EFFECTS OF GUANDINOETHANE SULFONATE ON CONTRACTION OF SKELETAL MUSCLE

C. Cuisinier, P. Gailly, M. Francaux and J. Lebacq
Faculty of medicine, Université catholique de Louvain, Louvain-la-Neuve, Belgium

Abstract: Guanidinoethane sulfonic acid (GES), a chemical and biological analog of taurine, decreases rat muscle taurine content when added to drinking water. Over the same period, GES appears in muscle[8]. GES supplementation is often used to study the effect of taurine depletion on physiological mechanisms, without taking into account the possible actions of GES. The purpose of the present study was to investigate the specific actions of GES on contraction of skeletal muscle. In mice EDL muscle, the time delay needed to observe a 20% force decrease after the end of a tetanic stimulation was higher in GES-supplemented than in control muscle. This observation in GES-supplemented muscle could be explained by the action of taurine or GES on several targets, beside others the rate of Ca^{2+} uptake by sarcoplasmic reticulum (SR) and the Ca^{2+} sensitivity of myofilaments. SR of rat EDL was isolated by successive centrifugations. The effect of 20 mM taurine or GES on the rate of Ca^{2+} uptake by SR was measured with the fluorescent Ca^{2+} indicator fura-2. The results show that the rate of Ca^{2+} uptake by SR is not modified in the presence of taurine or GES. The Ca^{2+} sensitivity of myofilaments was studied in chemically skinned fibers in the presence of 20 mM taurine or GES. Both taurine and GES increased the myofilament sensitivity to Ca^{2+}. Thus, the prolonged relaxation time of GES-supplemented muscle can be attributed to an increase in myofilament sensitivity to Ca^{2+}. This higher sensitivity is not due to a decrease in muscle taurine content but rather to an increased GES concentration.

INTRODUCTION

Taurine is highly concentrated in organs such as liver, brain, heart and skeletal muscle but relatively scarce in extracellular fluids[9]. The gradient

Taurine 4, edited by Della Corte *et al.*
Kluwer Academic / Plenum Publishers, New York, 2000.

across cell membranes is maintained by a regulated taurine transport system[4]. Several agonists able to compete with taurine transport have been discovered : ß-alanine, hypotaurine, guanidinopropionic acid and guanidinoethane sulfonic acid (GES)[8]. The latter is frequently used as a taurine-depleting agent when added 1% (w/v) to animal drinking water. Taurine depletion is accompanied by GES accumulation in tissues. In rat ventricular papillary muscle, GES supplementation induces a prolonged relaxation time after contraction. This observation was attributed to the taurine depletion[11]. To the best of our knowledge, the effects of taurine on contractility of skeletal muscle have never been studied. The results of the present study show that taurine depletion by GES supplementation prolongs the relaxation time of skeletal muscle. This increase cannot be explained by muscle taurine content decrease but is due to the presence of GES in muscle cells that modifies myofilament sensitivity to calcium.

MATERIALS AND METHODS

GES Supplementation of Mouse Skeletal Muscle

Male C57 B6 mice, aged 8 weeks, were separated into 2 groups. The GES group (n = 9) was provided with drinking water containing 1% (w/v) guanidinoethane sulfonic acid (GES) for 6 weeks. The control group (n = 10) had free access to pure water without any supplementation.

Mechanical Measurements

Extensor digitorum longus (EDL) muscles were removed from control and GES-supplemented animals, mounted on a force measuring system and immersed in continuously gassed (95% O_2- 5% water-saturated CO_2) Krebs solution (1 18 mM NaCl, 25 mM $NaHCO_3$, 5mM KCl, 1 mM $MgSO_4$, 2.5 mM $CaCl_2$ and 5 mM glucose). Optimal muscle length for maximal force development was determined and the muscles were stimulated for 400 ms at a frequency of 100 Hz by capacitor discharges of alternating polarity. The force developed during the tetanus and the relaxation period was recorded. The time delay needed to observe a 20% force decrease after the end of the stimulation ($t_{20\%}$) was calculated.

