Department of Biology[1], School of Medicine, Tehran University of Medical Sciences, Tehran, and Department of Pharmacology[2], Faculty of Science, Bu Ali Sina University, Hamedan, Iran

High sensitivity of chicken's skeletal muscle sarcoplasmatic reticulum to effects of diltiazem or verapamil on calcium uptake and release

N. Mirazi[1], M. J. Paydar[2], L. Vali[2], A. R. Dehpour[2]

Received July 25, 2005, accepted September 6, 2005

N. Mirazi, Assistant Professor, Department of Biology, Faculty of Science, Bu Ali Sina University, Hamedan, Iran
mirazi@basu.ac.ir

Pharmazie 61: 625–630 (2006)

In this study, the effects of two different calcium channel blockers, diltiazem and verapamil on calcium uptake and release from the membrane of heavy sarcoplasmic reticulum (SR) of chicken skeletal muscle were investigated. A fluorescent chelate probe technique was employed to determine calcium movement through the SR. Chlortetracycline was used as a fluorescent indicator which is able to penetrate the membrane, bind to the calcium on the inner face of the membrane and show an increase in fluorescence intensity when calcium uptake occurs. Addition of tris-ATP to the microsomes caused ATP-induced calcium uptake in a concentration dependent manner with half-maximal calcium uptake around 0.126 mM. Pretreatment of the medium containing sarcoplasmic reticulum with different concentrations of diltiazem or verapamil followed by added tris-ATP resulted a significant decrease in the fluorescence intensity of chlortetracycline, showing that these calcium channel blockers can diminish ATP-induced calcium uptake in a concentration-dependent manner. The maximum fluorescence intensity of tris-ATP falls to 50% in the presence of 1.75 μM diltiazem and 25 nM verapamil. In addition, diltiazem and verapamil can significantly induce rapid calcium release from the membrane of sarcoplasmic reticulum in a concentration-dependent manner. Therefore, membrane-bound or sequestered calcium in the sarcoplasmic reticulum may be targeted by these two calcium channel blockers in chicken skeletal muscle. Chicken SR is about 1000 times more sensitive to the effects of diltiazem on Ca^{2+} uptake and release than rabbit SR as shown previously.

1. Introduction

The availability of free calcium (Ca^{2+}) in the cytosol is determined by Ca^{2+} release and uptake by intracellular Ca^{2+} store sites such as sarcoplasmic reticulum (SR) and mitochondria, as well as by Ca^{2+} influx and efflux across cell membrane (Fusi et al. 2001; Gilchrist et al. 2003). Contraction and relaxation of skeletal muscle is regulated by rapid Ca^{2+} release and subsequent reuptake, respectively, from the SR (Abramson et al. 1993; Stoyanovsky et al. 1994). The coupling between the stimulation of a skeletal muscle cell by its motoneuron and the contractile response occurs through a unique mechanism, called excitation contraction (EC) coupling, in which depolarization of the plasma membrane triggers the release of Ca^{2+} from the SR (Gailly 2002).

The Ca^{2+}-ATPase in the intracellular Ca^{2+} store sites acts as an ion pump that transports Ca from the cytoplasm into the vesicular lumen to maintain a high gradient of Ca^{2+} across the membrane (Fusi et al. 2001; Gailly 2002; Gilchrist et al. 2003). Ca^{2+}-ATPase of skeletal muscle SR catalyses the most important step in muscle relaxation by coupling cleavage of ATP to transport of two Ca^{2+} ions into the lumen of SR (Abramson et al. 1993; Fusi et al. 2001; Stoyanovsky et al. 1994; Wingertzahn and Ochs 1998). Its activity largely determines the relaxation rate of the muscle and the energy requirement of the process accounts for up to 50% of the total ATP turnover during muscle activity (Simonides and Hardeveld 1990). Alterations in the total amount or specific activity of the SR Ca^{2+}-ATPase may be involved in various conditions and disorders that affect muscle performance and metabolism, such as malignant hyperthermia, thyroid dysfunction, muscular dystrophy and several forms of cardiac hypertrophy and failure (Simonides and Hardeveld 1990).

Ca^{2+} channel antagonists such as nifedipine, verapamil and diltiazem belong to three representative chemical classes: 1,4-dihydropyridines, phenylalkylamines and 1,5-benzothiazepines (Hagiwara et al. 2003). They are known to bind to distinct binding sites within the α_1 subunit of the L-type Ca^{2+} channel and to have a reciprocal allosteric interaction (Hagiwara et al. 2003). Ca^{2+} channel antagonists have also been used as specific probes for the pharmacological and structural characterization of Ca^{2+} channels (Hagiwara et al. 2003). To examine the role of extracellular Ca^{2+} in skeletal muscle contraction, numerous studies have been carried out with Ca^{2+} channel blockers (Curtis 1994). One of the approaches to revealing

the activity of Ca^{2+} channels is to follow Ca^{2+} translocation across the SR.

In our previous research, it was shown that diltiazem induces a rapid decrease in Ca^{2+} uptake in SR of rabbit skeletal muscle (Dehpour et al. 1998). In addition, it can accelerate the release of Ca^{2+} from the inner surface of SR (Dehpour et al. 1998). However, despite extensive studies both *in vitro* and *in vivo*, the underlying mechanism responsible for Ca^{2+} uptake and release from the SR in skeletal muscle is unknown. The theories have one thing in common: efflux of Ca^{2+} from the SR is presumed to be mediated by a Ca^{2+} channel distinct from the Ca^{2+} pump (Fill and Copello 2002; Martonosi and Pikula 2003).

