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Effect of taurine depletion on excitation-contraction coupling and Cl^- conductance of rat skeletal muscle

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Abstract

The pharmacological action of taurine on skeletal muscle is to stabilize sarcolemma by increasing macroscopic conductance to Cl^- (G_{Cl}), whereas a proposed physiological role for the amino acid is to modulate excitation-contraction coupling mechanism via Ca^{2+} availability. To get insight in the physiological role of taurine in skeletal muscle, the effects of its depletion were evaluated on voltage threshold for mechanical activation and G_{Cl} with the two intracellular microelectrode method in 'point' voltage clamp mode and current clamp mode, respectively. The experiments were performed on extensor digitorum longus muscle fibers from rats depleted of taurine by a chronic 4 week treatment with guanidinoethane sulfonate, a known inhibitor of taurine transporter. The treatment significantly modified the mechanical threshold of striated fibers; i.e. at each pulse duration they needed significantly less depolarization to contract and the fitted rheobase voltage was more negative by 10 mV with respect to untreated muscle fibers. In parallel, the treatment with guanidinoethane sulfonate produced a significant 40% lowering of G_{Cl}. In vitro application of 60 mM of taurine to such depleted muscles almost completely restored the mechanical threshold and increased G_{Cl} even above the value of untreated control. However, in vitro application of 60 mM of either taurine or guanidinoethane sulfonate to untreated control muscles did not cause any change of the mechanical threshold but increased G_{Cl} by 40% and 21%, respectively. Furthermore, 100 μM of the *S*-(−) enantiomer of 2-(*p*-chlorophenoxy)propionic acid almost fully blocked G_{Cl} but did not produce any change in the mechanical threshold of normal muscle fibers. The present results show that the large amount of intracellular taurine plays a role in the excitation-contraction coupling mechanism of striated muscle fibers. This action is independent from any effect involving muscle Cl^- channels, but it is likely mediated by the proposed ability of taurine to modulate Ca^{2+} availability through the interaction with the Ca^{2+} transporters present on sarcoplasmic reticulum.

Keywords: Taurine depletion; Skeletal muscle; Mechanical threshold; Excitation-contraction coupling; Cl^- conductance; Ca^{2+} handling mechanism

1. Introduction

Mammalian skeletal muscle has high intracellular levels of the sulfonic amino acid taurine (Huxtable, 1992). The muscle-specific biosynthesis of taurine is very little, being negligible the activity of cysteine sulfinic acid decarboxylase, the enzyme that catalizes the conversion of cysteine sulfinic acid to hypotaurine (Yamaguchi et al., 1973). Therefore, the intracellular millimolar concentrations are guaranteed by an high affinity proteic transporter able to use the micromolar concentrations of the amino acid in the plasma (Huxtable et al., 1979; Huxtable, 1992). Since in skeletal muscle taurine is mainly present as a free amino acid (Huxtable, 1992), it must have a remarkable and specific physiological role which accounts for its accumulation in muscle cells against gradient. Our previous studies showed that taurine applied in vitro at mM concentrations on skeletal muscle exerts a membrane stabilizing effect by specifically increasing membrane Cl^- conductance (G_{Cl}) (Conte Camerino et al., 1987). In addition, we found that taurine can counteract the low G_{Cl} in some forms of pharmacologically induced myotonias in rats (Conte Camerino et al., 1989), thus supporting its proposed therapeutical use to solve muscle membrane hyperexcitability of myotonic patients (Durelli et al., 1983). These effects are referred to as

