EDL, extensor digitorum longus; Rm, membrane resistance; Gm, total membrane conductance; GCl, resting chloride conductance; GK, resting potassium conductance; AP, action potential amplitude; Ith, threshold current; Lat, latency of the action potential; PKC, protein kinase C; 4- β -PDB, 4- β -phorbol 12,13-dibutyrate; GES, guanidinoethane sulfonate; CPP, 2-p-(chlorophenoxy) propionic acid; DMSO, dimethylsulfoxide; HPLC, high-performance liquid chromatography.

al., 1996a). Furthermore the excitation-contraction (e-c) coupling process of taurine-depleted muscles is significantly changed, the contraction occurring at more negative potentials with respect to normal controls (De Luca *et al.*, 1996a). We have demonstrated that the alteration of mechanical threshold in taurine-depleted muscle is not caused by the decrease of GCl, leading to hypothesize that taurine controls this function through other mechanisms (De Luca *et al.*, 1996a). For instance Huxtable and Bressler (1973) found that taurine enhances the rate of Ca^{++} and the total Ca^{++} sequestering capacity of sarcoplasmic reticulum isolated from rat skeletal muscle. This observation allowed us to propose a possible impairment of this process in taurine-depleted muscle that could result in an increase of cytosolic Ca^{++} responsible for the alteration observed in the l-c coupling mechanism (De Luca *et al.*, 1996a).

Taurine content declines slowly during late adulthood and decreases further during aging. Reduced taurine levels have been found in plasma, and some other tissues (atria, kidney and caudal artery) of 30-mo-old male Fischer 344 rats with respect to those of 8-mo-old rats of the same strain (Dawson and Wallace, 1992). Several abnormalities in the morphology and function of skeletal muscle have been reported during aging (Carmeli and Reznick, 1994). Among these we have found alterations in the electrical and contractile properties closely resembling those occurring in taurine-depleted muscle. We observed a specific reduction of GCl and a shift of the mechanical threshold toward more negative potentials in striated fibers of aged rats (De Luca and Conte Camerino, 1992; De Luca *et al.*, 1992, 1994). All these findings led to us to hypothesize that a reduction of muscle taurine content could occur during aging and this can play a role in the age-related changes of skeletal muscle function. We tested this hypothesis by evaluating the changes of taurine content in blood and skeletal muscle of aged rats and the effects of an *in vivo* treatment with taurine on the ionic conductances, excitability parameters and mechanical threshold of EDL muscle of aged rats. To better evaluate the potential benefit of the pharmacological treatment with this amino acid in the aged subject and to shed light on its mechanism of action we also performed *in vitro* pharmacological characterization of GCl of taurine-treated animals with the R-(+) enantiomers of CPP. In fact the age-related decrease of GCl is accompanied by a change in its sensitivity to this specific channel ligand so that R-(+) CPP produces different effects in aged *vs.* adult animals (De Luca *et al.*, 1992, 1996b). Also, by testing the sensitivity to phorbol esters in muscles of taurine-treated aged rats we evaluated the possible effect of taurine on the PKC-mediated phosphorylation of the chloride channels. The 4- β -phorbol 12,13-dibutyrate, able to activate a Ca^{++} and phospholipid-dependent PKC, is almost 20-fold more potent in reducing GCl in aged than in adult muscles suggesting an alteration during aging of the biochemical pathways modulating channel function (De Luca *et al.*, 1994). Recent studies have shown that taurine, by reducing cytosolic Ca^{++} levels and by inhibiting phosphoinositide turnover may inhibit PKC-catalyzed phosphorylation processes in rat brain (Li and Lombardini, 1991). For comparison the effects of taurine treatment were also evaluated on the adult (6-mo-old) rats.

Materials and Methods

Animals and Taurine Administration

Ten adult (6-mo-old) and 18 older (22-mo-old at the beginning of the experimental period) male Wistar rats of 400 to 500 g (Morini Laboratories, S. Polo D'Enza, Italy) were used for all experiments. The animals were maintained one per cage, with free access to food (Charles River, Calco, Italy, 4RF21), at a constant room temperature (20–22°C) and exposed to a light cycle of 12 hr/day (8.00 A.M.–8.00 P.M.) throughout the course of the experiments. Rats were subdivided in four groups: taurine-treated adult ($n = 5$) and taurine-treated aged ($n = 9$) rats receiving 1 g/kg taurine/day (Teofarma, Pavia, Italy); untreated adult ($n = 5$) and untreated aged ($n = 9$) rats receiving tap water were used as control. Taurine, dissolved in drinking water (2%) with sucrose (2%) was administered to the rats until they drunk the daily dose of 1 g/kg, contained in a volume of 20 to 25 ml. For the rest of the day they had free access to taurine-free drinking water. This treatment lasted 2 to 3 mo. Control rats also received sucrose (2%).

Tissue Preparation and HPLC Analysis

Trunk blood was collected in centrifuge tubes rinsed with 10 μl of ethylenediaminetetraacetic acid (150 mM). Part of blood was stored at -80°C until assay for taurine determination (Huxtable, 1992), and the other fraction was used for measuring glucose by hexokinase enzymatic method (Bondar and Mead, 1974). Tibialis anterior muscles were removed, washed in physiological solution, dried, weighed and homogenized with 10 ml of HClO_4 (0.4 N) per g tissue. The homogenized muscles were buffered with 80 μl K_2CO_3 (5.5 g/10 ml) for each ml of HClO_4 used. The homogenates were centrifuged at $600 \times g$ for 10 min at 4°C . The supernatants were stored at -80°C until assay. Derivatization with *o*-phthalaldehyde was performed as previously described and samples were processed for HPLC taurine determination (Lleu and Huxtable, 1992).