In this research, we followed Ca^{2+} uptake and release in terms of chlortetracycline (CTC) fluorescence to investigate the role of diltiazem and verapamil on isolated SR from chicken thigh muscle. We investigated the sensitivity of SR vesicles chicken skeletal muscle to the effects of Ca^{2+} channel blockers in comparison with the behavior of mammalian SR.

2. Investigations and results

The data were analyzed on the assumption that the increase in fluorescence intensity is proportional to the amount of the divalent cations inside the SR vesicles. CTC was used as a fluorescent chelate probe, which penetrates the membrane, binds to the Ca^{2+} accumulated on the inner side of the membrane and produces high intensity fluorescence (Jacob et al. 2003; Renard-Rooney et al. 1993).

Addition of a range of different concentrations of tris-ATP triggers concentration-dependent Ca^{2+} uptake by SR. The concentration of tris-ATP necessary for half-maximal Ca^{2+} uptake in SR vesicles is about 126 μM and maximum fluorescence intensity is induced by 0.5 mM tris-ATP (Fig. 1).

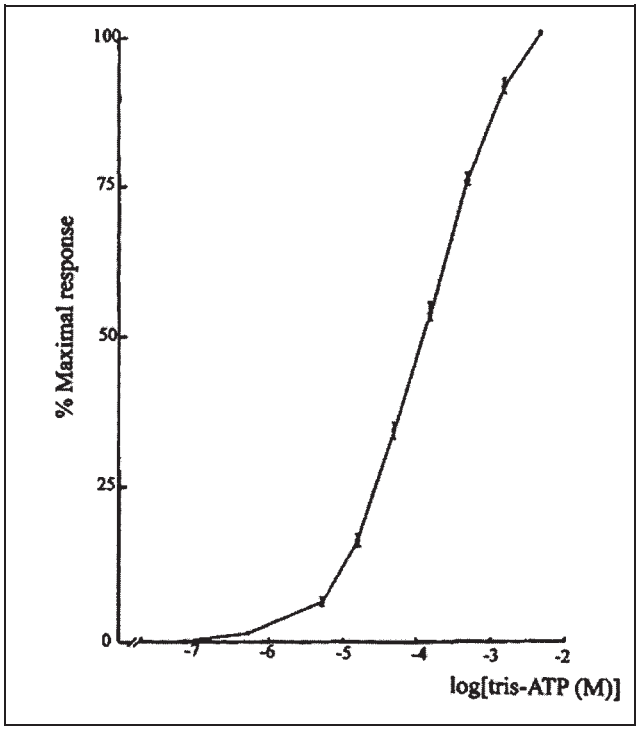

Fig. 1: Log concentration-response curve for the effects of 0.5 mM tris-ATP on calcium uptake by sarcoplasmic reticulum of chicken skeletal muscle. Half-maximal response is seen in 0.126 mM of tris-ATP. Shown as mean ± SEM (n = 6)

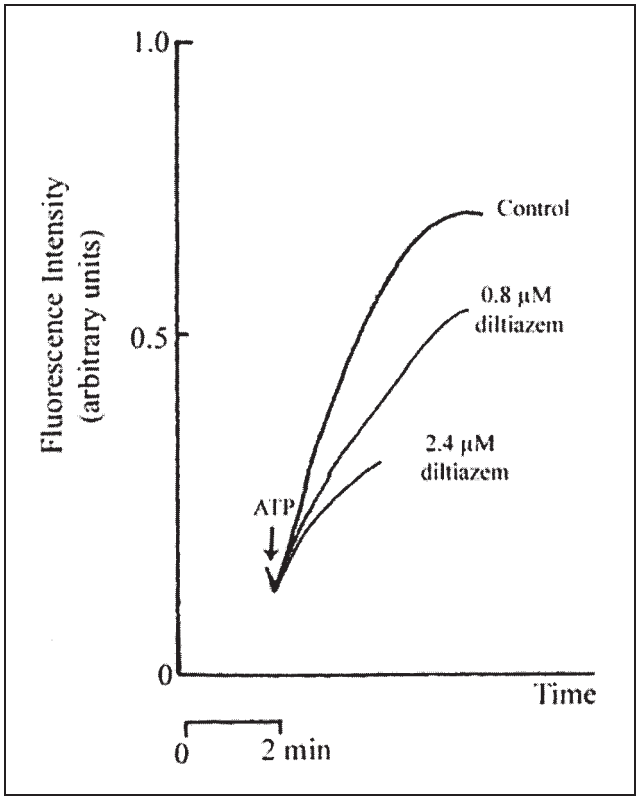

Fig. 2: The maximal calcium accumulation triggered by 0.5 mM tris-ATP decreases in SR vesicles pretreated with diltiazem. The medium contains either distilled water or diltiazem (0.8, 2.4 μM)