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'pharmacological' ones, i.e. mediated by the interaction of taurine with a low affinity site present on membrane phospholipids (Huxtable, 1992), likely nearby Cl^- channels. In support of this hypothesis we found that modification of taurine structure alters the taurine-like activity on muscle G_{Cl} (Pierno et al., 1994). However the pharmacological potential of the amino acid may be further corroborated by clarifying the 'physiological' role of its millimolar concentrations in skeletal muscle so to evaluate if abnormal intracellular level of taurine may cause tissue malfunction. This is of importance in consideration that, as a consequence of muscle fiber degeneration, the urinary excretion of taurine is increased in some dystrophic conditions, such as the Duchenne muscular dystrophy (Engel et al., 1994). Data obtained on heart allow to predict the possible physiological role of the amino acid in the contractile tissues. Indeed the taurine deficiency causes a severe dilated cardiomyopathy in the cats, which may be solved by taurine supplementation (Pion et al., 1987). Also, a taurine depletion by a chronic treatment with guanidinoethane sulfonate, a known inhibitor of taurine high affinity transporter (Huxtable et al., 1979), produces cardiac abnormalities in excitation-contraction coupling, characterized by a prolongation of action potential duration and of relaxation time and a decrease in isometric twitch force (Lake et al., 1991; Lake, 1992; Eley et al., 1994). These changes have been attributed to the multiple and complex actions that the amino acid exerts on the structures devoted to Ca^{2+} handling, in dependence of the functional state of the heart (Franconi et al., 1986; Huxtable, 1992). Thus in a low-Ca^{2+} medium, taurine exerts positive inotropic effects (Franconi et al., 1986; Takihara et al., 1986) by stimulating the Ca^{2+}-induced Ca^{2+} release by sarcoplasmic reticulum as well as the activity of Na^+/Ca^{2+} exchanger. Both actions are produced either directly or through the inhibition of the phospholipids methyltransferase enzyme present on the membranes (Panagia et al., 1987; Hamaguchi et al., 1991; Punna et al., 1994). On the contrary in the situation of Ca^{2+} overload, taurine can exert an antiarrhythmic action by stimulating Ca^{2+}-ATPase pumps on sarcoplasmic reticulum and sarcolemma (Harada et al., 1988, 1990; Steele et al., 1990; Huxtable, 1992). For the excitation-contraction coupling mechanism of skeletal muscle the influx of Ca^{2+} is not pivotal. Indeed the contraction is almost entirely sustained by the Ca^{2+} released from the ryanodine-sensitive channels of sarcoplasmic reticulum activated by the depolarization-induced conformational changes of the voltage sensor of the dihydropyridine receptor in the tranverse tubules (Rios and Pizarro, 1991; Gyorke et al., 1994). However the possibility that taurine may modulate excitation-contraction coupling also in skeletal muscle was proposed by Huxtable and Bressler (1973) upon the finding that taurine enhances the rate of Ca^{2+} uptake and the total Ca^{2+} sequestering capacity of sarcoplasmic reticulum isolated from rat skeletal muscle. Furthermore, in relation to its pharmacological role, taurine can control the excitation-contraction coupling by contributing to maintain the large resting G_{Cl} of sarcolemma. Indeed, a change of taurine content can produce an alteration of G_{Cl} that in turn would affect the duration of action potential and the frequency of depolarization, with a consequent change of the excitation pattern of the membrane before contraction (Bryant, 1979; Conte Camerino et al., 1987; De Luca et al., 1992a). In the present study we tested the hypothesis that the high intracellular taurine content in skeletal muscle may play a role in the excitation-contraction coupling process. At this aim we evaluated, by means of 'point' voltage clamp, the voltage threshold for mechanical activation of extensor digitorum longus muscle fibers from rats depleted of taurine by a chronic in vivo treatment with guanidinoethane sulfonate. In parallel, we evaluated the effect of taurine depletion on G_{Cl} at the aim of testing the possible G_{Cl}-mediated taurine effect on the excitation-contraction coupling mechanism in skeletal muscle.

2. Materials and methods

2.1. Experimental procedure

Male Wistar rats of 250 g were used for all the experiments. The chronically treated animals were maintained ad libitum on drinking water containing 1% guanidinoethane sulfonate for 4 weeks. Such a treatment has been previously found to decrease by at least 50% the intracellular concentration of taurine in skeletal muscle (Huxtable et al., 1979; De Luca et al., 1992a). The control rats received only drinking water ad libitum. The electrophysiological experiments were made in vitro on extensor digitorum longus muscle fibers at the end of the treatment period. The muscles were removed under urethane anesthesia (1.2 g/kg i.p.) from both guanidinoethane sulfonate-treated and untreated control rats. Soon after the removal of the muscle the rats, still anesthetized, were killed by a further i.p. injection of an urethane overdose. The extensor digitorum longus muscle was stretched to about 1.5 times its resting length on a 3 mm plastic rod in a temperature-controlled muscle chamber at 30°C and perfused with a physiological solution in the absence and in the presence of the test compounds.

