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Thienylhydrazone derivative increases sarcoplasmic reticulum Ca^{2+} release in mammalian skeletal muscle

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Abstract

3,4-Methylenedioxybenzoyl-2-thienylhydrazone (L-294) is a cardiac inotropic drug whose action is mediated by an increase in intracellular Ca^{2+} concentration as a result of enhanced Ca^{2+} accumulation in the sarcoplasmic reticulum. In the present study we tested whether this new thienylhydrazone derivative was effective in mammalian skeletal muscle. We investigated the effect of L-294 on the contractility of isolated skeletal muscle, on Ca^{2+} uptake and release by sarcoplasmic reticulum in skinned fibers and in membrane vesicles. L-294 increased in a dose-dependent manner tension of isolated rat soleus muscle. In skinned type I fibers, L-294 induced tension and did not alter sarcoplasmic reticulum loading with Ca^{2+}. L-294 reduced the threshold Ca^{2+} to induce Ca^{2+} release and did not affect the ATP-dependent accumulation of Ca^{2+} by sarcoplasmic reticulum vesicles. These results suggest that L-294 is an inotropic agent in skeletal muscle through an increase in the amount of Ca^{2+} released from the sarcoplasmic reticulum.

Keywords: LASSBio 294 (L-294); Skeletal muscle; Contractility; Ca^{2+}; Sarcoplasmic reticulum

1. Introduction

The new thienylhydrazine derivative, 3,4-methylenedioxybenzoyl-2-thienyl hydrazine (Fig. 1), named LASSBio 294 (L-294), is a positive cardiac inotropic agent whose mechanism is related to increased intracellular Ca^{2+} concentration in mammalian cardiac muscle (Sudo et al., 2001). The improvement of cardiac contractility by L-294 is due to an increase in the content of Ca^{2+} accumulated in the sarcoplasmic reticulum and could be useful for the treatment of mechanical dysfunction present in clinical syndromes of chronic heart failure. Chronic heart failure is also associated with abnormalities in skeletal muscle function (Drexler et al., 1992; Magnusson et al., 1996) that contribute to exercise intolerance and ventilatory failure (Mancini et al., 1992; McParland et al., 1995). Alterations in molecular and histological profiles could explain the impaired excitation–contraction coupling (ECC) of skeletal muscle in chronic heart failure. One finding is the increased proportion of slow-twitch fibers contributing to decreased force-generating capacity in skeletal muscle (Sieck and Fournier, 1989). Another finding is the decreased amount of Ca^{2+} available for force development consequent to a reduced activity of the sarcoplasmic Ca^{2+}-ATPase (Simonini et al., 1999). Since L-294 improved the contractile response in cardiac muscle, we hypothesized that this new agent could also affect skeletal muscle. The purpose of the present study was: (1) to determine whether L-294 has an inotropic effect on isolated slow-twitch skeletal muscle from Wistar rats; (2) to investigate the mechanism underlying that effect by examining Ca^{2+} uptake and release in chemically skinned type I fibers

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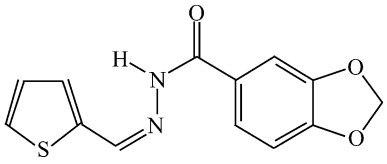

Fig. 1. Chemical structure of 3,4-methylenedioxybenzoyl-2-thienylhydrazone, LASSBio 294 (L-294).

and sarcoplasmic reticulum membrane vesicles from skeletal muscle.