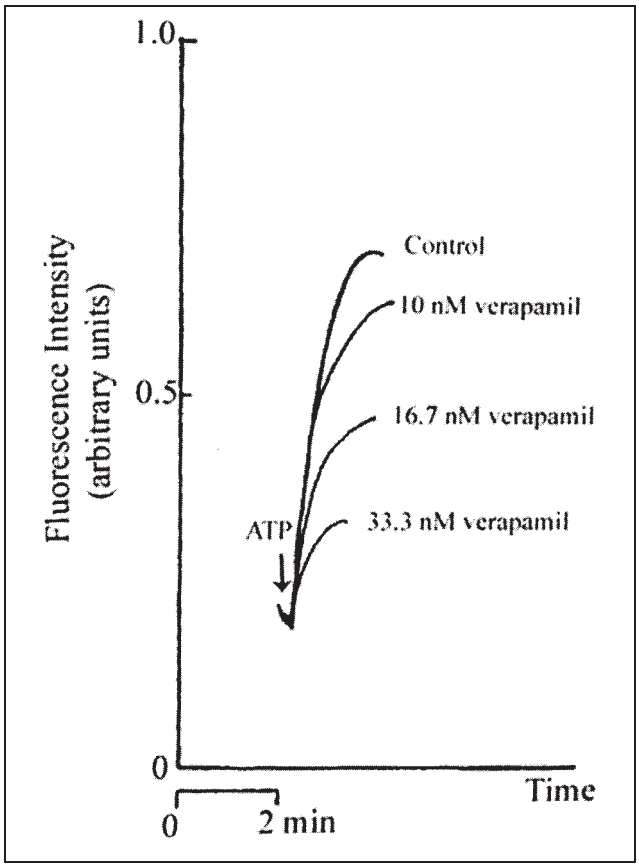

Fig. 3: The maximal calcium accumulation triggered by 0.5 mM tris-ATP decreases in SR vesicles pretreated with verapamil. The medium contains either distilled water or verapamil (10, 16.7, 33.3 nM)

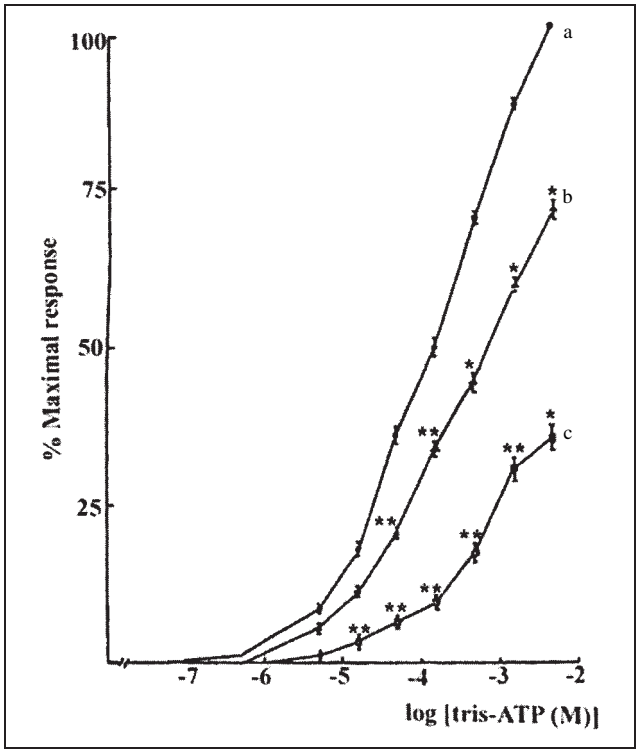

Fig. 4: Log concentration-response curves of tris-ATP effects on calcium accumulation:
a) in the absence of diltiazem
b) in the presence of 0.8 μM diltiazem
c) in the presence of 2.4 μM diltiazem
* $P < 0.05$ and ** $P < 0.01$
Shown as mean ± SEM (n = 6)

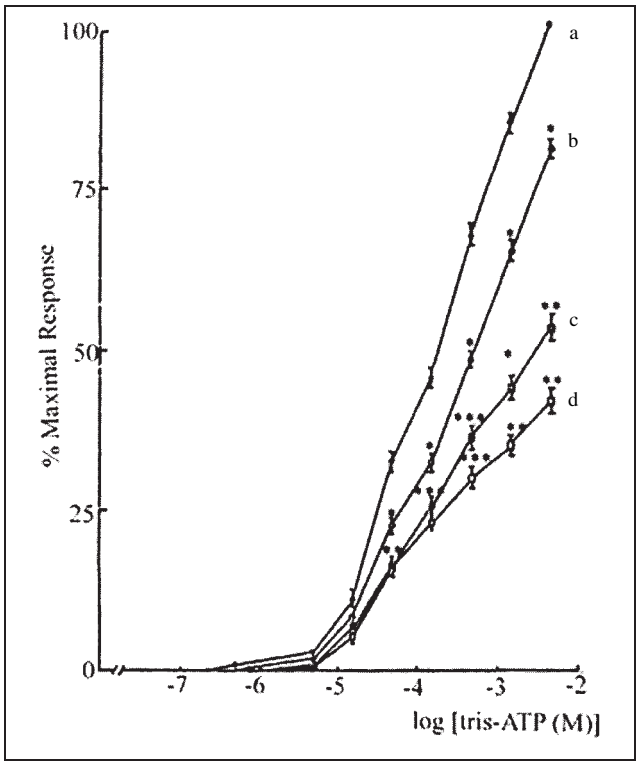

Fig. 5: Log concentration-response curves of tris-ATP effects on calcium accumulation:
a) in the absence of verapamil
b) in the presence of 10 nM verapamil
c) in the presence of 16.7 nM verapamil
d) in the presence of 33.3 nM verapamil
* $P < 0.05$, ** $P < 0.02$ and *** $P < 0.01$
Shown as mean ± SEM (n = 5)