Electrophysiological Experiments

Measurements of cable parameters, ionic conductances and excitability characteristics. Electrophysiological measurements were made *in vitro* at the end of the treatment period. The EDL muscles of both hindlimb were dissected under urethane anesthesia (1.2 g/kg, i.p.). Soon after the removal of the muscles the rats, still anesthetized, were killed by further i.p. injection of a urethane overdose. The muscles were placed in 25 ml muscle bath, maintained at 30°C and perfused with normal or chloride-free physiological solutions (Bryant and Conte Camerino, 1991) as detailed below. The membrane properties were obtained with the two intracellular microelectrode current clamp method in which a hyperpolarizing square-wave current pulse is passed through one electrode and the membrane voltage response is monitored at two distances from the current electrode (Bryant and Conte Camerino, 1991). The current pulse generation, the acquisition of the voltage records and the calculation of the fiber constants were done in real time under computer control as described in detail elsewhere (Bryant and Conte Camerino, 1991). From the experimentally determined values of input resistance, space constant and time constant and from an assumed myoplasmic resistivity (R_i) of $125 \Omega \times \text{cm}$ for both adult and aged fibers in agreement with previous studies (Boyd and Martin, 1959; De Luca *et al.*, 1992), calculated fiber diameter (dcalc), membrane resistance (R_m) and membrane capacitance (C_m) were then calculated (Bryant and Conte Camerino, 1991). The reciprocal of R_m from each fiber in normal physiological solution was assumed to be total membrane conductance (G_m), and the same parameter measured in chloride-free solution was considered to be potassium conductance (G_K). The mean chloride conductance (GCl) was calculated as the mean G_m minus the mean G_K . The excitability characteristics of the sampled fibers were determined by recording the intracellular membrane potential response to square-wave depolarizing constant

current pulses. In each fiber the membrane potential was set by a steady holding current to -80 mV, before passing the depolarizing pulse (Pierno *et al.*, 1994).

Measurements of mechanical threshold. The mechanical threshold of the fibers was determined using a two microelectrode "point" voltage clamp method as previously described (Dulhunty, 1988; Heiny *et al.*, 1990; De Luca and Conte Camerino, 1992). In brief, a voltage-sensing electrode (3 M KCl) and a current-passing electrode (2 M potassium citrate) were inserted within $50 \mu\text{m}$ of each other into the central region of a randomly selected superficial fiber that was continuously viewed using a stereomicroscope ($100\times$ magnification). The holding potential was set at -90 mV and depolarizing command pulses of variable duration were given at a rate of about 0.3 Hz. Tetrodotoxin ($3 \mu\text{M}$) was continuously present during recordings to prevent action potential generation (Dulhunty, 1988; Heiny *et al.*, 1990; De Luca and Conte Camerino, 1992). As a standard protocol the command-pulse duration was usually set sequentially to each of the following values: 500, 50, 5, 200, 20, 100 and 10 msec. At each duration, the command voltage was increased using an analogue control until contraction was just visible, and then backed down until the contraction just disappeared. A digital sample-and-hold millivoltmeter stored the value of the threshold membrane potential at this point. We estimated the uncertainty of any single measurement for a given fiber to be 1 to 2 mV. Particular care was taken to perform the measurements in any experimental condition in an identical fashion, with about the same length of time involved in each determination so as to exclude any effect on the mechanical threshold of intracellular citrate ions from the electrodes (Dulhunty, 1988). The threshold membrane potential V (mV) for each fiber was averaged at each pulse duration t and then the mean values plotted against duration gave us a "strength-duration" relationship. A fit estimate of the rheobase voltage (R) and of the time constant to reach the rheobase was obtained by a nonlinear least square algorithm using the following equation:

$$V = [H - R \exp(t/\tau)] / [1 - \exp(t/\tau)]$$

where H is the holding potential (mV), R is the rheobase (mV) and τ is the time constant (msec) (Miledi *et al.*, 1983; De Luca and Conte Camerino, 1992). In the fitting algorithm, each point was weighed by the reciprocal of the variance of that mean V and the best fit estimates of the parameters R and τ were made. The mechanical threshold values are expressed as the fitted rheobase (R) parameter \pm S.E. which was determined from the variance-covariance matrix in the nonlinear least square fitting algorithm.

Solutions and Drugs

The normal physiological solution had the following composition (in mM): NaCl 148; KCl 4.5; CaCl₂ 2.0; MgCl₂ 1.0; NaHCO₃ 12.0; NaH₂PO₄ 0.44 and glucose 5.55. The chloride-free solution was prepared by equimolar replacement of methylsulfate salts for NaCl and KCl and nitrate salts for CaCl₂ and MgCl₂. Both solutions were continuously gassed with 95% O₂ and 5% CO₂ (Bryant and Conte Camerino, 1991). To suppress spontaneous contraction of muscle preparations $1 \mu\text{M}$ tetrodotoxin was added to the chloride-free solutions. The pH of all the solutions used was carefully maintained between 7.2 to 7.3 during each experiment. To test the effects of R-(+)-2-p-(chlorophenoxy) propionic acid (R-(+)-CPP), stock solutions were prepared in 1% sodium bicarbonate solution. The final concentration was obtained by further dilution in normal physiological solution (De Luca *et al.*, 1992). 4- β -phorbol 12,13-dibutyrate (4- β -PDB; Sigma Chemical Co., St. Louis, MO), was dissolved in DMSO to produce concentrated stock solutions, to be added in microliter amounts to the bath solutions, as needed. A 0.5% DMSO solution, much stronger than the maximum DMSO concentration used (0.04%), was without effect on the parameters studied (De Luca *et al.*, 1994). The 4- β -PDB was tested at different concentrations (from 3 to 50 nM), no more than three doses being tested in each

preparation. The time of incubation of PDB was varied from 90 min (3 nM) to 30 min (50 nM) so as to reach a steady-state of drug effect (De Luca *et al.*, 1994).

