doi: 10.4149/gpb_2015026

**Review**

# Calcium *versus* strontium handling by the heart muscle

Michal Hendrych[1,2], Veronika Olejnickova[1] and Marie Novakova[1, 2]

[1] *Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
[2] *International Clinical Research Center – Center of Biomedical Engineering, St. Anne's University Hospital Brno, Pekarska 53, 656 91 Brno, Czech Republic*

**Abstract.** Calcium plays a crucial role in numerous processes in living systems, from both intracellular and intercellular signalling to blood clotting. Calcium can be replaced by strontium in various intracellular processes due to high level of their similarity and strontium thus may serve as a valuable tool for different experimental studies. On the other hand, strontium is also used in clinical medicine and is commonly taken to the human body with food and water. The negative cardiac side effects of strontium therapy of osteoporosis and bone metastases are well known, but still not fully explained. This fact explains enhanced interest in this element and its impact on human body. This article reviews effects of calcium and strontium on several biochemical and physiological processes, with special emphasis on cardiac muscle.

**Key words:** Strontium — Calcium — Cardiomyocyte — Sarcoplasmatic reticulum — Mitochondria

**Abbreviations:** AP, action potential; APD, action potential duration; ATP, adenosine triphosphate; CaMKII, $Ca^{2+}$/calmodulin-dependent kinase; CICR, calcium-induced calcium release; DHPRs, dihydropyridin receptor; $I_{si}$, slow inward current; mCU, mitochondrial $Ca^{2+}$ uniporter; mRyR, mitochondrial ryanodine receptor; NCX, $Na^+/Ca^{2+}$ exchanger; PKA, cAMP-dependent kinase; PMCA, plasmatic membrane $Ca^{2+}$ ATPase pump; PTP, permeability transition pore; RaM, rapid mode of mitochondrial $Ca^{2+}$ uptake; RANCL, receptor activator of nuclear factor Kappa B ligand; RF, recirculation fraction; ROS, reactive oxygen species; RyRs, ryanodine receptors; SERCA, sarcoplasmic reticulum $Ca^{2+}$ ATPase pump; SISR, strontium-induced strontium release; SR, sarcoplasmic reticulum.

## Introduction

Although strontium was discovered by Adair Crowford in 1790, the first report about its effects on the human body was published in 1909 by Lehnerdt (Lehnerdt 1909). Experiments on strontium's medical uses began in 1950, when its effects on the remineralisation of bone tissue were tested (Franěk et al. 2009). Later, strontium was employed in various clinical and experimental fields, including experimental cardiology. A question of its ability to replace calcium ions in certain cellular processes in cardiomyocytes was raised, because strontium's properties are close to those of calcium. An attempt to find the answer unleashed the first wave of experiments with strontium in the 1970s. Nowadays, strontium is used for treatment of osteoporosis. Since this treatment causes some side effects on the cardiovascular system, it has opened up new interest in this issue.

Correspondence to: Veronika Olejnickova, Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic
E-mail: veronika.olejnickova@mail.muni.cz

## Strontium, its forms and metabolism

Strontium (chemical symbol – $Sr^{2+}$) is a chemical element ranked alongside better known calcium, barium and radium in alkaline earth metals. Strontium occurs naturally only as minerals celestine ($SrSO_4$) and strontianite ($SrCO_3$) (Greenwood and Earnshaw 1997). Despite the fact that its properties are close to those of calcium, strontium is 2.2 times larger (Pors et al. 2004), less electronegative (Hesaraki et al. 2010), has lower ionization potential, lesser hydration radii

(Atwood and Suslick 1996) and is also pyrophorus. The difference between strontium and calcium is also expressed as different affinity to chelates. For example calcium has proven two times greater affinity to EGTA (Pors et al. 2004; Haase and Hartung 2009). On the other hand, strontium is better ligand for sulphates (Atwood and Suslick 1996). Its natural isotopes are all stable except for the $^{90}Sr$ isotope, which occurs naturally in trace amounts and is a source of β radiation emission (Kulveitová 2010).

Strontium occurs quite ordinarily in water and food, from which it is taken; a daily intake of strontium is about 2–4 mg (Franěk et al. 2009). It is absorbed mostly in jejunum, under influence of calcitriol and in the presence of calcium. The ratio of absorbed strontium/calcium is 60–70% (Pors et al. 2004). Strontium intestinal absorption through the enterocyte luminal membrane is provided by ECaC2 transporters, also known as TRPV6. Thus absorbed strontium binds to calmodulin, ubiquitous $Ca^{2+}$-binding protein and calbindin before its extrusion *via* plasmatic membrane $Ca^{2+}$ ATPase (PMCA) pump and predominant $Na^{+}/Ca^{2+}$ exchanger (NCX) transporters located in the basolateral side of the enterocytes (Weber et al. 2001; Wood et. al. 2011; Camargo et al. 2015). Its absorption drops down during ageing. The plasmatic level of strontium for a healthy person is up to 50 μg/l (Bianchi et al. 1999; Franěk et al. 2009). As with calcium, strontium is partially bound to plasmatic proteins in the blood stream. In comparison with calcium which is about 40–50% bound to plasmatic proteins, just about 25% of strontium is bound (Suchopár and Kučera 2005). This means that plasma levels of strontium are influenced by acid-base status and temperature in lower magnitude than of calcium. The dependence on the $H^{+}$ concentration is also analogous to calcium. The binding capacity of strontium to plasmatic proteins increases with pH (Berg et al. 1973). Although the exact ratio differs among the animal species (for instance in rat the $Ca^{2+}$-bound to $Sr^{2+}$-bound ratio is 1.13 and 1.29 for human, respectively), $Ca^{2+}$ binding to serum proteins is slightly greater in comparison with $Sr^{2+}$ in all animal species (Carr et al. 1962).

The main excretion route from the body is *via* the kidneys. Only approximately 4% is excreted through the bowel. In the renal glomerulus, strontium is at ease filtered into primary urine. Nevertheless, as compared to calcium, the tubular re-absorption of strontium is three times lower due to bigger molecule (Blake et al. 1989) and due to the competition with calcium for transporters ECaC1 and ECaC2. Moreover, strontium is also lesser transported *via* ATP-dependent transporters located in the basolateral membrane (Sugihira et al. 1992; Weber et al. 2001). The major amount of both calcium and strontium in the body, over than 99% of the total amount, is found in the bone (Dahl et al. 2001). As it is shown in several studies the hydroxyapatite solubility increases with increasing content of strontium (Christoffersen et al. 1997). Nevertheless, only a few strontium ions may be incorporated into the hydroxyapatite. Moreover, after treatment withdrawal strontium is rapidly decreased in the bone (Dahl et al. 2001). In a study on experimental intoxication with strontium development of hypocalcaemia caused by an excess in its excretion through the kidneys was established (Morohashi et al. 1994).