Two Ca^{2+} channel blockers, verapamil and diltiazem, were investigated for their effects on Ca^{2+} uptake and release. The SR suspension was pretreated with verapamil or diltiazem to give a final concentration range of 10–33.3 nM and 0.8–2.4 μM, respectively. Maximum fluorescence intensity induced by 0.5 mM tris-ATP, decreased in the presence of diltiazem (Fig. 2) or verapamil (Fig. 3). Even increasing the concentration of ATP did not alter the maximum level of Ca^{2+} uptake in vesicles pretreated by drugs (data not shown). That shows that subsequent Ca^{2+} uptake induced by tris-ATP is decreased because of the irreversible antagonistic effects of diltiazem or verapamil. As shown in Figs. 2 and 3, SR vesicles treated with Ca^{2+} channel blockers not only have a smaller capacity for Ca^{2+} uptake induced, but are also shown to have a relatively slower rate of Ca^{2+} uptake, since the curve of Ca^{2+} uptake is shifted to the right. The maximal response (Ca^{2+} uptake) triggered by 0.5 mM ATP decreased to 69.8% and 34.6% in SR vesicles pretreated with 0.8 and 2.4 μM diltiazem, respectively. In vesicles pretreated with 10, 16.7 and 33.3 nM verapamil, Ca^{2+} uptake was reduced to 80.4%, 53% and 41.2% of the maximum uptake. In Figs. 4 and 5, the log concentration of tris-ATP versus the fluorescent response is plotted for SR vesicles treated with diltiazem and verapamil, respectively. The concentration of tris-ATP necessary for half-maximal Ca^{2+} uptake in SR vesicles increases from 169 μM in the control to 794 μM in the presence of 0.8 μM diltiazem (Fig. 4) and from 199 μM in the control to 562 and 3470 μM in the presence of 10 and 16.7 nM of verapamil (Fig. 5). Thus, Ca^{2+} channel blockers reduce the efficacy of ATP and interfere with the function of ATP in Ca^{2+} uptake by SR, depending on their concentrations. This effect is not reversed by adding excessive amounts of ATP.

The fluorescence intensity which was increased by addition of tris-ATP is followed by a gradual decrease, because of spontaneous Ca^{2+} release from SR (Fig. 6a). However, the observed fluorescence signal after achieving a steady-state level is rapidly reversed by the subsequent

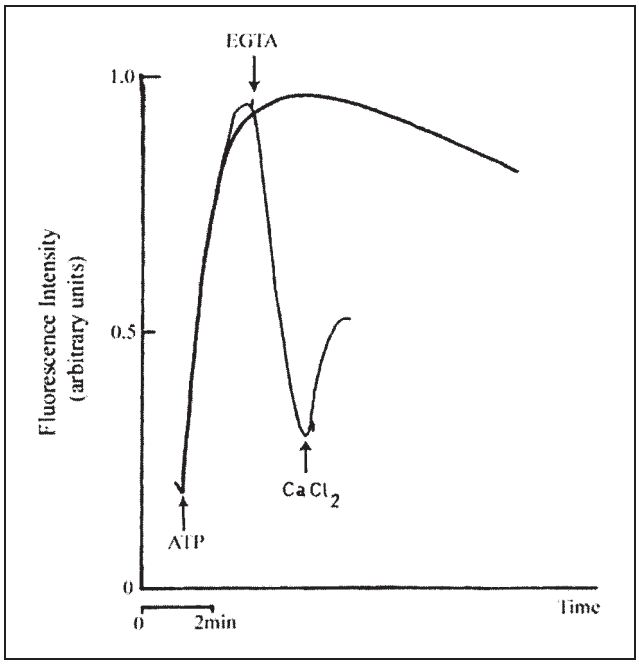

Fig. 6: ATP-dependant accumulation of calcium by SR is reversed either following spontaneous calcium release or following addition of 500 μM EGTA which is reversed again by 100 μM $CaCl_2$

addition of 500 μM EGTA, a Ca^{2+} chelator, to the assay medium. This effect is subsequently reversible by the addition of 100 μM $CaCl_2$ (Fig. 6b), demonstrating that CTC fluorescence is very sensitive to divalent cation complexation in the medium. Addition of Ethylene glycol-bis([beta]-aminoethyl-ether)-N,N,N′,N′-tetra-acetic acid (EGTA) creates a Ca^{2+} gradient in the SR membrane and causes release of Ca^{2+} from the SR membrane, and therefore a rapid decrease in the fluorescence of CTC occurs.

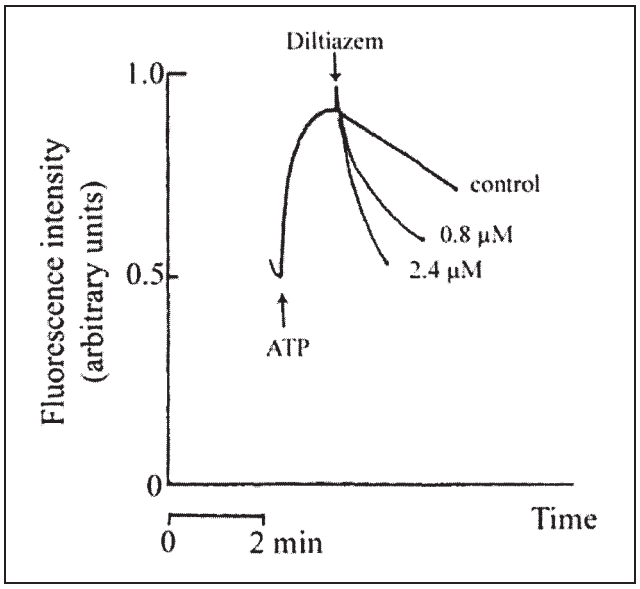

Fig. 7: CTC-fluorescence intensity triggered by 0.5 mM ATP decreased after addition of diltiazem. Calcium release is changed in a dose-dependent manner after addition of diltiazem (0.8, 2.4 μM)