2.2. Measurement 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 μm of each other into the central region of a randomly selected superficial fiber which was continuously viewed using a stereomicroscope (100 × 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. Below this rate the fibers could not be clamped for an appropriate length of time to perform the required measurements. Tetrodotoxin (3 μM) 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 ms. 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 1–2 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 experiments with each muscle lasted less than 2 h. The threshold membrane potential V (mV) for each fiber was averaged at each pulse duration t (ms) and then mean values plotted against duration giving 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:

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

were H is the holding potential (mV), R is the rheobase (mV) and τ is the time constant (Miledi et al., 1983; De Luca et al., 1992b). 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. We used this procedure in order to be able to incorporate all of our determination points and their associated errors into our estimate of R under each condition. The mechanical threshold values are expressed as the fitted rheobase (R) parameter ± standard error (S.E.) which was determined from the variance-covariance matrix in the nonlinear least square fitting algorithm. The statistical significance of the fitted rheobase values and their differences from each other were estimated by a Student's t distribution using these standard errors and 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.

2.3. Measurements of Cl^- conductance

The component Cl^- conductance (G_{Cl}) of extensor digitorum longus muscle fibers from guanidinoethane sulfonate-treated and untreated rats in the absence and in the presence of the test compounds was calculated from the membrane resistance (R_m) values measured by standard cable analysis 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. Current pulse generation, acquisition of the voltage records and calculation of fiber constants were done under computer control as detailed elsewhere (De Luca et al., 1992b, 1994; Pierno et al., 1994). The total membrane conductance, G_m, was $1/R_m$ in the normal physiological solution, whereas K^+ conductance G_K was $1/R_m$ in Cl^--free physiological solution. The mean G_{Cl} was calculated as the mean G_m minus the mean G_K (De Luca et al., 1992b, 1994; Pierno et al., 1994). The data are expressed as mean ± standard error of the mean (S.E.M.). The estimate of S.E.M. and N of G_{Cl} was calculated from the variance and N of G_m and G_K as described by Green and Margerison (1978). Significance between groups of means was evaluated by Student's t-test.

2.4. Solutions and drugs

The normal physiological solution had the following composition (mM): NaCl 148; KCl 4.5; $CaCl_2$ 2.0; $MgCl_2$ 1.0; NaH_2PO_4 0.44; $NaHCO_3$ 12 and glucose 5.55. The Cl^--free solution was made by equimolar substitution of methylsulphate salts for NaCl and KCl and nitrate salts for $CaCl_2$ and $MgCl_2$ (De Luca et al., 1992b, 1994). The physiological solution was continuosly bubbled with 95% O_2 and 5% CO_2 (pH 7.2). For in vitro application of taurine (Sigma, St.Louis, USA) and guanidinoethane sulfonate (kindly provided by prof. R.J. Huxtable, Tucson, AZ) on extensor digitorum longus muscle from guanidinoethane sulfonate-treated and control rats, the two drugs were dissolved in the physiological solutions in the mM concentrations needed. The use of equimolar concentration of sucrose allowed to establish that no effects were attibutable to the osmotic change of the solutions (Conte Camerino et al., 1987). Particular care has been taken to maintain the physiological pH at 7.2 when taurine was applied in

vitro to the muscle since its actions have been described to be strongly dependent upon external pH (Conte Camerino et al., 1987). Stock solutions of the *S*-(−) enantiomer of 2-(*p*-chlorophenoxy)propionic acid were prepared in 1% aqueous bicarbonate solution and the final concentration of 100 μM was obtained by further dilution in normal and Cl^--free solution to be tested on extensor digitorum longus muscle from untreated control rats (De Luca et al., 1992b).