**Usage in clinical practice**

Strontium has been used in low doses to treat osteoporosis, metastatic bone cancer and tooth hypersensitivity. For the treatment of osteoporosis stable strontium isotopes combined with ranelic acid are used. After peroral usage, just about 25% of strontium is absorbed (Suchopár and Kučera 2005). Strontium increases bone formation and decreases bone re-resorption by promoting preosteoblasts replication, collagen I and osteoprotegerin synthesis. Osteoprotegerin as a soluble receptor for bone growth factor RANKL (Receptor Activator of Nuclear factor Kappa B Ligand) is able to decrease bone resorption, inhibit osteoclast differentiation and evoke osteoclast apoptosis (Gallacher and Dixon 2010). In stomatology strontium is used as strontium chloride in toothpaste and, as a mechanical blocker of dentinal tubules, is able to solve tooth hypersensitivity (Karim and Gillam 2013). The artificial radioisotope $^{89}Sr$ is used to treat multiple bone metastases of solid tumours and multiple myeloma. The major usage of the artificial radioisotope $^{89}Sr$, rather than naturally occurring radioisotope $^{90}Sr$, is based on its markedly shorter half-life ($^{89}Sr$ – 50 days, $^{90}Sr$ – 28 years). Higher metabolic rate of bone metastases causes greater $^{89}Sr$ uptake, reaching 10-times higher concentrations than in healthy bone tissue; this ensures a local effect of the β radiation (Poršová et al. 2007).

**Usage in experiment**

In spite of the fact that the first experiment with strontium in medicine was only conducted in 1950, its ability to replace $Ca^{2+}$ in excitation-contraction coupling was observed up to 12 years later in frog skeletal muscle (Frank 1962). Following this, this characteristic of strontium was successfully proven by the experiment on heart muscle (Nayler 1965). In connection with these articles, Edwards confirmed the ability of strontium to fully activate ATPase in frog skeletal muscle, however with 30-times lesser affinity. Among others, Edwards described the existence of the $Sr^{2+}$ current in the sarcoplasmic reticulum (Edwards et al. 1966). The existence of an $Na^{+}$-independent $Sr^{2+}$ inward current was experimentally established in chicken hearts (Pappano and Sperelakis 1969), the origin of which was discovered several years later by voltage clamp method. Penetration of $Sr^{2+}$ into cardiomyocyte was experimentally proven through the same

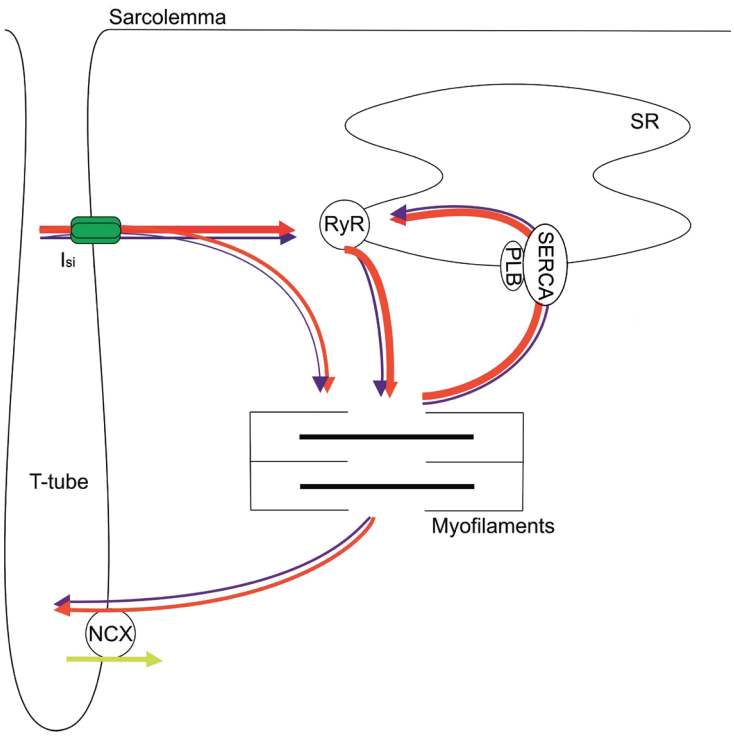


**Figure 1.** Calcium *vs.* strontium handling by the heart cell. Red arrow represents $Ca^{2+}$ current; blue arrow represents $Sr^{2+}$ current; yellow arrow represents $Na^{+}$ current. The different width of arrows implicates current magnitude. SR, sarcoplasmic reticulum; RyR, ryanodine receptor; PLB, phospholamban; SERCA, sarcoplasmic reticulum $Ca^{2+}$ ATPase pump; NCX, $Na^{+}$/$Ca^{2+}$ exchanger; $I_{si}$, slow inward current.

transport system as for $Ca^{2+}$ and its delayed inactivation was also proven (Kohlhardt et al. 1973). These experiments were followed by the set of investigations to establish the difference between divalent ions in $Ca^{2+}$ binding structures. The calmodulin as the central structure in $Ca^{2+}$-based intracellular signalling binds, except $Ca^{2+}$, also $Sr^{2+}$, $Mg^{2+}$, $Ba^{2+}$, and $Pb^{2+}$. Only $Pb^{2+}$ binds with higher affinity than physiological ligand, $Ca^{2+}$ (Lepsík and Field 2007; Kumar et al. 2012).

## An example how $Sr^{2+}$ replacement affects the dynamics of cellular processes – excitation contraction coupling in cardiomyocytes

Excitation-contraction coupling in cardiomyocyte is a process triggered by action potential and it leads to development of cardiac contraction. In this process, cytoplasmic availability of $Ca^{2+}$ is essential (Bers 2002). Heart muscle contraction is dependent on extracellular $Ca^{2+}$, which enters into a cell during the plateau phase of the action potential and on release of intracellular $Ca^{2+}$ pool located in the sarcoplasmic reticulum (Fabiato and Fabiato 1976). The both sources increase intracytoplasmic concentrations of $Ca^{2+}$ from $10^{-7}$ mol/l to $10^{-5}$ mol/l and thereby the binding to troponin C is reached. Despite that the overall cytosolic calcium levels reach 10 µmol/l, there are regions in the diad and on the SR surface where its levels could increase to much higher range (Hatano et al. 2012). The binding between troponin C and $Ca^{2+}$ leads to a decrease in the inhibiting effect of the troponin-tropomyosin complex and executes interaction between actin and myosin, followed by an ATP-dependent shift of the contractile proteins. Those intracellular changes become evident as the heart contracts (Bravený and Šumbera 1974).