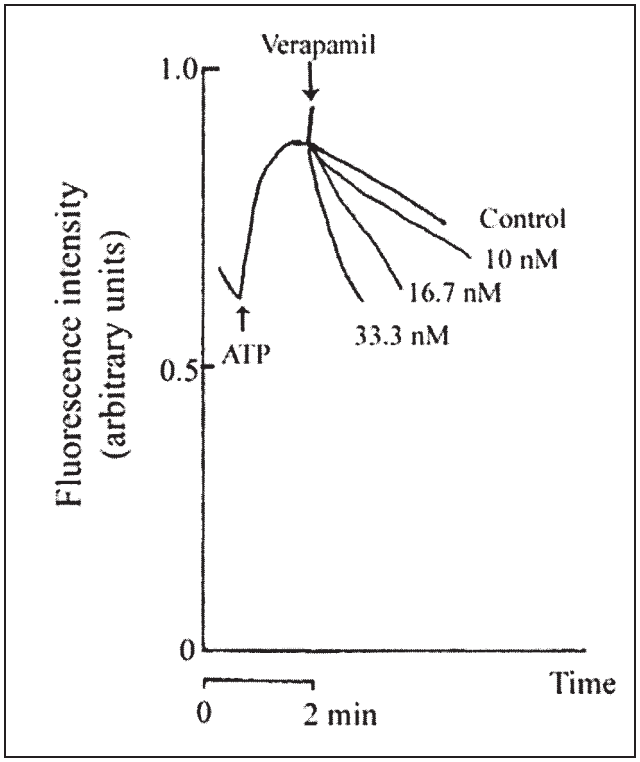

Fig. 8: CTC-fluorescence intensity triggered by 0.5 mM ATP decreased after addition of verapamil. Calcium release is reduced in a dose-dependent manner following addition of verapamil (10, 16.7, 33.3 nM)

We further characterized the effects of diltiazem or verapamil on Ca^{2+} release and compared it with spontaneous Ca^{2+} release from SR. The CTC fluorescence intensity triggered by tris-ATP decreased rapidly after addition of either 0.8–2.4 μM diltiazem or 10–33.3 nM verapamil in a concentration-dependent manner (Figs. 7, 8). Following addition of Ca^{2+} channel blockers, SR vesicles have been shown to have a relatively faster rate of Ca^{2+} release, since the Ca^{2+} release curve shifted to the left (Figs. 7, 8). Ca^{2+} release accelerated by diltiazem or verapamil was not reversed by $CaCl_2$ (data are not shown), in spite of the fact that Ca^{2+} release triggered by EGTA, was reversible following addition of $CaCl_2$ (Fig. 6).

3. Discussion

The present study, in which the CTC-fluorescence technique was employed as an intracellular probe for detection of divalent cations (Jacob et al. 2003), provides new and direct evidence for investigation of the effects of two Ca^{2+} channel blockers, diltiazem and verapamil, on Ca^{2+} movement across the SR vesicles of chicken skeletal muscle. Sarcoplasmic reticulum vesicles have a high capacity for Ca^{2+} uptake and release, which may be attributable to the high level of Ca^{2+}-ATP ase and Ca^{2+}-releasing channels in the vesicles (Fill and Copello 2002; Martonosi and Pikula 2003).

In accordance with our study, it has been reported that Ca^{2+} uptake in skeletal muscle SR vesicles is triggered by ATP (Fusi et al. 2001) in a concentration-dependent manner (Fig. 1). However, the current experiments unexpectedly showed huge differences in ATP-dependent Ca^{2+} uptake between species. The half-maximal Ca^{2+} uptake previously shown in SR vesicles of rabbit skeletal muscle was 42 μM of tris-ATP (Dehpour et al. 1998), but in chicken skeletal muscle, it was about 126 μM. It shows that rabbit SR is more sensitive to the effects of ATP than the SR obtained from chickens.

It appears that Ca^{2+} channel blockers may affect excitation-contraction coupling directly, independently of the modulation of the sarcolemmal Ca^{2+} current (Chattopadhyay et al. 1992; Zucchi and Ronca-Testoni 1997). Voltage-clamp studies have demonstrated the existence of a slow inward Ca^{2+} current in frog (Bohle 1992; Luttgau et al. 1987) and mammalian (Delbono 1992; Walsh et al. 1986) skeletal muscle. Ca^{2+} channel blockers which block this current (Bohle 1992; Gonzalez-Serratos et al. 1982; Luttgau et al. 1987; Walsh et al. 1986; Walsh et al. 1987), paradoxically appear to enhance mechanical activity (Dorrschedit-Kafer 1977; Gonzalez-Serratos et al. 1982). It has been reported that diltiazem potentiates twitch tension in bullfrog skeletal muscle (Walsh et al. 1988) as well as frog and mouse skeletal muscle fibers (Walsh et al. 1984). The mechanical potentiation produced by diltiazem in skeletal muscle contrasts with the inhibitory action of this drug in smooth and cardiac muscle (Dehpour et al. 1998). Hirata and Inamitsu observed stimulation of cation-induced Ca^{2+} release by diltiazem (Hirata and Inamitsu 1983). In skinned fibers, high concentrations (0.1–3 mM) of diltiazem or verapamil increased development of tension, which was interpreted as evidence of stimulation of spontaneous (Asayama et al. 1990) or Ca^{2+}-induced (Su 1988) Ca^{2+} release. It has also been reported that diltiazem activates skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase and causes the early release of Ca^{2+} after binding to its receptor (Wang et al. 1984). It has even been shown that verapamil binds to the α_1 subunit of the