3. Results

3.1. Effect of guanidinoethane sulfonate treatment on mechanical threshold and Cl^- conductance of rat skeletal muscle fibers

The threshold potential for contraction of extensor digitorum longus muscle fibers from both control and guanidinoethane sulfonate-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 in the present study (t = 30°C and rate of about 0.3 Hz) a constant rheobase value was almost fully reached at the longest pulses used, a behaviour which has been commonly seen with mammalian muscle fibers (De Luca and Conte Camerino, 1992). However, the mechanical threshold from guanidinoethane sulfonate-treated rats was significantly different from that of controls. Indeed, at each pulse duration, the muscle fibers from guanidinoethane sulfonate-treated animals needed a significantly less depolarization to contract with respect to controls (Table 1). Consequently, the strength-duration curve constructed as described in Methods was shifted towards more negative potentials in the guanidinoethane sulfonate-treated fibers with respect to that of untreated controls (Fig. 1). The voltage at rheobase estimated from the fit was −56.1 ± 0.9 mV for controls

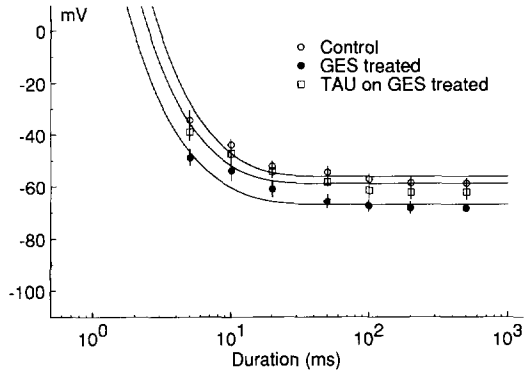

Fig. 1. Strength-duration curves for the threshold potentials of mechanical activation of extensor digitorum longus muscle fibers from rats untreated (control) and treated with guanidinoethane sulfonate (GES treated). The effect of in vitro application of 60 mM taurine on the muscles from guanidinoethane sulfonate-treated rats is also shown (TAU on GES treated). The experimental values, that are the same mean values ± S.E.M. listed in Table 1, have been fitted by using the equation described in Methods so to obtain the fitted values of rheobase voltage ± S.E. (R) and time constant ± S.E. (τ) detailed in the text.

and −67.0 ± 1.0 mV for guanidinoethane sulfonate-treated animals and the difference between these two values was highly significant with $P < 0.001$. However, the estimate of the time constant (τ) to reach the rheobase obtained from the fit of the available experimental points was not significantly different in the guanidinoethane sulfonate treated muscle (6.7 ± 0.82 ms) with respect to controls (6.5 ± 0.56 ms). Along with the more negative rheobase value, the guanidinoethane sulfonate-treated fibers had significantly lower values of Cl^- conductance (G_{Cl}) with respect to controls. Indeed G_{Cl} was 2917 ± 37 μS/cm² (n = 15) and 1973 ± 94 μS/cm² (n = 38) in control and treated fibers, respectively.

In vitro application of 60 mM taurine on extensor digitorum longus muscle fibers from guanidinoethane sulfonate-treated rats significantly restored the me-

Table 1
Effect of chronic treatment with guanidinoethane sulfonate on the mechanical threshold of rat extensor digitorum longus muscle fibers

Experimental condition	Duration (ms)						
	5	10	20	50	100	200	500
Control	−34.1 ± 3.8 (19)	−43.6 ± 1.9 (20)	−51.9 ± 1.9 (21)	−54.1 ± 2.0 (23)	−57.0 ± 2.0 (23)	−58.4 ± 2.2 (22)	−58.6 ± 1.7 (23)
GES-treated	−48.6 ± 3.1 [a] (14)	−53.6 ± 4.1 [a] (15)	−60.8 ± 3.3 [a] (16)	−65.6 ± 2.6 [a] (16)	−67.2 ± 2.4 [a] (16)	−68.1 ± 2.3 [a] (16)	−68.2 ± 1.5 [a] (18)
60 mM taurine on GES-treated	−38.7 ± 3.5 (11)	−47.0 ± 2.7 (13)	−53.8 ± 2.7 (15)	−58.0 ± 1.8 (13)	−61.3 ± 3.0 (14)	−62.0 ± 2.9 (15)	−62.0 ± 3.0 (13)