Statistical Analysis

The concentrations of taurine in blood and muscle are expressed as mean \pm S.E.M. from N number of animals. The electrophysiological data are expressed as mean \pm S.E.M. from n fibers of N EDL muscle preparations. The estimates for S.E.M. and N of GCl were obtained from the variance and from the number of fibers sampled for Gm and GK as described by Green and Margerison (1978). Significance between groups of means was evaluated by Student's t test. The IC₅₀ values for phorbol esters were estimated by fitting the logistic function to data points, as described in detail elsewhere (De Luca *et al.*, 1994). The statistical significance between the fitted values of rheobase was estimated by a Student's t distribution, using a number of degrees of freedom equal to the total number of threshold values determining the curves minus the number of means minus two for the free parameters (De Luca *et al.*, 1996a). Statistical differences between untreated aged and taurine-treated aged rats groups were also evaluated for significance using analysis of variance.

Results

General observations. Taurine treatment did not modify food consumption or body weight gain either in aged or adult animals. The 18 older rats used for the experiments were in good health with no impairment of hind limb movements or locomotor activity and no pathological sign was observed in any group of rats throughout the period of study. No mortality was observed in both taurine-treated and -untreated aged rats. However, blood glucose was found to be increased by 155 and 83% in untreated aged and taurine-treated aged rats, respectively, with respect to the adult group. The insulin-like action of taurine (Huxtable, 1992) may account for the reduced increase in blood glucose found in taurine-treated aged rats.

Plasma and skeletal muscle taurine content in adult and aged rats before and after chronic administration of taurine. As shown in table 1 tissue taurine content (determined by HPLC) was significantly lowered by 25% in the tibialis anterior muscle of untreated aged rats with respect to the untreated adult rats. In aged rats taurine administration significantly raised the muscular levels of the amino acid to the values found in the adult rat muscles. In contrast taurine treatment did not modify the taurine content in the muscle of adult rats. Similar taurine blood levels were found in both untreated adult and aged rats. The administration of taurine significantly increased the blood levels of taurine in the aged rats.

Effects of taurine chronic administration on the membrane ionic conductances and excitability parameters of muscle fibers from adult and aged rats. In agreement with previous findings (De Luca *et al.*, 1992; 1994) Rm of EDL muscle fibers was significantly higher in aged with respect to adult rats ($395 \pm 16 \Omega \times \text{cm}^2$, $n = 106$ and $335 \pm 8.5 \Omega \times \text{cm}^2$, $n = 74$, respectively, $P < .005$). The increase in Rm reflected a significant decrease of Gm that was almost completely attributable to a 16% decrease of GCl found in the aged rats (table 2). The GK was slightly higher in the aged with respect to the adult rats, as frequently occurs during aging (table 2) (De Luca *et al.*, 1994). Daily chronic treatment with 1 g/kg taurine produced a significant

TABLE 1
Effect of chronic taurine treatment on taurine concentration in blood and skeletal muscles of adult and aged rats

Tissue	Taurine Concentration			
	Adult	Taurine-treated adult	Aged	Taurine-treated aged
Muscle	2.63 ± 0.12 (4)	2.61 ± 0.19 (4)	1.95 ± 0.12 ^a (8)	2.41 ± 0.13 ^b (9)
Blood	0.36 ± 0.06 (4)	0.66 ± 0.09 (4)	0.39 ± 0.07 (5)	0.86 ± 0.05 ^b (5)

The values indicate the content of taurine in blood and tibialis anterior muscle from 6-mo-old adult untreated rats (adult), 6-mo-old adult rats orally administered with 1 g/kg/day taurine for 2 mo (taurine-treated adult), 24-mo-old aged untreated rats (aged) and 24-mo-old aged rats orally administered 1 g/kg/day taurine for 2 mo (taurine-treated aged). Taurine concentration was determined by HPLC analysis and expressed as mean ± S.E.M. for the number of animals given in parentheses.

Significantly different by Student's *t* test with respect to ^a adult (*P* < .01) and ^b aged (*P* < .025 for muscle; *P* < .001 for blood) values.

TABLE 2
Effects of chronic taurine treatment on membrane ionic conductances and excitability characteristics of extensor digitorum longus muscle fibers of adult and aged rats

Experimental Conditions	No. of Animals	<i>N</i>	GCl (μS/cm ²)	<i>N</i> 1	GK (μS/cm ²)	<i>N</i> 2	RP (-mV)	AP (mV)	Ith (nA)	Lat (msec)	<i>N</i> Spikes
Mean of Adult rats	5	74	2751 ± 69	30	373 ± 30	17	72 ± 2	102 ± 7	129 ± 6	5.3 ± 0.1	3.4 ± 0.5
Mean of taurine-treated Adult rats	5	60	3036 ± 71 ^a	37	313 ± 30	33	75 ± 1	106 ± 3	168 ± 8 ^a	5.5 ± 0.4	2.1 ± 0.2 ^a
Mean of aged rats	7	106	2380 ± 58 ^a	66	407 ± 19	25	71 ± 1	97 ± 4	75.3 ± 5 ^a	13.7 ± 0.8 ^a	2.1 ± 0.3 ^a
Taurine-treated rat	1	17	2690 ± 107 ^b	18	438 ± 48	6	71 ± 3	92.0 ± 1.4	128 ± 16 ^b	8.0 ± 0.9 ^b	3.3 ± 0.7
Taurine-treated rat	1	20	2763 ± 142 ^b	8	387 ± 49	6	77 ± 2	96.0 ± 4.4	123 ± 26 ^b	5.5 ± 0.7 ^b	3.4 ± 1.0
Taurine-treated rat	1	15	2326 ± 152	10	624 ± 53 ^b	5	74 ± 2	96.6 ± 8.0	50 ± 10 ^b	9.0 ± 0.3 ^b	1.2 ± 0.6
Taurine-treated rat	1	18	2899 ± 105 ^b	14	396 ± 25	6	74 ± 1	99.8 ± 5.1	152 ± 12 ^b	6.7 ± 0.5 ^b	3.0 ± 0.5
Taurine-treated rat	1	18	2687 ± 98 ^b	17	356 ± 19						
Taurine-treated rat	1	7	3499 ± 70 ^b	12	472 ± 49	4	75 ± 1	106 ± 3.0	240 ± 10 ^b	6.5 ± 0.8 ^b	2.0 ± 0.4
Taurine-treated rat	1	17	2901 ± 153 ^b	10	428 ± 46	6	72 ± 3	101 ± 4.7	179 ± 36 ^b	4.9 ± 0.4 ^b	1.1 ± 0.1
Mean of taurine-treated aged rats	7	112	2759 ± 50 ^b	89	435 ± 17	33	74 ± 1	99 ± 2.3	151 ± 13 ^b	6.5 ± 0.3 ^b	2.5 ± 0.3