Sarcoplasmic Reticulum Isolation and Calcium Uptake Measurements

Both EDL muscles of male Wistar rats aged 3 months were quickly removed and homogenized at 0°C in 1 ml of a buffer solution (200 mM sucrose, 10 mM sodium azide, 1 mM EDTA, 40 mM L-histidine, pH 7.8). The light fraction of sarcoplasmic reticulum (SR) was isolated according to the method of O'Brien[13]. The rate of Ca^{2+} uptake by the SR was determined with the Ca^{2+} fluorescent indicator fura-2[5,10]. The assay was initiated by injection of 15 µg SR in 200 µl assay medium which contained 10 µM Ca^{2+}, 5 µM fura-2 free acid, 20 mM Tris. HCl, 5 mM potassium oxalate, 5 mM $MgCl_2$, 5 mM Na_2ATP, 5 µM TPEN, 80 mM KCl, pH 7.0. The extravesicular Ca^{2+} concentration was measured for 200 s following initiation of the assay. Results are presented as the rate of Ca^{2+} uptake by isolated SR vesicles in the presence of different extravesicular free Ca^{2+} concentrations. Two conditions were tested by adding 20 mM taurine or 20 mM GES. The control conditions were conducted in presence of 20 mM sucrose.

Skinned Fiber Preparation and Ca^{2+} Sensitivity Determination

Bundles of rabbit psoas muscle were chemically skinned as described by Brenner[2]. Bundles of about 10 fibers were dissected and mounted on a force transducer. Sarcomere length was measured by laser diffraction[1] and adjusted to 2.2 µm. Fibers were stimulated by immersion in a solution containing 0.54 µM buffered free Ca^{2+}, 5 mM EGTA/CaEGTA, 10 mM imidazole, 5 mM MgATP, 10 mM caffeine, 20 mM taurine or 20 mM GES, pH 7.2 and ionic strength $\tau/2$ adjusted at 138 mM. Fiber bundles were relaxed between force measurements. The effects of 20 mM taurine (n = 6) and 20 mM GES (n = 6) on force developed by myofilaments were tested in a random order. In control conditions, taurine or GES was replaced by 20 mM KCl (n = 6). Results are presented as a percentage of maximal force developed in the presence of 10 µM Ca^{2+}.

Statistics

Data are presented in figures as means ± SD. Changes in variables of interest were assessed by a mean comparison test. Level of significance was set at $p < 0.05$ (*; ** and *** indicate $p < 0.01$ and $p < 0.001$ respectively).

RESULTS

Skeletal muscles of GES treated and control mice were isolated from the animals and stimulated in vitro. GES-treated muscles showed a significantly longer $t_{20\%}$ than control muscles ($p < 0.01$) (Figure 1).

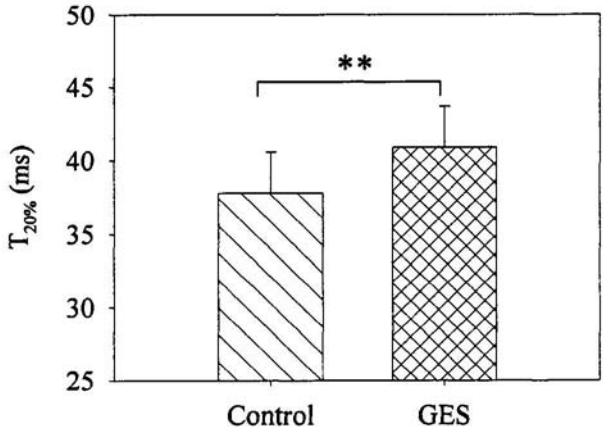

Figure 1. Time delay (ms) needed to observe a 20% force decrease after the end of the stimulation of control and GES supplemented mice EDL muscles (mean ± SD). ** $p < 0.01$.