The columns from left to right are as follows. Experimental conditions: the fibers sampled are from extensor digitorum longus muscles from four untreated rats (controls), from three rats treated chronically with guanidinoethane sulfonate as described in Methods (GES-treated) and from these latter preparations on which 60 mM taurine has been applied in vitro (60 mM taurine on GES-treated); for each experimental condition are shown the threshold membrane potential values obtained with depolarizing command pulse of duration ranging from 5 ms up to 500 ms. The values are expressed as means ± S.E.M. from the number of fibers shown in parentheses below each value. [a] Significantly different from control ($P < 0.025$ and less).

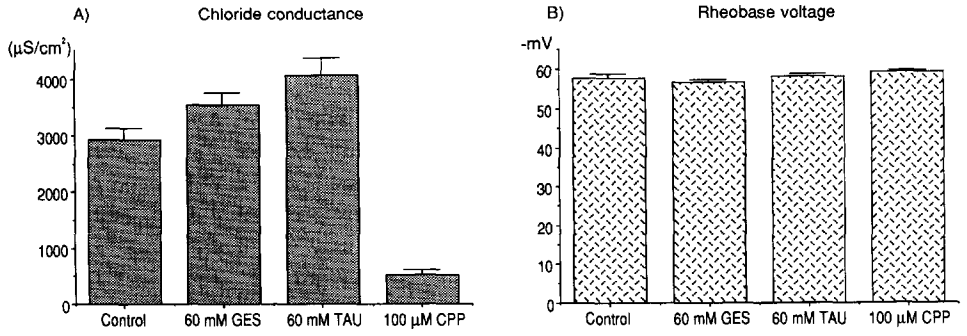

Fig. 2. Effect of in vitro application of 60 mM guanidinoethane sulfonate (60 mM GES), 60 mM taurine (60 mM TAU) and 100 μM of *S*-(−)-2-(*p*-chlorophenoxy)propionic acid (100 μM CPP) on resting Cl^- conductance (G_{Cl}) and rheobase voltage of extensor digitorum longus muscle fibers from untreated rats. For Cl^- conductance each bar is the mean ± S.E.M. from 13 to 64 fibers. The G_{Cl} values in the presence of guanidinoethane sulfonate, taurine and *S*-(−)-2-(*p*-chlorophenoxy)propionic acid shown in the figure were significantly different with respect to control ($P < 0.05$ and less). The rheobase voltages ± S.E. have been obtained from the fit of the experimental points listed in Table 2.

chanical threshold; in fact the strength-duration curve was shifted toward that of control (Fig. 1). The fitted values of rheobase and τ after taurine application on the depleted muscles were -58.8 ± 1.1 mV and 6.0 ± 0.7 ms, respectively. As it can be seen in Table 1, after in vitro application of taurine to guanidinoethane sulfonate-treated muscle, the potentials for contraction at each pulse duration were no more significantly different with respect to those of controls. Also the in vitro application of 60 mM taurine to the depleted muscles brought the G_{Cl} value to 3967 ± 217 $\mu S/cm^2$ ($n = 9$), a value which was significantly higher than that of untreated controls. Such wide increase in G_{Cl} may be due either to an increased affinity of the low affinity site as a consequence of the taurine depletion, or rather to a normalization of G_{Cl} upon the restoration of intracellular taurine content plus a further action of the amino acid on the low affinity site, detectable as the typical 40% increase in G_{Cl} over the value of untreated controls (Pierno et al., 1994).