## $Ca^{2+}$ influx into cardiomyocyte

Depolarization wave spreads across the cellular membrane and also in the T-tubules system. Terminal parts of the sarcoplasmic reticulum are attached to transversely running intracellular invaginations of the cellular membrane, which allows the signal to transfer from membrane to cytoplasm (Hatano et al. 2012). Voltage-dependent L-type $Ca^{2+}$ channels (dihydropyridine receptors – DHPRs) activated at –30 mV, are primarily located in the T-tubules region (Figure 1). The $Ca^{2+}$ slow inward current ($I_{si}$) which flows through L-type $Ca^{2+}$ channels is the major extracellular source of $Ca^{2+}$ (Callewaert 1992). Feedback regulation is mediated by $Ca^{2+}$ and calmodulin complex, inhibiting activity which is enhanced with an increasing $Ca^{2+}$ concentration in cytoplasm and by the membrane potential (Bers 2002). Through this channel $Sr^{2+}$ can also enter the intracellular space, whereas other bivalent ions such as Ni, Co and Mn inhibit it (Kohlhardt et al. 1973). $Ca^{2+}$ intracellular concentration is also affected by secondary active transport mediated by the $Na^{+}$/$Ca^{2+}$

exchanger (NCX) that transports 3 $Na^+$ ions in exchange for 1 ion of $Ca^{2+}$. Direction of the ion flux is affected by membrane potential as well as ion distribution. In forward mode NCX extrudes $Ca^{2+}$ from the cell, but conversely in reverse mode NCX brings $Ca^{2+}$ into the cell (Scriven and Moore 2013; Švíglerová et al. 2014). The NCX reversal mode is observed mainly during depolarization and initial fast re-polarization, when an abundant amount of $Na^+$ is found in the cell (Bers 2002). Exchanger activity also depends on quantity of ATP, which acts as an allosteric activator or indirectly acts through protein kinase A (PKA) or protein kinase C (PKC) (Schulze et al. 2003).

T-type $Ca^{2+}$ channels are low voltage-dependent channels (activated at –60mV), expressed especially in the embryonic period. In the adult heart, T-type $Ca^{2+}$ channels are located in the conduction system. Their expression is enhanced in pathological conditions such as hypertrophy or heart failure. T-type $Ca^{2+}$ channels also participate in enhancing intracytoplasmic concentrations of $Ca^{2+}$ but with lesser impact, in contrast with L-type $Ca^{2+}$ channels (Sipido et al. 1998; Ono and Iijima 2010).

Besides previously mentioned pathways, $Ca^{2+}$ can also penetrate into cardiomyocyte through alternative pathways such as voltage-dependent $Ca^{2+}$ releases, tetrodotoxin-sensitive $Ca^{2+}$ currents, the slip mode conductance in which $Na^+$ channels have altered preference for $Ca^{2+}$, and inositol-1,4,5-triphosphate receptors. The major role in releasing intracellular sources of $Ca^{2+}$ plays $I_{si}$, whereas the other ion currents have only a modulating function (Bers 2002).

**Sarcoplasmic reticulum**

The sarcoplasmic reticulum (SR) is a membrane-bound organelle able to store, release and draw $Ca^{2+}$ ions. The SR is an analogue of commonly occurring agranular endoplasmic reticulum and functions as a dynamic calcium governor in muscle that provides automatic feedback control for altering and maintaining myoplasmic and SR calcium levels (Rossi and Dirksen 2006). On the contrary to smooth muscle, where the SR is only slightly developed, its density in both types of striated muscle – skeletal and working myocardium – is really high (Guyton and Hall 2006). Because the cardiac SR in amphibians is developed at lower level as compared to mammals, $Ca^{2+}$ permeating from the extracellular compartments has more pronounced effect on enhancing cytoplasmic concentration of $Ca^{2+}$ in amphibian heart. The SR of mammals is, in contrast with amphibians, abundantly developed and its terminal parts are regularly attached to the T-tubules system. Nevertheless the cardiac SR of mammals, under physiological conditions, is not able to release sufficient amounts of $Ca^{2+}$ for full saturation of troponin C as it is known in the skeletal muscle (Bravený and Šumbera 1974; Kawata and Hatae 1977). The capacity of sarcoplasmic reticular $Ca^{2+}$ loading is sufficient to develop 50% to 90% of the maximal contraction force (Callewaert 1992). The remaining $Ca^{2+}$ for the contraction is $Ca^{2+}$ entry (Guyton and Hall 2006). Progressive development of the SR and the T-tubules system evolves during postnatal ontogenesis, therefore properties of mammalian newborn cardiomyocytes are closer to amphibians than adults (Vornanen 1985). The ability to accumulate and release $Ca^{2+}$ of the SR is increasing during its development. Simultaneously the sensitivity of the cardiac myofilaments to $Ca^{2+}$ is increasing and distribution of the sarcolemmal calcium is changing (Ošťádal et al. 2009).

On the SR membrane ligand-gated $Ca^{2+}$ channels, so-called ryanodine receptors (RyRs), are located. They are named by their antagonist, alkaloid ryanodine. Meanwhile 3 isoforms have been identified, diverging predominantly in expression as well as encoding by different genes. RyR1 represents dominant isoform in skeletal muscle; in cardiac muscle, RyR2 isoform dominates. RyR3 expression is not tissue specified (Shirokova and Niggli 2008). RyR1 and RyR2 are located particularly in the terminal cisternae of the cardiomyocyte's SR at 10–12 nm distance from the T-tubules system (Franzini-Armstrong et al. 1999) and are activated by $Ca^{2+}$ permeating especially through DHPRs. This action is called calcium-induced calcium release (CICR) (Fabiato and Fabiato 1976). RyR2 sensitivity to $Ca^{2+}$depends on intrasarcoplasmic $Ca^{2+}$ concentration (positive feedback). The insufficient SR loading is able to silence signals for $Ca^{2+}$ release (Shannon et al. 2000). RyR2 sensitivity can be also affected by some macromolecular complexes – inhibitors (for example calmodulin and sorcin). On the other side, $Ca^{2+}$/calmodulin-dependent kinase (CaMKII) promotes directly RyR2 sensitivity to $Ca^{2+}$ on the contrary to PKA indirect enhancing RyR2 sensitivity by increasing SR loading (Bers 2004). In the terminal cisternae of the SR a protein named calsequestrin is located. This is a $Ca^{2+}$-storage protein which binds $Ca^{2+}$ with high capacity (40–50 mol of $Ca^{2+}$/1 mol of calsequestrin) (Frank et al. 2001). A heart muscle isoform calsequestrin 2 is located in the vicinity of the RyR2 channels and is able to bind releasable $Ca^{2+}$. Calsequestrin 2 is connected with the RyR2 channels either directly or *via* proteins triadin and junction (Gaburjaková et al. 2013).