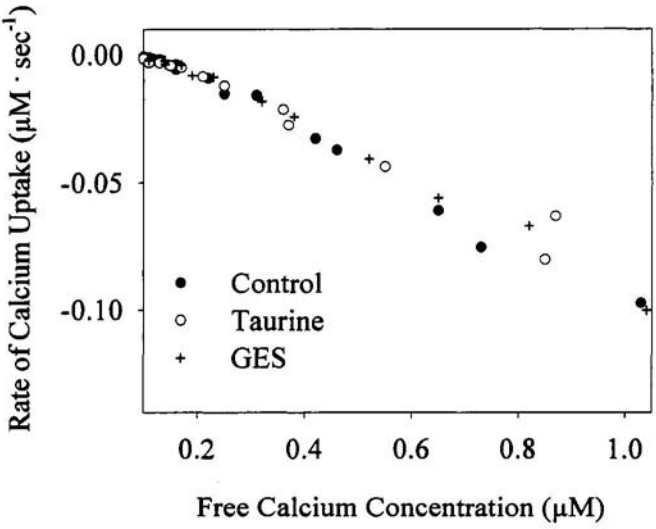

Figure 2. Rate of Ca^{2+} uptake by isolated SR vesicules in the presence of different extravesicular free Ca^{2+} concentration. The measure was conducted with the fluorescent Ca^{2+} indicator fura-2 in the presence of 20 mM taurine (o) or 20 mM GES (+). The control assay was conducted in the presence of 20 mM sucrose (●).

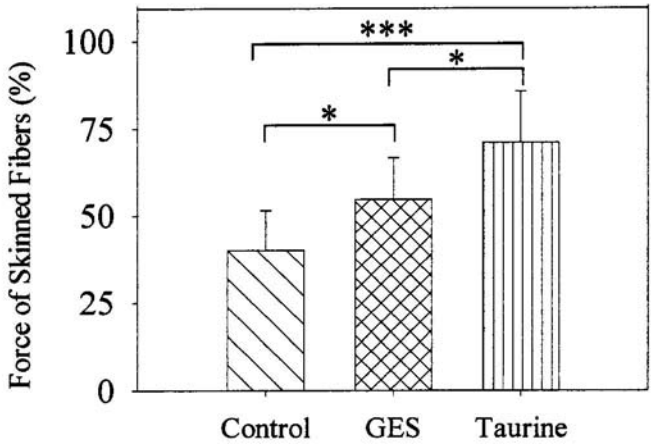

Figure 3. Force developed by bundles of chemically skinned rabbit psoas. Force was measured when fibers were activated in solutions containing 0.54 µM buffered free Ca^{2+} and 20 mM taurine or 20 mM GES. Results are expressed in percent of maximal force developed when fibers were submersed in solution containing 10 µM free Ca^{2+}. $*p < 0.05$, $***p < 0.001$.

Several muscle compartments were then isolated in order to explain this observation. First, SR vesicles were prepared as described previously. The effect of 20 mM taurine or GES on the rate of Ca^{2+} uptake by the SR was examined with the Ca^{2+} fluorescent indicator fura-2. Figure 2 shows that no difference in the rate of Ca^{2+} uptake was observed in the presence of 20 mM taurine or GES. Furthermore, effects of 20 mM taurine or GES on Ca^{2+} sensitivity of myofilaments were studied. Both taurine ($p < 0.001$) and GES ($p < 0.05$) increased the force developed by chemically skinned fibers activated in buffer solution containing 0.54 µM Ca^{2+}. The increase of myofilament sensitivity due to taurine was higher than the effect observed with GES ($p < 0.05$) (Figure 3).

DISCUSSION

Rats treated with GES show a decreased muscle taurine content (-2.1 µmol-g ww^{-1} in leg) and an increase of 2.7 µmol.g ww^{-1} in GES content[8]. The treatment also increases relaxation time of EDL muscle (Figure 1) which is consistent with a similar observation made on cardiac muscle[11]. To study the effects of GES supplementation on muscle contraction, both the effects of muscle taurine content decrease and those of GES increase have to be considered. Different targets could be responsible for this prolonged relaxation time after tetanic contraction, other than the rate of Ca^{2+}uptake by SR and the Ca^{2+} sensitivity of myofilaments. The former is not responsible