The columns from left to right are as follows: Experimental conditions and number of animals from which the electrophysiological parameters have been recorded. Single muscle preparation of taurine-treated aged are also given. *N* and *N*1 are the number of fibers sampled for GCl, resting chloride conductance and GK, resting potassium conductance, respectively and *N*2 is the number of fibers for each of the following parameter: RP, membrane resting potential; AP, amplitude of the action potential; Ith, threshold current; Lat, latency of the action potential; *N* spikes is the maximal number of action potentials elicitable by raising the intensity of a long-duration pulse. The values are expressed as mean ± S.E.M.

^a Significantly different from adults by Student's *t* test (*P* < .025 or less).

^b Significantly different from aged rats by Student's *t* test (*P* < .05 or less). The statistical analysis by analysis of variance between the untreated aged and the taurine-treated aged rats groups showed significant differences in GCl (*F* = 6.8; *dF* = 1/226; *P* < .01); Ith (*F* = 6.3; *dF* = 1/56; *P* < .025) and Lat (*F* = 18; *dF* = 1/56; *P* < .005).

decrease of *R*_m in the 24-mo-old rats with a mean value of 329 ± 7.6 Ω × cm² (*n* = 112) and a consequent restoration of GCl vs. the adult value (table 2). As shown in table 2, this effect occurred with a high incidence, indeed a significantly higher value of GCl with respect to the value found in the untreated aged rats was observed in six of seven aged treated rats studied. The seventh rat was unaffected by the treatment. The mean GK was not significantly modified by taurine treatment (table 2) and in one taurine-treated animal a significantly higher value of GK was observed. In the adult rats the taurine treatment slightly, but significantly, increased GCl by 10% with respect to the adult untreated rats, without modification of GK (table 2).

Excitability parameters were also recorded in adult and aged rats that did or did not receive the chronic treatment with taurine. The alteration of membrane excitability during aging was characterized by a decrease of the Ith necessary to elicit the first action potential and a marked increase of latency, the delay between the application of the depolarizing pulse and the onset of the action potential (Lat) (table 2). Moreover the firing capability (*N* spikes) decreased (table 2).

No significant differences were observed in the amplitude of the AP between adult and aged rats (table 2).

Taurine treatment produced in the aged rats an increase of Ith by 100%, reaching a value similar to that found in the untreated adult rats (table 2). Also the latency of the action potential was reduced by 50% to a value not significantly different from the adult one (table 2). The firing capability was increased by 19% after taurine treatment (table 2). The same treatment produced minor effects on the adult rats: Ith was increased by 30%, the latency was unchanged and the firing capability was reduced by 38% (table 2).

Effects of R-(+) enantiomer of 2-(p-chlorophenoxy) propionic acid on membrane chloride conductance of muscle fibers from untreated and taurine treated aged rats. It has been demonstrated (De Luca *et al.*, 1992) that GCl and consequently chloride channel function can be stereospecifically modulated by drugs such as the R-(+) enantiomer of CPP. As shown in figure 1 in adult rats the R-(+) CPP produced a typical biphasic effect, increasing GCl at low concentrations (3 μM) and decreasing it at higher concentrations (40–100 μM). In contrast, on four muscles from four

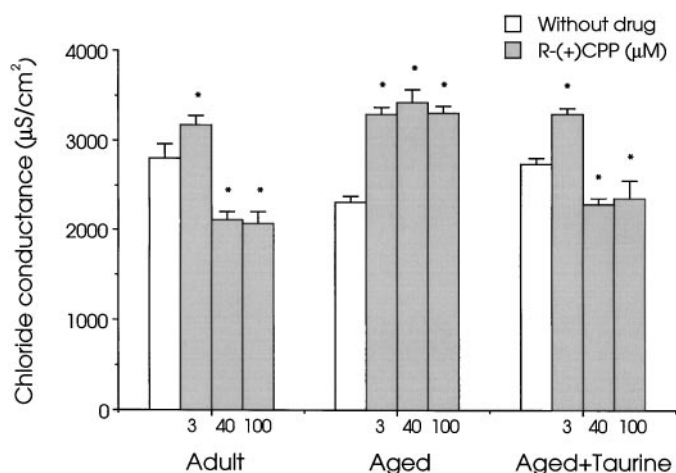


Fig. 1. Effects of *in vitro* application of the R-(+) enantiomer of 2-(p-chlorophenoxy) propionic acid (R-(+) CPP) on resting chloride conductance (GCl) of extensor digitorum longus muscle fibers of adult, aged and taurine-treated aged rats. Each bar represents the mean value of GCl obtained from 16–61 fibers of 2–4 muscle preparations. * Significantly different with respect to the related control values in the absence of R-(+) CPP (open bars; without drug) ($P < .05$ or less).

aged rats, R-(+) CPP did not produce the typical biphasic response, but increased GCl at all doses tested (fig. 1). Interestingly the *in vitro* application of R-(+)CPP on four muscles of four aged rats chronically treated with taurine restored the typical biphasic response observed in adult rats (fig. 1).