2. Materials and methods

2.1. Recording of isometric tension in isolated soleus muscle

These experiments were performed in accordance with the European Community Guidelines and the Animal Care and Use Committee at the Universidade Federal do Rio de Janeiro. Male Wistar rats (250–350 g) were anesthetized with ether and soleus muscles were dissected for isometric tension recording. One end of each muscle was attached to a force transducer (Grass, model FT-03) and the other end to a hook fixed at the bottom of the experimental chamber. The transducer signal was conditioned by a Cyberamp (Axon Instruments), digitized by a Digidata 1200 (Axon Instruments) and then displayed and stored on a computer for analysis using Axoscope software (Axon Instruments). The chamber was filled with Tyrode solution composed in mM of NaCl, 135; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 2.5; $NaHCO_3$, 15; Na_2HPO_4, 1; dextrose, 11 and oxygenated with carbogen gas (95%O_2 plus 5%CO_2) at 35.0° C. Muscles were field stimulated at 0.2 Hz. In one of the soleus, muscle twitches were observed before and after addition of increasing concentrations of L-294 (50–500 μM) to the Tyrode solution. L-294 was prepared in a stock solution of 50 mM dissolved in dimethyl sulfoxide (DMSO). A simultaneous control experiment was performed with the other soleus of the same animal to which DMSO alone was added to certify that it did not affect muscle contractility. Nine muscles were exposed to increasing concentrations of L-294 ranging from 50 to 500 μM.

2.2. Skinned muscle fiber preparation

The experimental protocol for the use of human tissue was approved by our Institutional Committee. Human skeletal muscle was obtained from consenting patients undergoing diagnostic muscle biopsies at the Brazilian Malignant Hyperthermia Diagnostic Center for contracture phenotyping protocol. Muscle fascicles were collected from negative phenotyped young adult male individuals (16–24 years) known to lack pre-existing skeletal muscle disease. Small bundles of human muscle (10 mm, length; 2 mm, width) were dissected and immersed in a relaxing solution containing a low Ca^{2+} concentration (composition below) at 2 °C for 24 h to obtain skinned skeletal fibers. This treatment destroys the sarcolemma and keeps the contractile proteins and sarcoplasmic reticulum membrane intact (Eastwood et al., 1979). After this procedure, the bundles were transferred to a relaxing solution with 50% glycerol v/v and stored at − 20 °C until tested. The ends of a single fiber were attached to two hooks, one connected to a micromanipulator and the other to a force transducer (Grass, model FT-03). The isometric tension was recorded with a Grass polygraph (model 7400). Throughout all experiments, the temperature was maintained at 22 ± 0.5 °C through a system composed of a temperature probe (Yellow spring, model 427) and a metal tube positioned inside the chamber. The probe was connected to a pump which perfused pre-chilled solution inside the metal tube when the temperature increased. The skinned fibers were activated by exposure to a washing solution containing 0.5 mM $CaCl_2$ to develop maximal tension followed by exposure to a relaxing solution to restore baseline tension. The composition of washing solution was the following (in mM): potassium propionate, 185; magnesium acetate, 2.5; imidazole propionate 10 and K_2Na_2ATP, 2.5 (pH 7.0). The relaxing solution had the same composition except that it contained 5 mM of 5 K_2-ethylene glycol-bis(beta-amino-ethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA). To investigate the effect of L-294 on type I fibers, we classified the skeletal fibers according to their response to

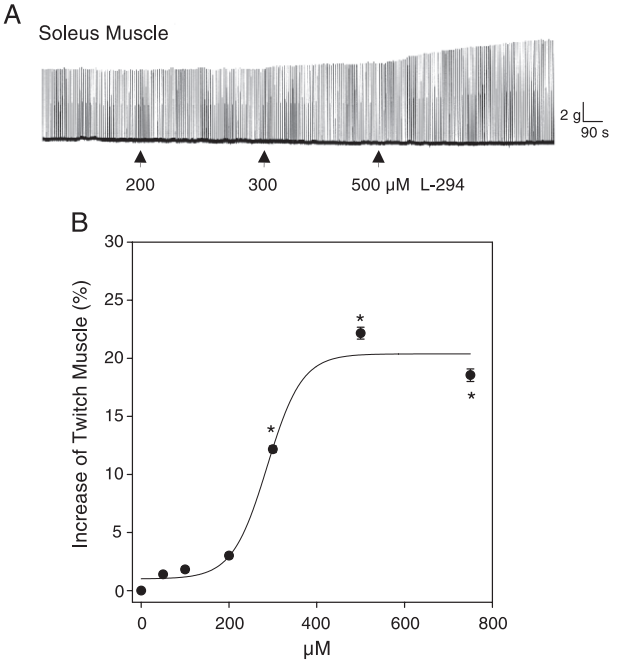

Fig. 2. Effect of L-294 exposure on the contractile response of isolated skeletal muscle. (A) Experimental tracing of twitches in a rat soleus muscle induced by field stimulation at 0.2 Hz before and after treatment with increasing concentrations of L-294. Arrows indicate the addition of the indicated concentration of L-294. (B) Relation between the increase in muscle twitches and L-294 concentration for nine experiments. Data shown represent means ± S.E.M. *$P < 0.05$.