for the $t_{20\%}$ increase, as neither GES nor taurine modify the rate of Ca^{2+} uptake by the SR (Figure 2). Huxtable' reported that when extravesicular Ca^{2+} concentration was 0.1 mM, taurine increased Ca^{2+} transport activity by rat skeletal muscle SR. Remtulla *et al.*[14] has shown in cardiac muscle that taurine did not affect ATP-dependent calcium uptake. The results of the present investigation suggest that the increase in $t_{20\%}$ is due to an increase in myofilament sensitivity to Ca^{2+} (Figure 3). Physiological taurine concentrations increase myofilament sensitivity to calcium in triton-skinned trabeculae[15], in skinned skeletal muscle of crayfish[3] and in mammalian skeletal muscle (Figure 3). Figure 3 shows that GES also increases myofilament sensitivity to Ca^{2+}, although the effect of taurine is larger than that of GES. Higher values of $t_{20\%}$ observed in GES supplemented muscles cannot be due to a decrease in taurine muscle content but rather to an increase in GES content which itself can explain the increased myofilament sensitivity to Ca^{2+} and the increased relaxation time. Nevertheless, other factors, involving for example energy supply changes could also slow down muscle relaxation after GES supplementation. Decrease in heart[12] and in skeletal muscle (own unpublished observations) creatine phosphate contents have been reported. Although GES is a possible substrate for creatine kinase[6], the energy flux through this reaction is probably reduced. Clearly, these assumptions would require further investigations.

REFERENCES

1. Allen, J.D., and Moss, R.L., 1987, Factors influencing the ascending limb ofthe sarcomere length-tension relationship in rabbit skinned muscle fibres. *J. Physiol.* 390: 119-136.
2. Brenner, B., 1998, Muscle mechanics II: skinned muscle fibres. In *Current methods in muscle physiology. Advantages, problems and limitations* (H. Sugi, ed.), Oxford University Press, Oxford, pp.22-69.
3. Galler, S., Hutzler, C., and Haller, T., 1990, Effects of taurine on Ca^{2+} -dependent force development of skinned muscle fibre preparations. *J. Exp. Biol.* 152: 255-264.
4. Ganapathy, V., and Leibach, F.H., 1994, Expression and regulation of the taurine transporter of cultured cell lines of human origin. In *Taurine in Health and disease* (R. Huxtable), Plenum Press, New York, pp.51-57.
5. Grynkiewicz, G., Poenie, M., and Tsien, R.Y., 1985, A new generation of Ca^{2+}indicators with greatly improved fluorescent properties. *J. Biol. Chem.* 260: 3440-3450.
6. Huxtable, R.J., 1992, Physiological actions of taurine. *Physiol. rev.* 72: 101-163.
7. Huxtable, R., and Bressler, R., 1973, Effect of taurine on a muscle intracellular membrane. *Biochimi. Biophys. Acta* 323: 573-583.
8. Huxtable, R.J., Laird, H.E., and Lippincott, S.E., 1979, The transport of taurine in the heart and the rapid depletion of tissue taurine content by guanidinoethyl sulfonate. *J. Pharmacol. Exp. Ther.* 211: 465-471.
9. Jacobsen J.G., and Smith L.H., 1968, Biochemistry and physiology of taurine and taurine derivatives. *Physiol. Rev.* 48: 424-511.

10. Kargacin, M.E., Scheid C.R., and Honeyman T.W., 1988, Continuous monitoring of Ca2+ uptake in membrane vesicules with fura-2. *Am. J. Physiol.* 245: C694-C698.
11. Lake, N., Splawinski, J.B., Juneai, C., and Rouleau, J.L., 1990, Effects of taurine depletion on intrinsic contractility of rat ventricular papillary muscles, *Can. J Physiol. Pharmacol.* 68: 800-806.
12. Mozaffari, M.S., Tan, B.H., Lucia M.A., and Schaffer S.W., 1986, Effect of drug-induced taurine depletion on cardiac contractility and metabolism, *Biochem. Pharm.* 35: 985-989.
13. O'Brien, P.J., 1990, Calcium sequestration by isolated sarcoplasmic reticulum: real-time monitoring using radiometric dual-emission spectrofluorometry and the fluorescent calcium-binding dye indo-l. *Mol. Cell. Biochem.* 94: 113-1 19.
14. Remtulla, M.A., Katz, S., and Applegarth D.A., 1978, Effect of taurine on ATP-dependent calcium transport in guinea-pig cardiac muscle. *Life Sciences.* 23: 383-390.
15. Steele, D.S., Smith, G.L., and Miller, D.J., 1990, The effects of taurine on calcium uptake by the sarcoplasmic reticulum and calcium sensitivity of chemically skinned rat heart. *J Physiol.* 422: 499-5 1 1.