RyR2 receptors were found to be densely clustered on the terminal cisternae of the SR, which allows collective gating of RyR2 channels, manifested by simultaneous openings and closings of the channels (Gaburjaková and Gaburjaková 2008). In the comparison with the single RyR2 channels which are also found on the SR, the couple gated channels exhibit doubled conductance (Ondriaš and Mojžišová 2002).

Based on the finding that cytoplasmic domains of the RyR2 channels are in contact, it was suggested that allosteric coupling allows "noisy" open state current levels and their

termination. Interaction between coupled RyR2 channels is described by a new indicator – the coupling stability, which is markedly higher in interaction between closed channels than between opened ones (Gaburjaková and Gaburjaková 2008). The coupled gating of RyR2 channel is enhanced by luminal $Ca^{2+}$ as well as in single gating RyR2 channel (Gaburjaková and Gaburjaková 2014). Recirculation fraction (RF) is the total amount of $Ca^{2+}$ released from SR during E-C coupling and its consequent sequestration into SR. In steady-state regular beats RF is composed of the constant amount of the $Ca^{2+}$ circulating at the intercellular level, completed by the auxiliary fraction entering through sarcolemma. Subsequent removing of $Ca^{2+}$ to the SR enables the relaxation of the cardiac muscle. This recirculation of the $Ca^{2+}$ is two-times more economical then its removal across sarcolemma (Syuu et al. 1998; Mizuno 2001).

**Relaxation mechanism(s)**

Three main $Ca^{2+}$ removal pathways from cytoplasm are: sarcoplasmic reticulum $Ca^{2+}$ ATPase pump (SERCA), plasmatic membrane $Ca^{2+}$ ATPase pump (PMCA), and NCX. SERCA is an ATPase located predominantly in central parts of the SR (Callewaert 1992) which pumps $Ca^{2+}$ from cytoplasm into the SR. The SERCA has a non-interchangeable role in inducing relaxation in the skeletal and heart muscles (Inesi and Tadini-Buoninsegni 2014). In the heart muscle the SERCA2a isoform is expressed, which transmits $Ca^{2+}$ in consumption of ATP at a rate of 2:1. In exchange for $Ca^{2+}$, $H^+$ ions permeate from the SR into the cytoplasm (Brini and Carafoli 2009). The SERCA has the greatest impression on removal of $Ca^{2+}$ from cytoplasm, for example in rat ventricular myocytes SERCA transposes about 90% of $Ca^{2+}$ (Bers 2002), whereas in a human or guinea pig SERCA transposes "only" 70% (Kohlhaas and Maack 2013). Phospholamban plays a crucial role in the regulation of SERCA activity (Tada and Katz 1982). Phosphorylation of phospholamban enhances SERCA's affinity to $Ca^{2+}$ and accelerates its efflux (lusitropic effect); it is mediated by $Ca^{2+}$/calmodulin-dependent kinase and cAMP-dependent kinase (PKA), which ensures β-adrenal stimulation (Simmerman and Jones 1998). SERCA's function is also modulated by intracellular concentration of $Mg^{2+}$, $Ca^{2+}$, ATP, and $H^+$ (Švíglerová et al. 2014). SERCA pumps $Ca^{2+}$ into the SR and according its concentration gradient it diffuses to the terminal parts of the SR (Bravený and Šumbera 1974).

PMCA is ATPase bound in the plasmatic membrane which pumps $Ca^{2+}$ into the extracellular compartment at a rate of 2 $Ca^{2+}$: 1 ATP. Its activity is modulated by calmodulin, which binds to C-terminal sequence and decreases the Michaelis constant (Guerini et al. 1998). Plasma membrane $Ca^{2+}$ ATPase modulation by $Sr^{2+}$ ions was studied in human erythrocytes (Graf et al. 1982). For its proper function, it requires $Mg^{2+}$ and shows low activity unless a second ion is added (it responds to second ion with the order $Ca^{2+} > Sr^{2+} >> Ba^{2+}$). However, the information about possible modulation of membrane $Ca^{2+}$ ATPase in cardiomyocytes by $Sr^{2+}$ is missing. The role of the NCX as an important transporter of calcium has been discussed above.

**Cardiac contraction**

Heart contraction is an active process leading to blood ejection into the circulatory system. Contraction is based on reversible cross-bridges formation and the reciprocal shift of the contractile proteins, actin and myosin, which are organized as thin and thick myofilaments (Figure 2). This interaction is modulated by the troponin-tropomyosin complex.

Thin myofilaments are composed of parallel pseudodouble helical filaments, so-called F-actin, from polymerized G-actin. Each strand of actin filament is associated with tropomyosin coiled-coil dimers and a troponin complex, which also directly interacts with actin. Each thin myofilament strand may be considered as a series of regulation units, consisting of seven G-actins, tropomyosin and one troponin complex (Farah and Reinach 1995). Cardiac troponin is a heterotrimer consisting of troponin C (receptor binding $Ca^{2+}$), troponin I (protein inhibiting interaction between actin and myosin), and troponin T (subunit anchoring troponin I and C to tropomyosin). Binding $Ca^{2+}$ to troponin C triggers a conformational change in troponin-tropomyosin complex and unfolds the myosin binding side on actin (Solaro and Rarick 1998).

Myosin represents the fundamental structure unit of thick myofilament. Each myosin molecule contains two heavy chains and four light chains. Heavy chain constitutes a myosin axial structure like a long α-helical rod structure separated into globular "head" regions. On each "head" region there is the light chain modulating the ATPase activity of the heavy chain. The myosin "head" region also contains a binding side for actin (Morkin 2000).

In cardiomyocytes there are thin and thick myofilaments regularly organized into the sarcomeres bounded by the Z-lines. Z-lines are built up from actin filaments, which pass through one sarcomere to another. In a transverse slice sarcomere has a hexagonal organization, where the axial structure represents a myosin filament surrounded by actin filaments in overlapping arrays, also called A-bands. During contraction the cross-bridges between actin and the "head" region of the myosin heavy chain are formed. The contraction is based on those reversible interactions and the bending of the "head" region, observed as sarcomere shortening and described by Huxley as the sliding filament

model. This model established that the length of myofilament is essentially constant during contraction and the sarcomere shortening is due to sliding filaments passing each other (Huxley and Niedergerke 1954; Huxley 1969; Bravený and Šumbera 1974).

**Mitochondria**

The mitochondria is an organelle, formed by a double membrane, frequently called an "energetic factory" of the cell, especially due to its ability to create a great amount of ATP. However, its main metabolic function relates to certain physiological and pathophysiological processes, such as cell cycle, cellular growth, apoptosis, necrosis, etc. Numerous experimental works published recently have shown its extraordinary role in the calcium handling processes. Its great importance is also shown by the fact that mitochondria occupies up to 37% of the cardiomyocyte volume (Barth et al. 1992). Therefore, we focus on mitochondria in this review in more detail.