solutions of pSr 5.2 and 4.4. Only type I fibers develop tension in the presence of pSr 5.2 and 4.4. These solutions consisted of (mM): potassium propionate, 152; imidazole propionate 10; K_2Na_2ATP, 2.5; magnesium acetate, 6.46 and 6.36 for pSr 5.2 and 4.4; K_2EGTA, 4.55 and 3.27; CaEGTA, 0.45 and 1.7; Sr^+ 0.0063 and 0.039 (pH 7.0). The pSr and pCa levels were based on appropriate mixtures of CaEGTA/K_2EGTA, calculated by the computer program described by Fabiato and Fabiato (1979) using the constants provided by Orentlicher et al. (1977). Dose–response curves for L-294-mediated Ca^{2+} release were obtained by exposing the skinned fibers to increasing concentrations of L-294 (25–200 μM) after sarcoplasmic reticulum preload. The same protocol was repeated in the presence of DMSO alone at the same concentration when in the presence of L-294. The *p*Ca of the solution used to load Ca^{2+} into the sarcoplasmic reticulum was 7.0. The solution was composed of (mM): potassium propionate, 172; magnesium acetate, 2.5; imidazole propionate 10; K_2Na_2ATP, 2.5; K_2EGTA, 4.2; CaEGTA, 8 (pH 7.0). Ca^{2+} loading into sarcoplasmic reticulum was evaluated by the tension induced by 20 mM caffeine after sarcoplasmic reticulum preload with a solution of *p*Ca 7.0 for 3 min (Figs. 2A and 3A).

2.3. Measuring Ca^{2+} release and uptake by sarcoplasmic reticulum membrane vesicles

The ryanodine receptor Ca^{2+} release channel of skeletal muscle (RyR1) is similar among humans, pigs, and rabbits in terms of its sensitivity to Ca^{2+} concentration, caffeine, activation by ATP, etc., suggesting that its mode of regulation may be similar among these species. So we decided to isolate a heavy sarcoplasmic reticulum fraction with high levels of ryanodine receptor protein from porcine longissimus dorsi muscle, which has been thoroughly characterized for the Ca^{2+} release protocol. According to methods previously described (Nelson, 1983; Zapata-Sudo et al., 1997), the heavy sarcoplasmic reticulum fraction pellet was stored at a final concentration of 20–30 mg/ml protein at −80 °C. For the Ca^{2+} induced Ca^{2+} release experiments, heavy fraction (50 μg) was added to a solution that contained in mM: KCl, 150; HEPES, 20 (pH 6.8); NaN_3, 5; adenosine triphosphate, 1; phosphocreatine, 5; creatine kinase, 20 μg/ml and arsenazo III, 0.02. Then, aliquots of $CaCl_2$ (5 nmol) were placed into the cuvette until the threshold for Ca^{2+} release at 30 °C was reached (Nelson and Nelson, 1990) (Fig. 6).

The rate of Ca^{2+} uptake into a light sarcoplasmic reticulum fraction was determined as previously described (Nelson et al., 1991). Briefly, the cuvette was filled with solution that contained in mM: KCl, 150; HEPES, 20 (pH 6.8); NaN_3, 5; K oxalate, 5; MgATP, 5; ruthenium red, 0.005; arsenazo III, 0.02. After introduction of light fraction vesicles (50 μg), 75 nmol of Ca^{2+} was added to the cuvette to start ATP-dependent uptake. Changes in the Ca^{2+} concentration for both protocols were determined as the difference in absorbance between 540 and 650 nm with a spectrophotometer (Beckmann DU 7500) using the indica-