3.2. Effect of in vitro application of guanidinoethane sulfonate, taurine and S-(−)-2-(p-chlorophenoxy)propionic acid on mechanical threshold and Cl^- conductance of rat skeletal muscle fibers

The chronic guanidinoethane sulfonate treatment affected in parallel mechanical threshold and G_{Cl}. In order to distinguish whether the effect on the mechanical threshold was directly due to the taurine depletion or rather mediated by an action on the low affinity site modulating G_{Cl} (Pierno et al., 1994), we tested the effects of both taurine and guanidinoethane sulfonate on the mechanical threshold in the situation of physiological taurine concentration (e.g. on normal untreated muscle). As expected, the in vitro application to nor-

Table 2
Effect of in vitro application of guanidinoethane sulfonate, taurine and *S*-(−)-2-(*p*-chlorophenoxy)propionic acid on the mechanical threshold of rat extensor digitorum longus muscle fibers

Experimental condition	Duration (ms)						
	5	10	20	50	100	200	500
Control	−34.0 ± 4.3 (8)	−47.0 ± 1.8 (9)	−52.8 ± 1.5 (12)	−54.4 ± 2.2 (9)	−59.8 ± 1.9 (10)	−60.2 ± 1.9 (9)	−60.0 ± 1.7 (10)
60 mM GES	−35.0 ± 1.2 (9)	−47.3 ± 0.9 (9)	−51.8 ± 0.7 (8)	−54.8 ± 1.6 (11)	−59.3 ± 0.8 (10)	−60.8 ± 1.1 (9)	−61.0 ± 1.8 (9)
60 mM Taurine	−34.8 ± 2.1 (9)	−45.5 ± 1.9 (9)	−52.0 ± 1.8 (10)	−54.6 ± 1.8 (11)	−59.0 ± 1.6 (10)	−59.5 ± 1.6 (10)	−59.4 ± 0.8 (10)
100 μM *S*-(−)-CPP	−34.4 ± 2.0 (5)	−47.1 ± 1.7 (7)	−52.4 ± 1.5 (7)	−55.5 ± 1.0 (7)	−59.4 ± 0.6 (7)	−60.4 ± 0.6 (7)	−60.4 ± 0.6 (7)

The columns from left to right are as follows. Experimental conditions: guanidinoethane sulfonate (GES) and taurine at the concentration of 60 mM and *S*(−)-2-(*p*-chlorophenoxy)propionic acid (CPP) at the concentration of 100 μM were applied in vitro on extensor digitorum longus muscles from untreated rats (controls). For each experimental condition are shown the threshold membrane potential values obtained with depolarizing command pulse of duration ranging from 5 ms up to 500 ms. The values are expressed as means ± S.E.M. from the number of fibers shown in parentheses below each value.

mal muscle of 60 mM of either guanidinoethane sulfonate or taurine produced an increase in G_{Cl} related to their relative potency on the low affinity site (Pierno et al., 1994). Indeed guanidinoethane sulfonate increased G_{Cl} by 25% against the 40% increase observed with an equimolar concentration of taurine (Fig. 2A). The effects of in vitro application of either taurine and guanidinoethane sulfonate on mechanical threshold of extensor digitorum longus muscle from normal untreated animals are shown in Table 2. As it can be seen, neither 60 mM guanidinoethane sulfonate nor 60 mM taurine produced significant changes in the voltage threshold for mechanical activation at any pulse duration. Consequently no differences were found in the fitted values of the rheobase voltage (Fig. 2B) or in the values of τ; indeed τ was 6.8 ± 0.61 ms in controls and 5.8 ± 0.23 ms and 6.3 ± 0.37 ms in the presence of 60 mM guanidinoethane sulfonate and taurine, respectively. To further establish if the reduction of G_{Cl} could have been responsible of the effect on the mechanical threshold observed in guanidinoethane sulfonate-treated muscle fibers, we tested the action of a specific blocker of skeletal muscle Cl^- channels, the *S*-(−) isomer of 2-(*p*-chlorophenoxy)propionic acid (De Luca et al., 1992b) on the mechanical threshold of normal muscle fibers. At the concentration of 100 μM the *S*-(−)-2-(*p*-chlorophenoxy)propionic acid produced an almost complete block of G_{Cl} (Fig. 2A). Nonetheless this compound had no significant effects on the voltage threshold values at any pulse duration and on the fitted rheobase voltage with respect to controls (Table 2, Fig. 2B). Also the τ value was completely unaffected being 6.8 ± 0.32 ms.