Mitochondria are able to intake $Ca^{2+}$ from cytoplasm and also to extrude $Ca^{2+}$ back into the cytoplasm (Figure 3). Until the discovery of signalling microdomains, mitochondrial $Ca^{2+}$ cycle magnitude, which is influenced by plasmatic $Ca^{2+}$ concentration changes, was considered as minimal (approximately only about 1–2% of cellular $Ca^{2+}$ cycle) (Dedkova and Blatter 2008). The signalling microdomains consist of mitochondria and SR terminal cisternae with RyRs at average distance of 15–20 nm. Thereby mitochondria are exposed to high $Ca^{2+}$ concentrations, which exceed low transporter affinity (Chen et al. 2012). More recent estimates calculate on up to 26% of released $Ca^{2+}$ from SR, which are absorbed into mitochondria (Dedkova and Blatter 2008).

$Ca^{2+}$ enters mitochondria through three pathways: mitochondrial $Ca^{2+}$ uniporter (mCU), rapid mode of mitochondrial $Ca^{2+}$ uptake (RaM) (O'Rourke and Blatter 2009) and mitochondrial ryanodine receptors (mRyR1) (Dedkova and Blatter 2008). mCU is a voltage-dependent ion channel located on the inner mitochondrial membrane, which, by contrast to outer membrane, is ion non-permeable. The channel is activated by negative membrane potential and this activation leads to $Ca^{2+}$ influx into the mitochondrial matrix according to an electrochemical gradient. For this channel rapid inactivation is characteristic and moreover offers great selectivity for $Ca^{2+}$ (O'Rourke and Blatter 2009). RaM is active mainly in the initial phase of extramitochondrial $Ca^{2+}$ concentration peak and is approximately 300-times faster than mCU. For fully activation of mRyR1, which is identical to the skeletal muscle RyR1, lower $Ca^{2+}$ concentration is needed than for mCU. Those transporters were proposed as the physiological mechanism of mitochondrial $Ca^{2+}$ influx, whereas mCU operates under pathological conditions (Dedkova and Blatter 2008). Finally, after intensive research effort, mCU was confirmed as principal physiological transporter. This conclusion was based on the discovery of signalling microdomains and describing high local $Ca^{2+}$ concentration near to mCU (Kohlhaas and Maack 2013).

Increased $Ca^{2+}$ concentration in mitochondria acts as an activator of the tricarboxylic acid cycle essential enzymes: pyruvate-dehydrogenase and α-ketoglutarat-dehydrogenase.

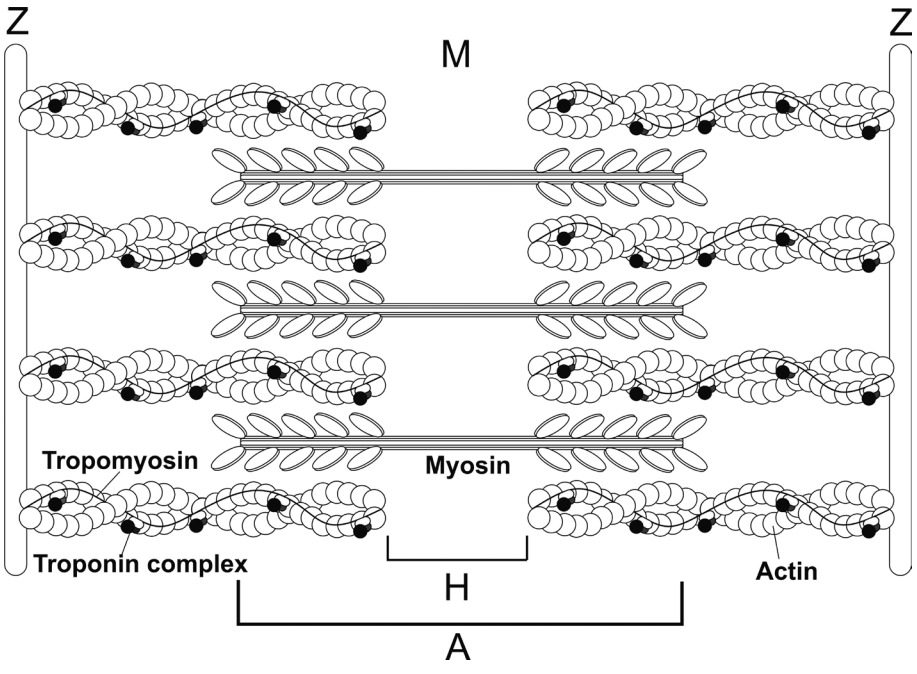


**Figure 2.** Contractile apparatus. Z, Z-disk; M, M-line; H, H-zone; A, A-band. Troponin complex consists of Troponin T represented by the white ellipsoid, Troponin I represented by the grey ellipsoid and Troponin C represented by the black circle.

Furthermore, $Ca^{2+}$ directly stimulates ATP-synthase function. Both pathways lead to ATP production and also $O_2$ consumption enhancement. With enhanced $O_2$ consumption ROS production is linked. Massive ROS production is well described during mitochondrial $Ca^{2+}$ overloading when it leads to cytochrome c release, caspases activation and permeability transition pore (PTP) activation (Maack and O'Rourke 2007).

$Ca^{2+}$ mitochondrial release is realised by two mechanisms, $Na^+$-dependent and $Na^+$-independent. $Na^+$-dependent $Ca^{2+}$ release is mediated by the mitochondrial $Na^+/Ca^{2+}$ exchanger (mNCX). mNCX transports 3 $Na^+$ ions into the mitochondrial matrix in exchange for 1 ion of $Ca^{2+}$ to the cell cytoplasm. This transport is also associated with the mNHX, which transports $Na^+$ back to the cytoplasm in for $H^+$. $Na^+$-independent transport is mediated *via* PTP. PTP is a high conductance transporter and is activated by massive ROS production, depolarization or mitochondrial $Ca^{2+}$ overloading (Dedkova and Blatter 2013).