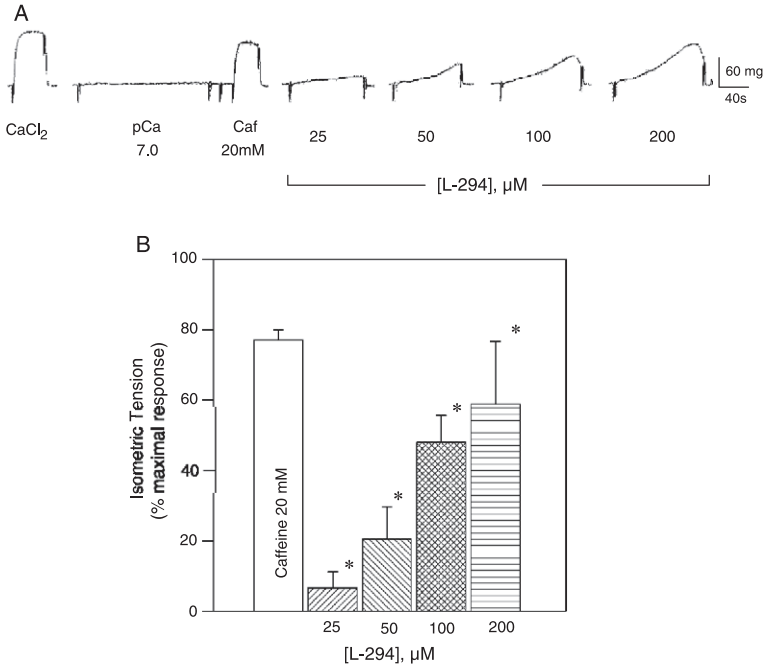

Fig. 3. L-294 induced tension in chemically skinned human skeletal fibers. (A) Maximal tension was induced by exposure to $CaCl_2$ (0.5 mM) followed by relaxation in the presence of relaxing solution containing a high concentration of EGTA. Any Ca^{2+} stored in the sarcoplasmic reticulum was released with 20 mM caffeine in relaxing solution. Then, the fiber was exposed twice to washing solution and after sarcoplasmic reticulum loading with Ca^{2+} (*p*Ca 7.0) for 3 min, contractures were observed in the presence of caffeine (20 mM) or L-294 (25–200 μM). (B) Bars represent the amplitudes of tension induced by caffeine and L-294 relative to the maximal activated response to $CaCl_2$. Data represent the means ± S.E.M. of nine experiments.

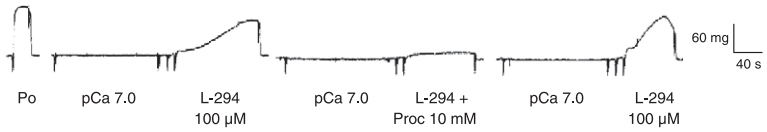

Fig. 4. Ca^{2+} release from sarcoplasmic reticulum induced by LASSBio 294 (L-294) in a skeletal skinned fiber. Representative tracing showing the maximal response (Po) of the skinned fiber and the L-294-induced tension after sarcoplasmic reticulum loading with Ca^{2+} in the absence and presence of procaine (10 mM). The loading cycle was initiated by exposure to a solution of *p*Ca 7.0 for 3 min followed by two washes with wash solution. Note that procaine prevented Ca^{2+} release from sarcoplasmic reticulum induced by L-294.

tor dye arsenazo III. The same assays were used in the absence and presence of L-294, 100 μM.

2.4. Statistics

All data are expressed as means ± S.E.M. and differences between means were evaluated by Student's *t*-test with statistical significance when $P < 0.05$.

3. Results

3.1. Effect of L-294 on contractility of isolated soleus muscle

Fig. 2A shows representative isometric twitches recorded from soleus muscle at 0.2 Hz stimulation in the absence and presence of increasing concentrations of L-294. An increase in peak tension was observed with 300 and 500 μM L-294. The average results from nine muscles are shown in Fig. 2B. The inotropic effect was dependent on L-294 concentration and the maximal response was observed at 500 μM, with a significant ($P < 0.05$) increase of 22.2 ± 0.4%. The contraction and relaxation kinetics were compared before and after exposure to L-294. The contraction time was slower in the presence of 500 μM L-294. At that concentration, time to peak contraction increased significantly from 36.0 ± 2.7 to 54.0 ± 5.9 ms ($P < 0.01$). The decay times were similar with 168.3 ± 28.6 and 184.3 ± 20.0 ms in the absence and presence of L-294 (500 μM), respectively. L-294 effects on contractility were totally reversible after washout of the preparations.