Effects of phorbol esters on membrane chloride conductance of muscle fibers of untreated and taurine treated aged rats. The *in vitro* application of 4- β -PDB, a well known PKC activator, tested in the range from 3 to 50 nM, produced a concentration-dependent block of GCl which was much greater in EDL muscle fibers from four aged rats than in those from adults (fig. 2). Indeed the concentrations required for half-maximal block of GCl (IC_{50}) were 25.6 ± 1.7 and 9.06 ± 0.44 nM in adult and aged EDL muscle, respectively. Moreover at a concentration of 50 nM, 4- β -PDB pro-

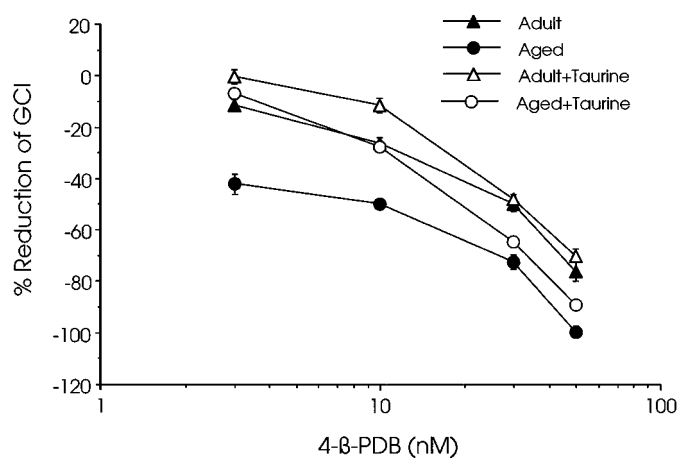


Fig. 2. Effects of *in vitro* application of 4- β -phorbol 12,13 dibutyrate (4- β -PDB) on resting chloride conductance (GCl) of extensor digitorum longus muscle fibers from untreated adult and aged rats (filled symbols) and taurine-treated adult (Adult + Taurine) and aged (Aged + Taurine) rats (open symbols). The mean values of GCl obtained at each concentration of 4- β -PDB (from 16–35 fibers of 2–4 muscles) have been normalized *vs.* the group-related mean values of GCl recorded in the absence of phorbol ester. Thus each point is the normalized percent change \pm S.E.M. of GCl in the presence of 4- β -PDB.

duced a complete block of GCl (99%) in aged muscle fibers, whereas the same concentration produced a 76% block of GCl in the adult (fig. 2). The block of GCl produced by 4- β -PDB in four taurine-treated aged rats was similar to that observed in the adult, particularly at the lower concentrations (3 and 10 nM), with an IC_{50} of 18.4 ± 0.6 nM (fig. 2). The *in vitro* application of 4- β -PDB on EDL muscle fibers from taurine-treated adult rats produced a concentration-dependent block of GCl similar to that found in the adult untreated rats, except for the two lower doses, which were less effective in blocking GCl (fig. 2). Thus, the half-maximal concentration of 4- β -PDB in taurine treated adult rats was 31 ± 1.1 nM.

Effects of taurine chronic administration on the mechanical threshold of muscle fibers of aged rats. The threshold potential for contraction of extensor digitorum longus muscle fibers from both control and taurine-treated rats showed the typical dependence on command pulse duration; *i.e.*, it was the more negative the longer the duration of the pulse. Under the experimental conditions used ($t = 30^\circ\text{C}$ and rate of about 0.3 Hz), a constant rheobase value was almost fully reached at the longest pulses used, a behavior commonly seen with mammalian muscle fibers (De Luca and Conte Camerino, 1992; De Luca *et al.*, 1996a). In line with previous results (De Luca and Conte Camerino, 1992), in our experiments the aged fibers needed significantly less depolarization to contract with respect to those of adult at each pulse duration, and the resulting strength-duration curve obtained from five aged rats was clearly shifted toward more negative potentials with respect to that of the four adult animals tested (fig. 3). The voltage at rheobase (R) estimated from the fit of the experimental points was significantly different with respect to that of the adults, although the time constant (τ) to reach the rheobase was slightly longer, although not significantly, in the aged rats compared to the adults (table 3).

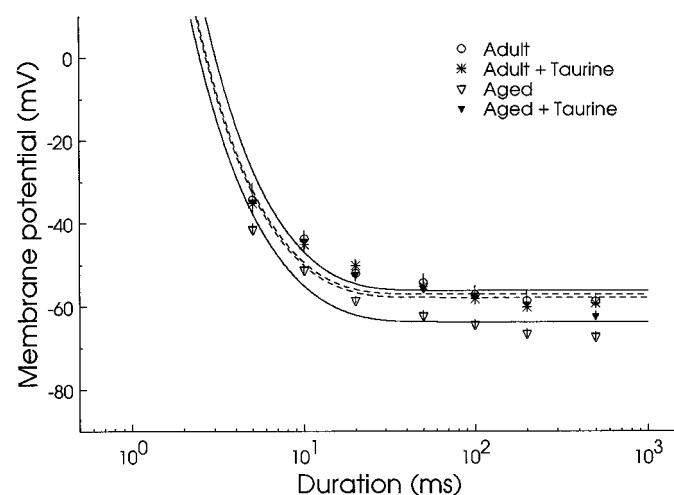


Fig. 3. Strength-duration curves for the threshold potentials for mechanical activation of extensor digitorum longus muscle fibers from untreated adult (number of animals = 4) and aged ($N = 5$) rats (adult and aged; continuous lines) and taurine-treated adult (adult + taurine; $N = 4$) and aged (aged + taurine; $N = 5$) rats (dashed lines). Each point is the mean value \pm S.E.M. of the threshold potential (in mV) recorded at each pulse duration from 18 to 48 fibers. The curves fitting the experimental points have been obtained using the equation described in "Methods." The fitting procedure allowed also to obtain the following calculated values \pm S.E. of the rheobase voltage (R) and of the time constant (τ) in each experimental condition showed in table 3.