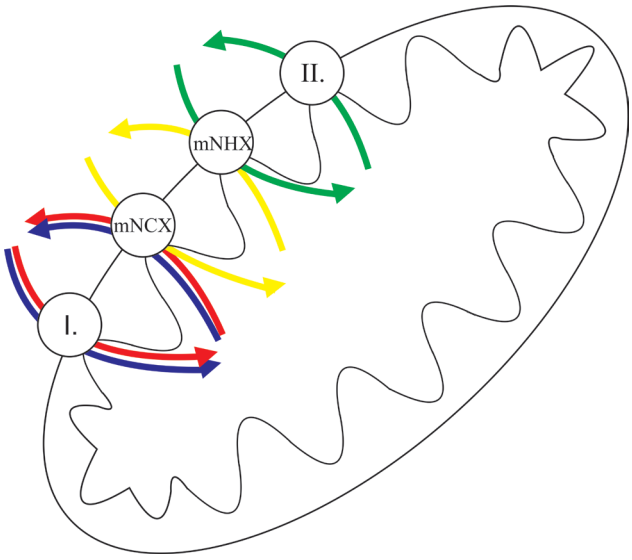


**Figure 3.** Calcium *vs.* strontium handling by the mitochondria. Red arrow represents $Ca^{2+}$ current; blue arrow represents $Sr^{2+}$ current; yellow arrow represents $Na^+$ current; green arrow represents $H^+$ current. mNCX, mitochondrial $Na^+/Ca^{2+}$ exchanger; mNHX, mitochondrial $Na^+/H^+$ exchanger; I., $Ca^{2+}$ enters mitochondria through three pathways: mitochondrial $Ca^{2+}$ uniporter (mCU), rapid mode of mitochondrial $Ca^{2+}$ uptake (RaM) and mitochondrial ryanodine receptor (mRyR1). II., $H^+$ pump.

## $Sr^{2+}$-induced $Sr^{2+}$ release

$Sr^{2+}$ is well known for its ability to replace $Ca^{2+}$ in several biochemical and physiological functions in cardiomyocyte (Figure 1). $Sr^{2+}$ permeates into cardiomyocyte through voltage-dependent L-type $Ca^{2+}$ channels and NCX in reversal mode located on the plasmatic membrane (Niggli 1989); transport *via* the NCX – as well as in physiological conditions – has lower impact (Štengl and Pučelík 1999). $Sr^{2+}$ completely replaces $Ca^{2+}$ as a physiological carrier for $I_{si}$. This $Sr^{2+}$ slow inward current is characterised by delayed inactivation, which is more prolonged at higher concentrations. $Sr^{2+}$ is probably not able to replace $Ca^{2+}$ in $Ca^{2+}$-dependent L-type $Ca^{2+}$ channels inactivation. Increased $Sr^{2+}$ concentration also augments slow inward current and besides decreases threshold potential. On the other side, activation kinetics is not altered (Kohlhardt et al. 1973).

Frequency-dependent prolongation of action potential duration (APD) was observed as the effect of $Sr^{2+}$, simultaneously with characteristic change of AP shape. The predominantly plateau phase was characteristically changed, accompanied by relatively fast terminal depolarization. These changes are markedly pronounced with decreasing stimulation frequency (Štengl and Pučelík 1999) and with increasing $Sr^{2+}$ concentration. Increasing extracellular $Sr^{2+}$ concentration further shifts plateau phase to more positive values of membrane potential (Gonzalez and Vassalle 1989). These characteristic AP changes are caused on one hand by the delayed inactivation of $I_{si}$, based on $Ca^{2+}$-dependent L-type $Ca^{2+}$ channels inactivation mentioned before, and on the other hand at least partly by the altered $K^+$ conductance of the repolarizing channels (Štengl and Pučelík 1999). $K^+$ conductance of repolarizing channels in physiological conditions is stimulated by increasing $Ca^{2+}$ concentration (Tohse et al. 1987). As a $K^+$ channel activator $Sr^{2+}$ is less effective (Gonzalez and Vassalle 1989). Moreover, the inwardly rectifying $K^+$ conductance blockage was observed in frog skeletal muscle in the presence of $Sr^{2+}$ and $Ba^{2+}$. In these voltage-dependent blockages $Sr^{2+}$ is about 400-times less effective that $Ba^{2+}$ in concentrations of up to 5 mM (Standen and Stanfield 1978). The selective $I_{si}$ conductance blocker nifedipine suppresses $Sr^{2+}$ effect on AP (Štengl and Pučelík 1999).

SERCA pumps $Sr^{2+}$ from cytoplasm into the SR, where it is sequestrated and prepared to be released. SERCA affinity to $Sr^{2+}$ is about 25-times lower that to $Ca^{2+}$ (Spencer and Berlin 1997), and consequently the loading period is prolonged (Vornanen 1985). $Sr^{2+}$ ability to activate RyR2 receptors and in this way to release ions from SR is about 10-times lower in comparison to $Ca^{2+}$. This fact can be explained by decreased positive feedback of SR loading in replacement $Ca^{2+}$ to $Sr^{2+}$. Releasing of only 20% of the SR $Sr^{2+}$ load during $Ca^{2+}$ influx also supports such an explanation (Spencer and Berlin 1997). Some authors describe the inability of $Sr^{2+}$ to release $Sr^{2+}$ ($Sr^{2+}$-induced $Sr^{2+}$ release, SISR) from the SR when $Ca^{2+}$ is fully replaced with $Sr^{2+}$ (King and Bose 1983; Niggli 1989), whereas others describe the existence of SISR (Zoghbi et al. 2004).

Another way of $Sr^{2+}$ removal from cytoplasm (except of transport by SERCA into SR) represents pumping into the mitochondria. Mitochondrial transporters' affinity to $Sr^{2+}$ is close to that of $Ca^{2+}$ (Saris and Åkerman 1980). Both $Sr^{2+}$ and $Ca^{2+}$ are able to promote activity of essential mitochondrial tricarboxylic acid cycle enzymes but with 10-times lower effectiveness of strontium (McCormack and Osbaldeston 1990). $Sr^{2+}$ is extruded from mitochondria by the mitochondrial NCX (Hunter et al. 1980) and from cytoplasm by the sarcoplasmic NCX (Gonzalez and Vassalle 1990). Contrary to $Ba^{2+}$, $Sr^{2+}$ current *via* NCX is nearly indistinguishable from the calcium's one (Haase and Hartung 2009). Even though $Na^+/K^+$-ATPase naturally transports monovalent cations, strontium binds to its intracellular transport side and increases the apparent $K_m$ for $Na^+$ (Gatto et al. 2007).

In summary, the circulation of $Sr^{2+}$ between SR and cytosolic compartment is significantly slower in comparison with $Ca^{2+}$ movement. Due to delayed inactivation of $I_{si}$ and alteration or even blockade of $K^+$ conductance of the repolarizing channels $Sr^{2+}$ prolongs action potential duration. The $Sr^{2+}$ also affects the shape of AP when terminal phase of plateau exhibits faster depolarization and shifts the plateau phase to the more positive values of membrane potential.