4. Discussion

The results of the present study showed that a depletion of taurine content in skeletal muscle, produced by an in vivo treatment with the taurine-transporter inhibitor guanidinoethane sulfonate, affected the excitation-contraction coupling mechanism, in that the rheobase voltage for contraction was shifted towards more negative potentials. In parallel a significant decrease in G_{Cl} was observed. It was likely to attribute both effects to the reduction of taurine content since they have been reversed upon in vitro application of taurine, which likely acted by restoring the intracellular concentration of the amino acid. It was of our interest to verify this hypothesis and to evaluate the correlation between the two altered parameters. Thus we found that when the intracellular concentration of taurine was within the physiological range, as in untreated muscles, in vitro application of either taurine or guanidinoethane sulfonate did not change the mechanical threshold; however the typical pharmacological action on the low affinity site, i.e. the increase in G_{Cl} was clearly detectable. This observation suggests that the change of excitation-contraction coupling in taurine-depleted fibers was not due to the interaction of these compounds with the low affinity site (Huxtable, 1992; Pierno et al., 1994). Also 100 μM of *S*-(−)-2-(*p*-chlorophenoxy)propionic acid, which almost completely blocked G_{Cl}, did not modify the mechanical threshold of normal muscle fibers. In the light of these findings one can rule out any effect on mechanical threshold of mammalian striated fibers directly attributable to Cl^- channels or indirectly related to an alteration of membrane excitability in consequence of a decrease in sarcolemmal G_{Cl} (Heiny et al., 1990). Consequently, the mechanism by which taurine ensures appropriate excitation-contraction coupling in skeletal muscle is likely related to an action of the amino acid on those intracellular structures preposed to Ca^{2+} handling and contraction. It is known that the rheobase is the voltage at which the release of Ca^{2+} from sarcoplasmic reticulum equals the speed at which the Ca^{2+} is buffered to stop contraction (Dulhunty, 1988; Walsh et al., 1988). Any pathological or pharmacological intervention able either to increase the release of Ca^{2+} from sarcoplasmic reticulum to a greater extent than the uptake or to reduce the uptake, would shift the rheobase voltage in a negative direction as a result of higher cytosolic Ca^{2+} concentration (Dulhunty, 1988; Walsh et al., 1988). The identification of the specific intracellular site involved in the mechanical threshold change of taurine-deficient striated fibers is not an easy task since the large amount of evidence on cardiac tissue suggest that the amino acid can maintain the intracellular Ca^{2+} availability at physiological levels and thus control excitation-contraction coupling of the heart, by acting at different sites (Huxtable, 1992). However the available information can allow us to discriminate among the possible hypotheses about the role of taurine in the control of excitation-contraction coupling mechanism of skeletal muscle, taking into account the well known differences of this process with that occurring in cardiac muscle (Rios and Pizarro, 1991). As far as the release of Ca^{2+} is concerned the taurine deficiency may have affected either the voltage sensor of L-type Ca^{2+} channels in the transverse tubules or the Ca^{2+} release channels of sarcoplasmic reticulum. No data are available about the effects of taurine on the charge movements, the electrophysiological parameter that quantifies the conformational changes of the voltage sensor of L-type Ca^{2+} channels upon depolarization (Rios and Pizarro, 1991). However, studies on cardiac tissue suggest that taurine, in contrast with most of the drugs affecting the voltage sensor (Walsh et al., 1988; Heiny et al., 1990), modulates L-type Ca^{2+} channels in relation to the external Ca^{2+} concentration, making unlikely a relevant and