The chronic treatment with taurine ameliorated the mechanical threshold for contraction changed by aging. Indeed the strength-duration curve constructed with the values obtained from five different animals was shifted toward that of adults (fig. 3). As it can be seen in table 3, the effect of taurine treatment was present in each of the rat tested; thus the fitted values of rheobase and τ were similar to that of the untreated adult and significantly different from that of the aged untreated rats (table 3).

The taurine treatment did not produce appreciable alterations of the mechanical threshold for contraction in the four adult rats examined. Indeed R and τ were not significantly different with respect to the related untreated controls (fig. 3; table 3).

Discussion

Previous results obtained with aged Fisher 344 rats suggested that during aging the plasma taurine level may fluctuate in relation to dietary intake and renal function, whereas tissues such as brain and heart can retain more stable taurine levels provided that the high-affinity taurine transporter works properly (Dawson and Wallace, 1992). Our experiments performed on aged Wistar rats have shown no decrease in taurine content in the blood but a significant reduction of its level in skeletal muscle with respect to adult rats. Chronic taurine administration markedly raised taurine levels in blood and produced a significant increase of the amino acid muscle content toward the adult value, showing that the tissue fall can be pharmacologically counteracted. These findings suggest that the age-related decline in muscle taurine content may be due to alterations of the transport system responsible for the uptake of taurine inside the fibers (Huxtable, 1992; Jhiang *et al.*, 1993). One possible mechanism for the reduced taurine influx during aging can be related to the biochemical modulation of its transporter. In fact recent studies in Ehrlich cells have proposed that the transport β -system accounting for taurine accumulation, is inhibited after phosphorylation on specific sites by phorbol esters-activated PKC (Mollerup and Lambert, 1996). It is notable that an overactivity of PKC can occur in skeletal muscle fibers during aging (De Luca *et al.*, 1994). A volume-

activated taurine efflux through selective channels, already described in many tissues (Kirk and Kirk, 1993; Ballatori *et al.*, 1995; Moorman *et al.*, 1995), may be also hypothesized.

Whatever the mechanism underlying the cellular decrease of taurine during aging, our results show that it plays a role in the alteration of the electrical and contractile properties observed in this situation. Interestingly from a therapeutic point of view, the chronic treatment with the amino acid, raised the muscle taurine content *vs.* the adult value, although functional parameters were ameliorated. The taurine treatment improved the e-c coupling mechanism, *i.e.*, the rheobase voltage for contraction, abnormally negative in striated fibers of aged subjects, was shifted toward the adult value. In parallel, the lower GCl found in old rats was increased toward the adult value and the excitability parameters related to GCl were similarly ameliorated. The ability of intracellular taurine level to control both the mechanical threshold and GCl of muscle fibers had been already observed by inducing a pharmacological taurine deficiency in rats (De Luca *et al.*, 1996a). The lowering of mechanical threshold could be in part related to the decrease in GCl either affecting the membrane resistance or enhancing the calcium entry during the prolonged action potential duration (Bianchi, 1992). The contribution of the action potential duration cannot be evaluated in our recordings of mechanical threshold for the need to block action potential rise with tetrodotoxin, although we have previously ruled out the contribution of changes of membrane resistance, due to low GCl, in the alteration of mechanical threshold (De Luca *et al.*, 1996a). All these observations open two main possibilities for the mechanism of action of taurine on muscle function: the intracellular taurine content can affect independently different cellular functions or rather act on a unique step able to modulate various effectors. Our findings and the data available in the literature favor the latter hypothesis. It has been long claimed that taurine exerts a direct modulatory effect on the e-c coupling in the heart by acting on Ca^{++} availability for contraction (for review see Huxtable, 1992). In skeletal muscle taurine has been found to stimulate Ca^{++} uptake and storage capacity of sarcoplasmic reticulum (Huxtable and Bressler, 1973). A direct ability of taurine to increase Ca^{++}

TABLE 3

Effects of chronic taurine treatment on the mechanical threshold of extensor digitorum longus muscle fibers of adult and aged rats

Experimental Conditions	No. of Rats Tested	Rheobase Voltage (mV)	Time Constant (msec)
Adult rats	4	-56.1 \pm 0.9	6.50 \pm 0.56
Taurine-treated adult rats	4	-57.8 \pm 0.4	6.09 \pm 0.2
Aged rats	5	-63.7 \pm 0.4 ^a	7.17 \pm 0.29
Taurine-treated aged rat	1	-55.0 \pm 0.8 ^b	6.10 \pm 0.30
Taurine-treated aged rat	1	-58.5 \pm 0.4 ^b	6.25 \pm 0.44
Taurine-treated aged rat	1	-58.3 \pm 0.5 ^b	5.89 \pm 0.70
Taurine-treated aged rat	1	-56.2 \pm 0.9 ^b	5.96 \pm 0.26
Taurine-treated aged rat	1	-57.1 \pm 0.4 ^b	6.18 \pm 0.20
Taurine-treated aged rats	5	-57.0 \pm 0.4 ^b	6.00 \pm 0.20 ^b

For each of the experimental condition are shown the rheobase voltage (R) and the time constant (τ) values (\pm S.E.) calculated by fitting the threshold membrane potential values obtained at the various duration of depolarizing command pulse as described in "Materials and Methods." The threshold membrane potential values used for the fitting process are mean \pm S.E.M. from 18 to 48 fibers from the number of animals showed in the table, and are the same of figure 3. For taurine-treated aged rats group, the fitted values of R and τ of each rat are also shown. In this case the threshold membrane potential values used for the fitting process are mean \pm S.E.M. from 5 to 10 fibers. Statistical evaluation for significance between fitted values has been made by Student's *t* test, as described in "Materials and Methods."