## Strontium effects on contraction

Although $Sr^{2+}$ is able to replace $Ca^{2+}$ in the contractile apparatus activation due to its binding to troponin C, its affinity to troponin C is up to 4-times lower (Zoghbi et al. 2004). Both AP as well as contraction duration are prolonged upon $Sr^{2+}$ replacement. Contraction under $Sr^{2+}$ has characteristic biphasic shape, especially at lower stimulation rates – fast and slow component. The fast component develops as twitch contraction and passes to tonic contraction which represents the slow component. Moreover, relaxation is prolonged (Niggli 1989).The first fast component has markedly higher amplitude as compared to the second slow component, which is more frequency-dependent. The slow component frequency dependence determines its disappearance at higher frequencies (above 1.5 Hz), whereas with decreasing frequency increases its amplitude, contraction force and also time to peak of contraction. The fast component is based on cation release from the SR. Suppressing the fast component by ryanodine (the selective RyR receptor blocker) established this conclusion. The slow component is based on influx of $Sr^{2+}$ from extracellular space mainly through the L-type $Ca^{2+}$ channels. Influx through the NCX in reversal mode has only a negligible impact on development of the slow component (Štengl and Pučelík 1999). Not only onset of contraction is prolonged, but also relaxation is prolonged as well as the contraction force is decreased (Niggli 1989).

Adding $Ca^{2+}$ into the perfusion solution leads to competition between $Sr^{2+}$ and $Ca^{2+}$ ions and inhibits changes occurring under $Sr^{2+}$. Due to enhanced inactivation of $I_{si}$, decreased time to peak of contraction, APD and decreased tonic contraction force was observed. On the other side, the replacement of $Na^+$ with sucrose enhances $Sr^{2+}$ influx into the cardiomyocyte caused by delayed $I_{si}$ inactivation, which enhances $Sr^{2+}$ effect (Gonzalez and Vassalle 1989). Exactly the same impact have cardiac glycosides, which increase intracellular $Na^+$ concentration. This intervention preferentially enhances the slow component (King and Bose 1983). In case of premature stimulus, extrasystole acquires the shape of a biphasic contraction with a higher slowly component in comparison to steady-state beats. Postextrasystolic contraction exhibits only a potentiated fast component (Bravený and Šumbera 1974).

## Conclusion

This review summarises known calcium and strontium impacts on the cardiac cell, their membrane transport mechanisms, and effects on contraction and relaxation processes. Although natural strontium intake to the human body in food and water is negligible, it is impossible to dismiss its effects at the cellular level, nominally in the cardiovascular system, which is the target of side effects of the strontium compounds used for example in osteoporosis treatment.

**Conflict of interest:** The authors declare no conflict of interest.

**Acknowledgement:** The financial support from grant projects GAP102/12/2034, MUNI/A/1326/2014, and European Regional Developmental Fund – Project FNUSA-ICRC (No. CZ.1.05/ 1.1.00/ 02.0123) is acknowledged.

## References

Atwood J. L., Suslick K. S. (1996): Supramolecular Reactivity and Transport: Bioinorganic Systems. Elsevier, Oxford

Barth E., Stämmler G., Speiser B., Schaper J. (1992): Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man. J. Mol. Cell. Cardiol. **24,** 669–681 http://dx.doi.org/10.1016/0022-2828(92)93381-S

Berg D., Oberhausen E., Muth H. (1973): Interaction of 47Ca, 85Sr, 133Ba and 226Ra with serum proteins. Biophysik **10,** 309–319 http://dx.doi.org/10.1007/BF01194226

Bers D. M. (2002): Cardiac excitation-contraction coupling. Nature **415,** 198–205 http://dx.doi.org/10.1038/415198a

Bers D. M. (2004): Macromolecular complexes regulating cardiac ryanodine receptor function. J. Mol. Cell. Cardiol. **37,** 417–429