3.2. Effect of L-294 on Ca^{2+} loading and release from sarcoplasmic reticulum in skinned, human skeletal muscle fibers

Fig. 3A shows a representative recording indicating that the functions of the contractile system and the sarcoplasmic reticulum were intact after chemical skinning of skeletal fibers. Initially, the single fiber was exposed to $CaCl_2$ (0.5 mM) to induce a maximal contractile response. After the sarcoplasmic reticulum was preloaded with solution containing 0.1 μM Ca^{2+} (*p*Ca 7.0) for 3 min, caffeine (20 mM) induced tension, the amplitude of which corresponded to the amount of Ca^{2+} accumulated in the sarcoplasmic reticulum. Exposure of the Ca^{2+} preloaded fiber to L-294 (25–200 μM) induced a dose-dependent contracture. This effect of L-294 was totally reversed after washout (not shown). L-294, 100 μM, produced tension 48.0 ± 7.7% of maximal response (Fig. 3B). No response was observed in the presence of DMSO alone. To confirm that L-294 could induce Ca^{2+}

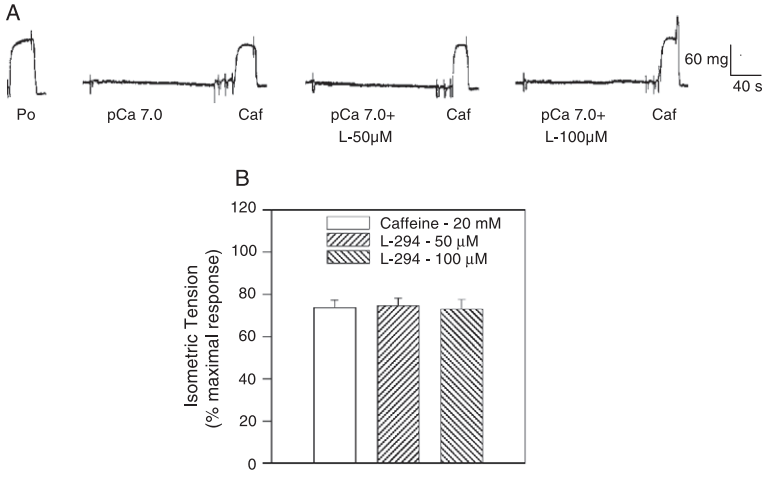

Fig. 5. Effect of L-294 on Ca^{2+} loading into the sarcoplasmic reticulum. (A) Representative tracing showing the maximal response (Po) of the skinned fiber and the caffeine-induced tension after sarcoplasmic reticulum loading with Ca^{2+} in the absence or presence of L-294. (B) Summarized data for the lack of effect of L-294 on the uptake of Ca^{2+} into the sarcoplasmic reticulum. Values are means ± S.E.M. of nine experiments.

release from sarcoplasmic reticulum, we observed its effect in the presence of procaine (10 mM), which is recognized as an effective inhibitor of Ca^{2+} release through skeletal muscle RyR1 (Fig. 4). L-294-induced contractures were completely inhibited in the presence of procaine.

The Ca^{2+} loaded into the sarcoplasmic reticulum was estimated by the caffeine response of the fibers after sarcoplasmic reticulum preload with Ca^{2+} for 3 min. In the trace shown in Fig. 5A, caffeine (20 mM) induced a contracture which was 80% of the maximal response of the fiber. The caffeine-induced tension was not altered when L-294 (50–100 μM) was present during sarcoplasmic reticulum loading with Ca^{2+} (Fig. 5B). This finding indicates that L-294 did not affect the maximal amount of Ca^{2+} loaded into the sarcoplasmic reticulum.