^a Significantly different with respect to adult rats ($P < .001$).

^b Significantly different with respect to the untreated-aged rats ($P < .001$).

binding to membrane phospholipids has also been observed (Huxtable, 1992). Furthermore in muscle of aged rats we found a reduction of taurine content while Larsson and Salviati (1989) described an impairment of the Ca^{++} sequestration capacity of sarcoplasmic reticulum, resulting in higher level of cytosolic Ca^{++} . The relationship between cytosolic Ca^{++} and mechanical threshold is supported by the finding that *in vitro* application of the calcium ionophore A23187 to striated fibers shifts contraction toward more negative potentials (Morgan and Bryant, 1977). These observations corroborate that a taurine deficiency, such as that occurring naturally during aging, leads to an increase in cytosolic Ca^{++} able to affect e-c coupling mechanism, and that this situation can be counteracted by the amino acid administration through a restoration of intracellular taurine content. The proposed increase of Ca^{++} availability, occurring in taurine-depleted muscles of aged rats, can in turn account in a positive feed-back loop for the decrease in GCl observed in this situation. In fact GCl is reduced by application of the calcium ionophore A23187 as well as by the activation of a Ca^{++} dependent PKC (De Luca *et al.*, 1994). If this hypothesis is correct one would expect an ability of taurine to act on the biochemical modulatory pathway of the chloride channel (De Luca *et al.*, 1994). Accordingly, we found that the taurine administration reduced the potency of 4- β -PDB in decreasing GCl in aged rats, so that the block of GCl resulting from the phorbol ester-induced-PKC activation was similar to that observed in normal adults. Moreover Li and Lombardini (1991) found that taurine inhibits PKC catalyzed phosphorylation processes in rat brain by reducing the cytosolic Ca^{++} levels and by inhibiting phosphoinositide turnover. The taurine treatment also restored the sensitivity of the chloride channels to a specific channel ligand. As generally observed in adult rats, the R-(+) CPP produced in taurine-treated aged muscle an increase of GCl at low concentrations and a decrease of it at the higher ones, in contrast with the lack of this biphasic response observed in aged untreated muscle (De Luca *et al.*, 1992). We have recently observed a suppression of the biphasic response by the R-(+) CPP in adult animals after muscle pretreatment with phorbol esters, *i.e.*, after induction of channel phosphorylation, suggesting that the phosphorylated channel may have a different pharmacological sensitivity (De Luca *et al.*, 1996b). These findings again corroborate the ability of the taurine treatment to restore chloride channel function and pharmacology by acting on Ca^{++} and PKC-mediated biochemical pathway.

A direct action of taurine on chloride channels can also contribute to the effects observed after chronic treatment. In fact the administration of taurine to adult rats increased the amino acid level in blood but not in skeletal muscle and no remarkable effects were observed on the electrical and contractile properties. However the taurine-treated adult rats showed a slight increase of GCl along with the modification of the related excitability parameters, similarly to what observed when taurine is applied *in vitro* on skeletal muscle. Furthermore, lower concentrations of 4- β -PDB were less effective in reducing GCl of taurine-loaded adult rats with respect to untreated adults and this is also observed when 4- β -PDB is applied *in vitro* on adult EDL muscle previously incubated with taurine (18–30 mM) (unpublished observations). We already described that *in vitro* application of tau-

rine in the mM range produces a specific increase of skeletal muscle GCl, by acting on a low-affinity site (Conte Camerino *et al.*, 1987; Pierno *et al.*, 1994). Thus a direct pharmacological action of taurine on chloride channel of aged rat muscle, synergic with the effects produced by the restoration of the muscle taurine content, cannot be excluded. At the moment the verification of this hypothesis is made difficult by the fact that the chloride channels accounting for the large GCl in skeletal muscle cannot be studied by patch clamp methodology in native muscle fibers (Pusch *et al.*, 1994).

Our results add new evidences about the importance of maintaining appropriate level of intracellular taurine for skeletal muscle function. In particular the aminoacid supplementation may ameliorate muscle performance in aged subject. Taking into account that this physiological compound is almost free of side effects, the results of our *in vivo* studies corroborate its therapeutical potential in pathophysiological situations such as aging.