dihydropyridine receptor with a K_d of 30 nM, whereas the concentration required to block muscle contraction is 30 μM (Valdivia et al. 1990). These contradictory findings appear to be the result of multiple sites of action of these drugs in skeletal muscle (Su 1988). There is some evidence that Ca^{2+} channel blockers can penetrate the cell membrane and accumulate in the cytosol, where they may interact with intracellular Ca^{2+} binding proteins (Dehpour et al. 1998; Catterall 2003; Walsh et al. 1984).

In our study, after pretreatment of SR with diltiazem or verapamil, the maximum uptake triggered by 0.5 mM ATP was significantly reduced, while, in addition, the rate of Ca^{2+} uptake was decreased. Thus, these two Ca^{2+} channel blockers can interfere with the ATP-induced Ca^{2+} uptake process, and as increasing the ATP concentration did not change the maximum Ca^{2+}, a non-competitive antagonistic effect is probably detected. In accordance with our study, it has been found that verapamil and diltiazem inhibited Ca^{2+} uptake in both skeletal and cardiac muscle SR due to inhibition of Ca^{2+} ATPase activity (Colvin et al. 1982; Wang et al. 1984). On the contrary, other investigations have shown either no effect or stimulation of SR Ca^{2+} uptake by verapamil or diltiazem (Zucchi et al. 1992; Zucchi 1996). This might be due to differences in species or drug concentrations.

Furthermore, in agreement with other studies (Dehpour et al. 1998; Hirata and Inamitsu 1983; Su 1988), we confirmed that these Ca^{2+} channel blockers have the capacity to trigger the release of Ca^{2+} from isolated SR vesicles at low concentrations. Ca^{2+} release by Ca^{2+} channel blockers was not reversed by addition of excess $CaCl_2$. Different results have been reported by Zucchi, who observed a reduction in Ca^{2+} release from cardiac or skeletal muscle SR with verapamil and diltiazem at high concentrations (Zucchi et al. 1992; Zucchi 1996). It should be pointed out that these studies did not clarify whether the effect of Ca^{2+} antagonists was mediated by dihydropyridine receptors or, rather, involved a different intracellular target (Zucchi 1996). In fact, these drugs may act on intracellular organelle systems pertinent to Ca^{2+}-transport activities (Dehpour et al. 1998). Chicken SR is about 1000 times more sensitive to the effect of diltiazem on Ca^{2+} uptake and release than rabbit SR which is affected by diltiazem in the range of 0.6–2 mM (Dehpour et al. 1998). The presumed mechanism is probably differences in the receptor protein between the species.

As shown in Fig. 6 by gradual disappearance of the fluorescence signal, Ca^{2+} is even released spontaneously in skeletal muscles (Gyorke and Gyorke 1996; Lacampagne et al. 1998). It has been proposed that spontaneous events may result from the activation of SR Ca^{2+} release channels by Ca^{2+}-induced Ca^{2+} release (Lacampagne et al. 1998). In both species, treatment with 500 μM EGTA resulted in a rapid disappearance of the CTC-fluorescence intensity indicating rapid Ca^{2+} release from the inner surface of the SR (Dehpour et al. 1998), whereas drug-induced Ca^{2+} release was determined by a gradual diminution of fluorescence signal. EGTA chelates external Ca^{2+} and induces a Ca^{2+} gradient, which is directed towards the extravesicular medium and induces Ca^{2+} release that is reversible by addition of $CaCl_2$. Therefore, Ca^{2+} release is directly dependent on extravesicular Ca^{2+} concentration which is important in skeletal muscle contraction.

Taken together, the evidence supports the hypothesis that the induction of Ca^{2+} release triggered by EGTA is completely different from triggered by Ca^{2+} channel blockers (Dehpour et al. 1998). On the other hand, the presence of binding sites for phenylalkylamines in the Ca^{2+} release channel was confirmed (Valdivia et al. 1990). Such a finding has not yet been seen for diltiazem, but it may possibly exist for diltiazem also.

In conclusion, Ca^{2+} channel blockers may inhibit ATP-triggered Ca^{2+} uptake and potentiate Ca^{2+} release in chicken skeletal muscle. Our results support the suggestion that these drugs probably interfere with ATP at its site of action on involved intracellular proteins. The targets involved may perhaps be Ca^{2+} binding proteins such as Ca^{2+} ATPase or Ca^{2+} release channel in skeletal muscle (Dehpour et al. 1998), although direct proof of this finding requires measurement of the precise Ca^{2+} concentration.