3.3. Effect of L-294 on Ca^{2+} release and uptake in sarcoplasmic reticulum membrane vesicles

We next tested whether L-294 altered Ca^{2+} release through the RyR1 present in the sarcoplasmic reticulum membranes. Under control conditions, Ca^{2+} release from the skeletal heavy sarcoplasmic reticulum fraction was induced following a Ca^{2+} preload of 72.5 ± 2.5 (n = 6) nmol Ca^{2+}/mg protein. A significant reduction in the amount of Ca^{2+} preload required for Ca^{2+} release was observed in the presence of L-294 (Fig. 6). At L-294, 50 μM, the threshold was reduced to 52.5 ± 2.5 nmol Ca^{2+}/mg protein (n = 6). No further significant decrease in Ca^{2+} preload for Ca^{2+} release was observed at concentrations higher than 50 μM. At 500 μM, Ca^{2+} release was induced by 55.0 ± 5.0 nmol Ca^{2+}/mg protein (n = 6).

To examine L-294 effects on Ca^{2+} pump activity, we compared ATP-dependent Ca^{2+} accumulation in the absence and presence of the drug. In light sarcoplasmic reticulum fraction, the rate of Ca^{2+} transport was not significantly altered by L-294 from 50 to 500 μM. The Ca^{2+}-loading rate of the skeletal sarcoplasmic reticulum vesicle was 18.3 ± 0.8 (n = 12) and 20.2 ± 1.2 (n = 6) nmol Ca^{2+}/mg protein/s in the absence and presence of 500 μM L-294, respectively. These results suggest that the increase in the contractile response of skeletal muscle induced by L-294 was not mediated by the Ca^{2+} transport ATPase but possibly by an increase in the release of Ca^{2+} from the sarcoplasmic reticulum. DMSO alone did not interfere with the uptake or the release of Ca^{2+} from sarcoplasmic reticulum vesicles.

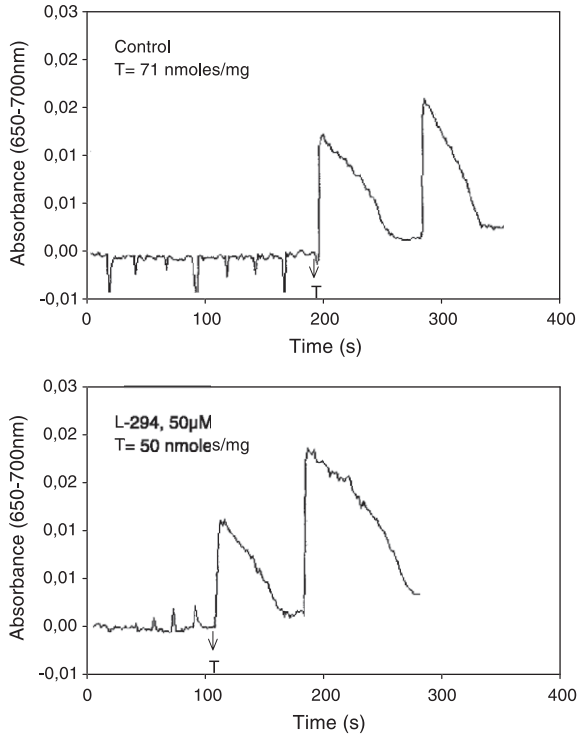

Fig. 6. Effect of L-294 on the calcium load threshold (T) for the pig sarcoplasmic reticulum membrane preparation. Upper panel: Representative tracing of Ca^{2+} release from isolated sarcoplasmic reticulum membrane vesicles. Serial additions of Ca^{2+} were followed by uptake of Ca^{2+} until the Ca^{2+} release channel was activated. The opening of the Ca^{2+} release channels induced by a critical amount of Ca^{2+} loaded in the sarcoplasmic reticulum lumen was followed by Ca^{2+} release and its subsequent return to the sarcoplasmic reticulum lumen by SERCA1. Lower Panel: Same procedure was repeated in the presence of 50 μM L-294. Note that L-294 reduced T, indicating that it can increase the sensitivity of Ca^{2+} receptors to Ca^{2+}.