specific role of the amino acid at this level (Sawamura et al., 1990). Also the lack of effect on the mechanical threshold observed by us upon the pharmacological decrease in G_{Cl} allow to exclude an involvement of the voltage sensor brought about by the higher frequency of depolarization linked to the taurine depletion-induced decrease in G_{Cl} (De Luca et al., 1992a). The release of Ca^{2+} from cardiac sarcoplasmic reticulum is directly stimulated by taurine (Steele et al., 1990; Punna et al., 1994), accounting for a delay of this process in the taurine-depleted cardiac muscle fibers (Lake, 1992). Therefore a similar situation occurring in taurine-depleted skeletal muscle would have produced changes of mechanical threshold opposite to those we actually observed. The effect of taurine depletion on excitation-contraction coupling of skeletal muscle may be related to a decreased ability of sarcoplasmic reticulum to buffer Ca^{2+} and to stop contraction. In fact Huxtable and Bressler (1973) have described a taurine-dependent increase in the rate and amount of Ca^{2+} sequerested by sarcoplasmic reticulum vesicles isolated from skeletal muscle. Since taurine has no effect on the low affinity Ca^{2+} sites (Huxtable, 1992) such as calsequestrin (Lake, 1992), the major Ca^{2+} sequestering proteins of sarcoplasmic reticulum, the effect observed on the vesicles of sarcoplasmic reticulum has been related to the ability of taurine to stimulate the high affinity ATP-dependent Ca^{2+} uptake. At this regard it has been found that taurine stimulates the Ca^{2+} pumps in a manner strongly dependent on the presence of both Mg^{2+} and ATP, suggesting a specific role of the amino acid on some of the crucial steps of the active Ca^{2+} transport (Harada et al., 1988, 1990; Huxtable, 1992). Since the Ca^{2+} extrusion pump on sarcolemma plays a minor role in the excitation-contraction coupling of skeletal muscle (Dulhunty, 1988; Walsh et al., 1988), taurine depletion could have likely determined the shift of the mechanical threshold observed by slowing the Ca^{2+} uptake by sarcoplasmic reticulum. A decrease in Ca^{2+} availability of sarcoplasmic reticulum due to a long-term inhibition of pump activity in the depleted muscle could also play a role, as a result of higher resting level of cytosolic Ca^{2+}. Similar alterations of the Ca^{2+} sequestering transporters have been proposed time by time as the mechanism by which taurine-depleted heart muscle cells have a markedly prolonged relaxation time (Lake et al., 1991), an enhanced toxicity by Ca^{2+} overload of doxorubicin (Harada et al., 1988, 1990) and a spontaneous activity even at low concentration of external Ca^{2+} (Lake, 1992). On the contrary, the decreased strength of cardiac contraction also observed was attributed to a decreased sensitivity of the myofilaments to Ca^{2+} in consequence of the taurine depletion (Steele et al., 1990; Lake, 1992; Eley et al., 1994). This latter observation allows to rule out that the shift of the mechanical threshold towards more negative potentials observed in the taurine-depleted muscles is due to an effect of taurine on the proteins of the contractile machinery. More generally a disfacilitation of Ca^{2+} binding to phospholipids during taurine deficiency (Huxtable, 1992), can also increase the availability of free Ca^{2+} for contraction. Although addressed studies will allow to better evaluate the Ca^{2+} handling structure that is a target of taurine action in skeletal muscle, it is to underline that such an increase in Ca^{2+} availability in taurine-depleted muscle can also account for the decrease in G_{Cl} observed in this situation. Indeed we have previously found that a pharmacological increase in intracellular Ca^{2+} by the Ca^{2+} ionophore A23187, either directly or via the activation of Ca^{2+} and phospholipid dependent protein kinase C produce a decrease in G_{Cl} (De Luca et al., 1994). Interestingly Li and Lombardini (1991) have proposed that taurine may exert a negative control of protein kinase C in the cortex, likely via Ca^{2+}, so that in the situation of taurine depletion one could observe a facilitation of enzyme activity. These results add new evidence about the importance of maintaining appropriate level of intracellular taurine to ensure muscle performance. It is interesting to undeline that in aged rats, where the concentration of taurine is reduced in both plasma and tissues (Dawson and Wallace, 1992), we have found that the mechanical threshold is significantly shifted towards negative potentials, similarly to what was observed in the present experiments after taurine depletion (De Luca and Conte Camerino, 1992). This novel role of taurine to directly control the excitation-contraction coupling of striated fibers should be taken into account also for the possible pharmacological use of this safe amino acid in those severe muscle disorders characterized by fiber necrosis upon elevation of myoplasmic Ca^{2+}, especially if accompanied by a loss of taurine content (Engel et al., 1994).

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