4. Experimental

4.1. HSR preparation

Gallus domesticus chicken (850 ± 150 g) were obtained from a local market and housed in an environmentally controlled room (temperature 20–25 °C) with reserved light-dark cycles. All experiments (muscle sampling) were initiated at approximately the same time each day to avoid large diurnal variations in muscle. On the day of experiment, animals were sacrificed and the thigh muscle (Stratagene) was rapidly excised, dissected and placed in ice-cold buffer solution (100 mM KCl and 5 mM Tris, pH 7.4). Tendons were removed and the fresh muscle tissue was diluted 1 : 4 (wt/vol) in buffer containing 100 mM KCl and 5 mM Tris (pH 7.4) and mechanically homogenized with an Omni-Mixer 17106 at 16,000 rpm, for 4 × 30-s bursts, at 0–4 °C. A heavy fraction of SR was prepared by a centrifugation method described previously (Martonosi 1968). Briefly, the homogenate was centrifuged at 1000 × g for 20 min in a RC_2-B rotor (Sorvall). The supernatant was centrifuged again at 8000 g for 20 min. This step was repeated once more and then the new supernatant was recentrifuged at 28000 × g for 60 min. The pellets were suspended in a solution containing 50 mM histidine, 350 mM sucrose, 30 μM chlortetracycline and 500 μM $MgCl_2$ (pH 6.8). The assay medium contained Ca^{2+} only at the levels of impurity that could originate from the chemicals. The final protein concentration was adjusted to 0.5 mg/ml by the Lowry method (Lowry et al. 1951).

4.2. Drugs and chemicals

Tris-ATP, diltiazem, verapamil and EGTA were obtained from Sigma (St. Louis, MO, USA).

4.3. Fluorescence probe technique

Ca^{2+} uptake and release were measured in muscle homogenate, using the Ca^{2+} fluorescent probe, CTC (Dehpour et al. 1998; Jacob et al. 2003; Renard-Rooney et al. 1993). Fluorescence intensity was recorded with an Aminco-Bowman spectrophotofluorometer in the Department of Pharmacology, Tehran University of medical sciences (TUMS). Excitation and emission wavelengths were scanned and then fixed at 390 and 520 nm, respectively. For stabilizing the baseline levels of CTC fluorescence, SR microsomes were preincubated in histidine buffer for 30 min at room temperature. In our experiments, Ca^{2+} uptake by SR vesicles was analyzed at different concentrations of ATP (0.1 μM to 10 mM). The assay was initiated by injection of Tris-ATP into the fluorescence cuvette. Shortly after the addition of Tris-ATP, active Ca^{2+} uptake and SR loading was initiated. The final volume of the homogenate and histidine buffer mixture was 2 ml. Once the SR was loaded with Ca^{2+} and a steady-state was achieved, spontaneous Ca^{2+} release was initiated. Either 20 μl demineralized water as control or 20 μl of the Ca^{2+} channel blockers, verapamil or diltiazem, were added to give a final concentration range of 10–33.3 nM and 0.8–2.4 μM, respectively. They were added under two separate protocols: either pre-incubated with the assay mixture prior to injection of Tris-ATP to study the uptake process, or injected after active loading of the SR, just before the release process. The fluorescence intensity was immediately recorded as a function of time. The time for mixing Tris-ATP and initiating of fluorescence measurement was less than 5 s. While the dilution effect was seen to occur in almost all the assays performed, it was noted that the contribution of the homogenate to the total fluorescence level was negligible.

4.4. Data analysis

All data are presented as mean ± SEM and significances were tested by Student's t-test. Statistical significances were denoted by * $p < 0.01$, ** $p < 0.02$ and *** $p < 0.05$.

References

Abramson JJ, Milne S, Buck E, Pessah IN (1993) Porphyrin induced calcium release from skeletal muscle sarcoplasmic reticulum. Arch Biochem Biophys 301: 396–403.

Asayama J, Tatsumi T, Miyazaki H, Omori I, Shirayama T, Inoue D, Nakagawa M (1990) Effect of diltiazem on spontaneous cyclic Ca^{2+} release from sarcoplasmic reticulum. Eur J Pharmacol 175: 309–315.

Bohle T (1992) The effect of the benzothiazepine diltiazem on force and Ca^{2+} current in isolated frog skeletal muscle fibres. J Physiol 445: 303–318.

Catterall WA, Striessnig J, Snutch TP, Perez-Reyes E (2003) International Union of Pharmacology. XL. Compendium of voltage-gated ion channels: calcium channels. Pharmacol Rev 55: 579–581.

Chattopadhyay RN, Roy RK, Das AK, Chaudhuri S, Lahiri HL, Maiti SP, Das MM (1992) Comparative studies of the effects of calcium channel blockers on isolated skeletal muscle preparation. Indian J Pharmacol 24: 233–234.

Colvin RA, Pearson N, Messineo FC, Katz AM (1982) Effects of Ca channel blockers on Ca transport and Ca ATPase in skeletal muscle and cardiac sarcoplasmic reticulum vesicles. J Cardiovasc Pharmacol 4: 935–941.

Curtis BA (1994) Effect of diltiazem upon a rapidly exchanging calcium compartment related to repriming in frog skeletal muscle. J Muscle Res Cell Motil 15: 49–58.

Dehpour AR, Mousavizadeh K, Gerayesh-Nejad S (1998) Calcium release by diltiazem from isolated sarcoplasmic reticulum of rabbit skeletal muscle. Gen Pharmacol 31: 463–468.

Delbono O (1992) Calcium current activation and charge movement in denervated mammalian skeletal muscle fibres. J Physiol 451: 187–203.

Dorrschedit-Kafer M (1977) The action of D600 on frog skeletal muscle: facilitation of excitation-contraction coupling. Pflugers Arch 369: 259–267.

Fill M, Copello JA (2002) ryanodine receptor calcium release channels. Physiol Rev 82: 893–922.

Fusi F, Tzankova V, Valoti M, Pessina F, Sgaragli G (2001) 3,5-Di-t-butyl-4-hydroxyanisole (DTBHA) activation of rat skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase. Biochem Pharmacol 62: 1613–1619.