4. Discussion

In a previous study we demonstrated that the safrole derivative, L-294, is a novel potent cardioinotropic compound that could have a beneficial effects in conditions of ventricular dysfunction (Sudo et al., 2001). We hypothesized that the inotropic effect of L-294 in cardiac muscle was due to a change in the process of Ca^{2+} loading into the sarcoplasmic reticulum. As a consequence, the amount of Ca^{2+} that could be released from the sarcoplasmic reticulum into the sarcoplasm during the excitation–contraction coupling process (ECC) was increased. The potential clinical advantage of L-294 over other classical cardioinotropic agents is the stability of EKG parameters demonstrated in isolated rat hearts and in vivo dogs experiments (Sudo et al., 2001).

Skeletal muscle fatigue is an early and very frequent symptom that occurs simultaneously with chronic heart failure. The mechanism of fatigue is not completely understood but is probably related to an intrinsic alteration of ECC in skeletal muscle fibers. The recent correlation of muscle fatigue with chronic heart failure and the finding that L-294 is a potent cardioinotropic agent led us to investigate the effect of this drug in mammalian skeletal muscle. As we previously observed in the heart (Sudo et al., 2001), twitches of rat soleus muscle were increased in the presence of L-294. However, the maximal effect was smaller than that achieved in ventricular muscle (ca. 22% versus 100% increase above control). The effect of L-294 on skeletal

muscle is in agreement with the previous finding described by Gonzalez-Serratos et al. (2001) in single muscle fiber from *Rana pipiens*. Those authors demonstrated that the increase in muscle force induced by L-294 was dose and frequency-dependent. At 0.1 Hz, 25 μM L-294, increased muscle twitches to about 30% of control but this effect was not observed at frequencies higher than 30 Hz. An interesting finding of this study is related to the reduction of muscle fatigue in the presence of L-294. The time of decline of muscle maximal tension in response to repetitive short tetanic stimulation was prolonged by L-294 in a dose-dependent manner. Also, recovery from fatigue tetanic stimulation was significantly accelerated by L-294 (Gonzalez-Serratos et al., 2001).

Our findings in cardiac muscle showed the possible role of the sarcoplasmic reticulum transport process in the increased contractile response induced by L-294. The effects of L-294 on the Ca^{2+} transport function of the Ca^{2+} pump of skeletal muscle and on the Ca^{2+} release channel were indirectly evaluated in skinned fibers and sarcoplasmic reticulum membrane vesicles preparations. L-294 did not change the Ca^{2+} uptake rate by sarcoplasmic or endoplasmic reticulum type Ca^{2+} pumps (SERCA1) in light sarcoplasmic reticulum fraction vesicles and also did not interfere with the caffeine response of skinned fibers when present during sarcoplasmic reticulum loading with Ca^{2+}. Thus, alteration of Ca^{2+} transport into the sarcoplasmic reticulum and the total amount of Ca^{2+} available for muscle contraction is unlikely to be responsible for the observed L-294 effects. One possible action of L-294 on skeletal muscle is to alter the gating properties of the RyR1. Our results tend to support this site of action because L-294 alone induced tension in sarcoplasmic reticulum Ca^{2+}-preloaded skinned fibers. This L-294-induced tension was abolished in the presence of procaine, a local anesthetic that inhibits Ca^{2+} release from sarcoplasmic reticulum. So, the increase in the contractile force induced by L-294 can be attributed to a release of Ca^{2+} from sarcoplasmic reticulum.

Another interesting observation was the slow time course of tension induced by L-294 in skinned fibers (see Fig. 3A). Several compounds interacting with RyR1 open the Ca^{2+} channel at different rates that can be explained by different mechanisms. As an example, the rate of binding of the natural compound ryanodine is slow and can be modified by previous activation of the Ca^{2+} release channels (Sudo and Nelson, 1997). The binding of caffeine to this receptor is faster. We assume that the binding rate of L-294 to RyR1 is slower than that of caffeine, and that the simultaneous activation of Ca-ATPase causes a slow increase in tension in the presence of L-294. The effects of L-294 in intact or in skinned fiber preparations were completely reversed after washout. These results suggest that the association rate is slow and the dissociation rate is fast when compared to that of ryanodine binding to RyR1 (Sudo and Nelson, 1997).