References

- Ballatori N, Truong TA, Jackson PS, Strange K and Boyer JL (1995) ATP depletion and inactivation of an ATP-sensitive taurine channel by classic ion channel blockers. *Mol Pharmacol* **48**:472–476.
- Bianchi CP (1992) Role of calcium channels of the sarcolemma and sarcoplasmic reticulum in skeletal muscle functions, in *Excitation-Contraction Coupling in Skeletal, Cardiac and Smooth Muscle* (Frank GB, Bianchi CP and ter Keurs HED eds) *Adv Exp Med Biol* Vol 311, pp 237–244, Plenum Press, New York.
- Bondar RJL and Mead DC (1974) Evaluation of glucose-6-phosphatase dehydrogenase from *Leuconostoc mesenteroides* in the hexokinase method for determining glucose in serum. *Clin Chem* **20**:586.
- Boyd IA and Martin AR (1959) Membrane constants of mammalian muscle fibers. *J Physiol* **147**:450–457.
- Bryant SH and Conte Camerino D (1991) Chloride channel regulation in the skeletal muscle of normal and myotonic goats. *Pflügers Arch* **417**:605–610.
- Carmeli E and Reznick AZ (1994) The physiology and biochemistry of skeletal muscle atrophy as a function of age. *Proc Soc Exp Biol Med* **206**:103–113.
- Conte Camerino D, Franconi F, Mambrini M, Bennardini F, Failli P, Bryant SH and Giotti A (1987) The action of taurine on chloride conductance and excitability characteristics of rat striated muscle fibers. *Pharmacol Res Commun* **19**:685–701.
- Dawson R and Wallace DR (1992) Taurine content in tissues from aged Fischer 344 rats. *Age* **15**:73–81.
- De Luca A and Conte Camerino D (1992) Effects of aging on the mechanical threshold of rat skeletal muscle fibers. *Pflügers Arch* **420**:407–409.
- De Luca A, Tortorella V and Conte Camerino D (1992) Chloride channels of skeletal muscle from developing, adult and aged rats are differently affected by enantiomers of 2-(p-chlorophenoxy) propionic acid. *Naunyn-Schmiedeberg Arch Pharmacol* **346**:601–606.
- De Luca A, Tricarico D, Pierno S and Conte Camerino D (1994) Aging and chloride channel regulation in rat fast-twitch muscle fibers. *Pflügers Arch* **427**:80–85.
- De Luca A, Pierno S and Conte Camerino D (1996a) Effect of taurine depletion on excitation-contraction coupling and Cl^- conductance of rat skeletal muscle. *Eur J Pharmacol* **296**:215–222.
- De Luca A, Pierno S, Tortorella V and Conte Camerino D (1996b) Age-dependent modification of phosphorylation state of skeletal muscle chloride channel controls its biophysical and pharmacological properties. *Fund Clin Pharmacol* **10**:184.
- Dulhunty A (1988) Internal citrate ions reduce the membrane potential for contraction threshold in mammalian skeletal muscle fibers. *Biophys J* **53**:609–615.
- Green JR and Margerison D (1978) *Statistical Treatment of Experimental Data*, pp 86–90, Elsevier, New York.
- Heiny JA, Jong D, Bryant SH, Conte Camerino D and Tortorella V (1990) Enantiomeric effects on e-c coupling in frog skeletal muscle by a chiral phenoxy carboxylic acid. *Biophys J* **57**:147–152.
- Huxtable RJ (1992) The physiological actions of taurine. *Physiol Rev* **72**:101–163.
- Huxtable RJ and Bressler R (1973) Effect of taurine on a muscle intracellular membrane. *Biochim Biophys Acta* **323**:573–583.
- Jiang SM, Fithian L, Smanik P, McGill J, Tong Q and Mazzaferri EL (1993) Cloning of the human taurine transporter and characterization of taurine uptake in thyroid cells. *FEBS Lett* **318**:139–144.
- Kirk K and Kirk J (1993) Volume-regulatory taurine release from a human lung cancer cell line. Evidence for amino acid transport via a volume-activated chloride channel. *FEBS Lett* **336**:153–158.
- Larsson L and Salviati G (1989) Effect of age on calcium transport activity of sarcoplasmic reticulum in fast- and slow-twitch rat muscle fibres. *J Physiol* **419**:253–264.
- Lehmann-Horn F and Rüdell R (1995) Hereditary nondystrophic myotonias and periodic paralyses. *Curr Opin Neurol* **8**:402–410.
- Li Y-P and Lombardini JB (1991) Inhibition by taurine of the phosphorylation of specific synaptosomal proteins in the rat cortex: Effects of taurine on the stimulation of calcium uptake in mitochondria and inhibition of phosphoinositide turnover. *Brain Res* **553**:89–96.

- Lleu P-L and Huxtable RJ (1992) Phospholipid methylation and taurine content of synaptosomes from cerebral cortex of developing rat. *Neurochem Int* **21**:109–118.
- Miledi R, Parker I and Zhu PH (1983) Calcium transients studied under voltage clamp control in frog twitch muscle fibres. *J Physiol (Lond)* **340**:649–680.
- Mollerup J and Lambert IH (1996) Phosphorylation is involved in the regulation of the taurine influx via the β -system in Ehrlich ascites tumor cells. *J Membrane Biol* **150**:73–82.
- Moorman JR, Ackerman SJ, Kowdley GC, Griffin MP, Mounsey JP, Chen Z, Cala SE, O'Brian JJ, Szabo G and Jones LR (1995) Unitary anion currents through phospholemman channel molecules. *Nature* **377**:737–740.
- Morgan KG and Bryant SH (1977) The mechanism of action of dantrolene sodium. *J Pharmacol Exp Ther* **201**:138–147.
- Pierno S, Tricarico D, De Luca A, Campagna F, Carotti A, Casini G and Conte Camerino D (1994) Effects of taurine analogues on chloride channel conductance of rat skeletal muscle fibers: A structure-activity relationship investigation. *Naunyn-Schmiedeberg Arch Pharmacol* **349**:416–421.
- Pion PD, Kittleson MD, Rogers QR and Morris JG (1987) Myocardial failure in cats associated with low plasma taurine: A reversible cardiomyopathy. *Science* **237**:764–768.
- Pusch M, Steinmeyer K and Jentsch TJ (1994) Low single channel conductance of the major skeletal muscle chloride channel, ClC-1. *Biophys J* **66**:149–152.
- Sturman JA (1993) Taurine in development. *Physiol Rev* **73**:119–147.

Send reprint requests to: Prof. Diana Conte Camerino, Unità di Farmacologia, Dipartimento Farmacobiologico, Facoltà di Farmacia, Università di Bari, Via Orabona, 4, Campus, 70125 Bari, Italy.