Gailly P (2002) New aspects of calcium signaling in skeletal muscle cells: implications in Duchenne muscular dystrophy. Biochim Biophys Acta 1600: 38–44.

Gilchrist JS, Palahniuk C, Abrenica B, Rampersad P, Mutawe M, Cook T (2003) RyR1/SERCA1 cross-talk regulation of calcium transport in heavy sarcoplasmic reticulum vesicles. Can J Physiol Pharmacol 81: 220–233.

Gonzalez-Serratos H, Valle-Aguilera R, Lathrop AD, Delcarmen Garcia M (1982) Slow inward calcium currents have no obvious role in muscle excitation-contraction coupling. Nature 298: 292–294.

Gyorke I, Gyorke S (1996) Adaptive control of intracellular Ca^{2+} release in C2C12 mouse myotubes. Pflugers Arch 431: 838–843.

Hagiwara M, Adachi-Akahane S, Nagao T (2003) High-affinity binding of [^{3}H]DTZ323 to the diltiazem-binding site of L-type Ca^{2+} channels. Eur J Pharmacol 466: 63–71.

Hirata M, Inamitsu T (1983) Effects of diltiazem on the release of calcium from the canine fragmented cardiac sarcoplasmic reticulum. Jpn J Pharmacol 33: 991–997.

Jacob J, Chandran D, Sasidharan R, Kuruvila L, Madhusudan UK, Rao NL Benerjee D (2003) Chlortetracycline, a fluorescent probe for pH of calcium stores in cells. Curr Sci 84: 671–674.

Lacampagne A, Klein MG, Schneider MF (1998) Modulation of the frequency of spontaneous sarcoplasmic reticulum Ca^{2+} release events (Ca^{2+} sparks) by myoplasmic [Mg^{2+}] in frog skeletal muscle. J Gen Physiol 111: 207–224.

Lowry OH, Rosebrough NJ, Farr AL, Randall FJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275.

Luttgau HC, Gottschalk G, Berwe D (1987) The effect of calcium and Ca antagonists upon excitation-contraction coupling. Can J Physiol Pharmacol 65: 717–723.

Martonosi A (1968) Sarcoplasmic reticulum. IV. Solubilization of microsomal adenosine triphosphatease. J Biol Chem 243: 71–81.

Martonosi AN and Pikula S (2003) The network of calcium regulation in muscle. Acta Biochim Pol 50: 1–30.

Renanrd-Rooney DC, Hajnoczky G, Seutz MB, Schneider TG, Thomas AP (1993) Imaging of inositol 1,4,5-trisphosphate-induced Ca^{2+} fluxes in single permeabilized hepatocytes. Demonstration of both quantal and nonquantal patterns of Ca^{2+} release. J Biol Chem 268: 23601–23610.

Simonides WS and Hardeveld C (1990) An assay for sarcoplasmic reticulum Ca^{2+}-ATPase activity in muscle homogenates. Anal Biochem 191: 321–331.

Stoyanovsky DA, Salama G, Kagan VE (1994) Ascorbate/iron activates Ca^{2+}-release channels of skeletal sarcoplasmic reticulum vesicles reconstituted in lipid belayers. Arch Biochem Biophys 308: 214–221.

Su JY (1988) Intracellular mechanism of verapamil and diltiazem action on striated muscle of the rabbit. Naunyn-Schmiedebergs-Arch-Pharmacol 338: 297–302.

Valdivia HH, Valdivia C, Ma J, Coronado R (1990) Direct binding of verapamil to the ryanodine receptor channel of sarcoplasmic reticulum. Biophys J 58: 471–481.

Walsh KB, Bryant SH, Schwartz A (1984) Diltiazem potentiates mechanical activity in mammalian skeletal muscle. Biochem Biophys Res Commun 122: 1091–1096.

Walsh KB, Bryant SH, Schwartz A (1986) Effect of calcium antagonist drugs on calcium current in mammalian skeletal muscle fibers. J Pharmacol Exp Ther 236: 403–407.

Walsh KB, Bryant SH, Schwartz A (1987) Suppression of charge movement by calcium antagonists is not related to calcium channel block. Pflugers Arch 409: 217–219.

Walsh KB, Bryant SH, Schwartz A (1988) Action of diltiazem on excitation-contraction coupling in bullfrog skeletal muscle fibers. J Pharmacol Exp Ther 245: 531–536.

Wang T, Tasi L, Schwartz A (1984) Effect of verapamil, diltiazem and nislodipine on sarcoplasmic reticulum. Eur J Pharmacol 100: 253–261.

Wingertzahn MA, Ochs RS (1998) Control of calcium in skeletal muscle excitation-contraction coupling: implications for malignant hyperthermia. Mol Genet Metab 65: 113–120.

Zucchi R, Limbruno U, Ronca-Testoni S, Yu G, Galbani P, Ronca G, Mariani M (1992) Effects of verapamil, gallopamil, diltiazem and nifedipine on sarcoplasmic reticulum function in rat heart. Cardioscience 3: 167–172.

Zucchi R (1996) Effect of gallopamil on excitation-contraction coupling. Gen Pharmacol 77: 749–753.

Zucchi R, Ronca-Testoni S (1997) The sarcoplasmic reticulum Ca^{2+} channel/ryanodine receptor: modulation by endogenous effectors, drugs and disease states. Pharmacol Rev 49: 1–51.