In summary, we hypothesize that L-294 could be considered an important compound for the treatment of chronic heart failure due to a combination of cardio and skeletal muscle inotropic effects and also by reducing symptoms of fatigue. The mechanism of action of L-294 in cardiac cells was to increase Ca^{2+} accumulation in the sarcoplasmic reticulum and in skeletal muscle to Ca^{2+} release from the sarcoplasmic reticulum into the sarcoplasm. Complementary studies should be performed to clarify the exact site and nature of the interaction between L-294 and RyR1.

Acknowledgements

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References

Drexler, H., Riede, U., Munzel, T., Konig, H., Funke, E., Just, H., 1992. Alterations of skeletal muscle in chronic heart failure. Circulation 85, 1751–1759.

Eastwood, A.D., Wood, D.S., Bock, K.L., Sorenson, M.M., 1979. Chemically skinned mammalian skeletal muscle. I. The structure of skinned rabbit psoas. Tissue Cell. 11, 553–566.

Fabiato, A., Fabiato, F., 1979. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J. Physiol. 75, 463–505 (Paris).

Gonzalez-Serratos, H., Chang, R., Pereira, E.F.R., Castro, N.G., Aracava, Y., Melo, P.A., Lima, P.C., Fraga, C.A.M., Barreiro, E.J., Albuquerque, E.X., 2001. A novel thienylhydrazone, (2-thyenylidene)3,4-methylenedioxylhydrazine, increases inotropism and decreases fatigue of skeletal muscle. J. Pharmacol. Exp. Ther. 299, 558–566.

Magnusson, G., Kaijser, L., Rong, H., Isberg, B., Sylven, C., Saltin, B., 1996. Exercise capacity in heart failure patients: relative importance of heart and skeletal muscle. Clin. Physiol. 16, 183–195.

Mancini, D.M., Henson, D., Lamanca, J., Levine, S., 1992. Respiratory muscle function and dyspnea in patients with chronic congestive heart failure. Circulation 86, 909–918.

McParland, C., Resch, E.F., Krishnan, B., Wang, Y.M., Cujec, B., Gallagher, C.G., 1995. Inspiratory muscle weakness in chronic heart failure-role of nutrition and electrolyte status and systemic myopathy. Am. J. Respir. Crit. Care Med. 151, 1101–1107.

Nelson, T.E., 1983. Abnormality in calcium release from skeletal sarcoplasmic reticulum of pigs susceptible to malignant hyperthermia. J. Clin. Invest. 72, 862–870.

Nelson, T.E., Nelson, K.E., 1990. Intra- and extraluminal sarcoplasmic reticulum membrane regulatory sites for Ca^{2+}-induced Ca^{2+} release. FEBS Lett. 263, 92–294.

Nelson, T.E., Lin, M., Volpe, P., 1991. Evidence for intraluminal Ca^{2+} regulatory site defect in the sarcoplasmic reticulum from malignant hyperthermia pig muscle. J. Pharmacol. Exp. Ther. 256, 645–649.

Orentlicher, M., Brandt, P.W., Reuben, J.P., 1977. Regulation of tension in skinned muscle fibers: effect of high concentrations of Mg-ATP. Am. J. Physiol. 233, C127–C134.

Sieck, G.C., Fournier, M., 1989. Diaphragm motor unit recruitment during ventilatory and nonventilatory behaviors. J. Appl. Physiol. 66, 2539–2545.

Simonini, A., Chang, K., Yue, P., Long, C.S., Massie, B.M., 1999. Expression of skeletal muscle sarcoplasmic reticulum calcium-ATPase is reduced in rats with postinfarction heart failure. Heart 81, 303–307.

Sudo, R.T., Nelson, T.E., 1997. Changes in ryanodine-induced contractures by stimulus frequency in MHS and MHN dog skeletal muscle. J. Pharmacol. Exp. Ther. 282, 1331–1336.

Sudo, R.T., Zapata-Sudo, G., Barreiro, E.J., 2001. The new compound, LASSBio 294, increases the contractility of intact and saponin-skinned cardiac muscle from Wistar rats. Br. J. Pharmacol. 134, 603–613.

Zapata-Sudo, G., Sudo, R.T., Lin, M., Nelson, T.E., 1997. Calcium-sensitizing function for the dipeptide carnosine in skeletal muscle contractility. Cell. Physiol. Biochem. 7, 81–92.