http://dx.doi.org/10.1016/j.yjmcc.2004.05.026
Bianchi M. l., Barletta L., Ardissino G., Daccó V., Claris-Appiani A., Schmitt C., Mehls O. (1999): No difference in intestinal strontium absorption after an oral or an intravenous **1,**25(OH)2D3 bolus in normal subjects. J. Bone Miner. Res. **14,** 1789–1795
http://dx.doi.org/10.1359/jbmr.1999.14.10.1789
Blake G. M., Zivanovic M. A., Lewington V. J. (1989): Measurements of the strontium plasma clearance rate in patients receiving 89Sr radionuclide therapy. Eur. J. Nucl. Med. **15,** 780–783
http://dx.doi.org/10.1007/bf00255497
Bravený P., Šumbera J. (1974): Stažlivost myokardu a její stupňování na buněčné úrovni. Avicenum, Praha (in Czech)
Brini M., Carafoli E. (2009): Calcium pumps in health and disease. Physiol. Rev. **89,** 1341–1378.
http://dx.doi.org/10.1152/physrev.00032.2008
Callewaert G. (1992): Excitation-contraction coupling in mammalian cardiac cells. Cardiovasc. Res. **26,** 923–932
http://dx.doi.org/10.1093/cvr/26.10.923
Camargo M. B. R., Vilaça T., Hayashi L. F., Rocha O. G. F., Lazaretti-Castro M. (2015): 25-Hydroxyvitamin D level does not reflect intestinal calcium absorption: an assay using strontium as a surrogate marker. J. Bone Miner. Metab. **33,** 319–328
http://dx.doi.org/10.1007/s00774-014-0592-8
Carr C. W., Singer L., Spurrell F. A. (1962): The relative binding of calcium and strontium with serum proteins and other substances. Proc. Soc. Exp. Biol. Med. 110 80-83
http://dx.doi.org/10.3181/00379727-110-27430
Chen Y., Csordás G., Jowdy C., Schneider T. G., Csordás N., Wang W., Liu Y., Kohlhaas M., Meiser M., Bergem S., et al. (2012): Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle Ca(2+) crosstalk. Circ. Res. **111,** 863–875
http://dx.doi.org/10.1161/CIRCRESAHA.112.266585
Christoffersen J., Christoffersen M. R., Kolthoff N., Bärenholdt O. (1997): Effects of strontium ions on growth and dissolution of hydroxyapatite and on bone mineral detection. Bone **20,** 47–54
http://dx.doi.org/10.1016/S8756-3282(96)00316-X
Dahl S. G., Allain P., Marie P. J., Mauras Y., Boivin G., Ammann P., Tsouderos Y., Delmas P. D., Christiansen C. (2001): Incorporation and distribution of strontium in bone. Bone **28,** 446–453
http://dx.doi.org/10.1016/S8756-3282(01)00419-7
Dedkova E. N., Blatter L. A. (2013): Calcium signaling in cardiac mitochondria. J. Mol. Cell. Cardiol. **58,** 125–133
http://dx.doi.org/10.1016/j.yjmcc.2012.12.021
Dedkova E. N., Blatter L. A. (2008): Mitochondrial Ca2+ and the heart. Cell Calcium **44,** 77–91
http://dx.doi.org/10.1016/j.ceca.2007.11.002
Edwards C., Lorković H., Weber A. (1966): The effect of the replacement of calcium by strontium on excitation-contraction coupling in frog skeletal muscle. J. Physiol. **186,** 295–306
http://dx.doi.org/10.1113/jphysiol.1966.sp008035
Fabiato A., Fabiato F. (1976): Dependence of calcium release, tension generation and restoring forces on sarcomere length in skinned cardiac cells. Eur. J. Cardiol. **4** (Suppl.), 13–27
Farah C. S., Reinach F. C. (1995): The troponin complex and regulation of muscle contraction. FASEB J. **9,** 755–767
Franěk T., Průša R., Kukačka J., Kizek R. (2009): Strontium in the laboratory medicine. Klin. Biochem. Metab. **17,** 239–244
Frank G. B. (1962): Utilization of bound calcium in the action of caffeine and certain multivalent cations on skeletal muscle. J. Physiol. **163,** 254–268
http://dx.doi.org/10.1113/jphysiol.1962.sp006972
Frank K. F., Mesnard-Rouiller L., Chu G., Young K. B., Zhao W., Haghighi K., Sato Y., Kranias E. G. (2001): Structure and expression of the mouse cardiac calsequestrin gene. Basic Res. Cardiol. **96,** 636–644
http://dx.doi.org/10.1007/s003950170016
Franzini-Armstrong C., Protasi F., Ramesh V. (1999): Shape, size, and distribution of Ca2+ release units and couplons in skeletal and cardiac muscles. Biophys. J. **77,** 1528–1539
http://dx.doi.org/10.1016/S0006-3495(99)77000-1
Gaburjaková J., Gaburjaková M. (2014): Coupled gating modifies the regulation of cardiac ryanodine receptors by luminal Ca(2+). Biochim. Biophys. Acta **1838,** 867–873
http://dx.doi.org/10.1016/j.bbamem.2013.11.005
Gaburjaková J., Gaburjaková M. (2008): Effect of luminal Ca2+ on the stability of coupled gating between ryanodine receptors from the rat heart. Acta Physiol. **193,** 219–227
http://dx.doi.org/10.1111/j.1748-1716.2008.01837.x
Gaburjaková M., Bal N. C., Gaburjaková J., Periasamy M. (2013): Functional interaction between calsequestrin and ryanodine receptor in the heart. Cell. Mol. Life Sci. **70,** 2935–2945
http://dx.doi.org/10.1007/s00018-012-1199-7
Gallacher S. J., Dixon T. (2010): Impact of treatments for postmenopausal osteoporosis (bisphosphonates, parathyroid hormone, strontium ranelate, and denosumab) on bone quality: a systematic review. Calcif. Tissue Int. **87,** 469–484
http://dx.doi.org/10.1007/s00223-010-9420-x
Gatto C., Arnett K. L., Milanick M. A. (2007): Divalent cation interactions with Na,K-ATPase cytoplasmic cation sites: implications for the para-nitrophenyl phosphatase reaction mechanism. J. Membr. Biol. **216,** 49–59
http://dx.doi.org/10.1007/s00232-007-9028-x
Gonzalez M. D., Vassalle M. (1989): Electrical and mechanical effects of strontium in sheep cardiac Purkinje fibres. Cardiovasc. Res. **23,** 867–881
http://dx.doi.org/10.1093/cvr/23.10.867
Gonzalez M. D., Vassalle M. (1990): Strontium induces oscillatory potentials in sheep cardiac Purkinje fibers. Int. J. Cardiol. **27,** 87–99
http://dx.doi.org/10.1016/0167-5273(90)90195-B
Graf E., Verma A. K., Gorski J. P., Lopaschuk G., Niggli V., Zurini M., Carafoli E., Penniston J. T. (1982): Molecular properties of calcium-pumping ATPase from human erythrocytes. Biochemistry **21,** 4511–4516
http://dx.doi.org/10.1021/bi00261a049
Greenwood N. N., Earnshaw A. (1997): Chemistry of the Elements. Butterworth-Heinemann, Oxford
Guerini D., Garcia-Martin E., Zecca A., Guidi F., Carafoli E. (1998): The calcium pump of the plasma membrane. Acta Physiol. Scand. **163,** 265

Guyton A. C., Hall J. E. (2006): Textbook of Medical Physiology. Elsevier Ins., Philadelphia

Haase A. Hartung K. (2009): Pre-steady-state kinetics of Ba-Ca exchange reveals a second electrogenic step involved in Ca2+ translocation by the Na-Ca exchanger. Biophys. J. **96,** 4571–4580 http://dx.doi.org/10.1016/j.bpj.2009.03.017

Hatano A., Okada J., Hisada T., Sugiura S. (2012): Critical role of cardiac t-tubule system for the maintenance of contractile function revealed by a 3D integrated model of cardiomyocytes. J. Biomech. **45,** 815–823 http://dx.doi.org/10.1016/j.jbiomech.2011.11.022

Hesaraki S., Alizadeh M., Nazarian H., Sharifi D. (2010): Physicochemical and in vitro biological evaluation of strontium/calcium silicophosphate glass. J. Mater. Sci. Mater. Med. **21,** 695–705 http://dx.doi.org/10.1007/s10856-009-3920-0

Hunter D. R., Komai H., Haworth R. A., Jackson M. D., Berkoff H. A. (1980): Comparison of Ca2+, Sr2+, and Mn2+ fluxes in mitochondria of the perfused rat heart. Circ. Res. **47,** 721–727 http://dx.doi.org/10.1161/01.RES.47.5.721

Huxley A. F., Niedergerke R. (1954): Structural changes in muscle during contraction; interference microscopy of living muscle fibres. Nature **173,** 971–973 http://dx.doi.org/10.1038/173971a0

Huxley H. E. (1969): The mechanism of muscular contraction. Science **164,** 1356–1365 http://dx.doi.org/10.1126/science.164.3886.1356

Inesi G., Tadini-Buoninsegni F. (2014): Ca(2+)/H (+) exchange, lumenal Ca(2+) release and Ca (2+)/ATP coupling ratios in the sarcoplasmic reticulum ATPase. J. Cell Commun. Signal. **8,** 5–11 http://dx.doi.org/10.1007/s12079-013-0213-7

Karim B. F. A., Gillam D. G. (2013): The efficacy of strontium and potassium toothpastes in treating dentine hypersensitivity: A systematic review. Int. J. Dent. **2013**

Kawata H., Hatae J. (1977): Contractile behavior of cardiac ventricular muscle in strontium solution. Jpn. J. Physiol. **27,** 167–184

King B. W., Bose D. (1983): Mechanism of biphasic contractions in strontium-treated ventricular muscle. Circ. Res. **52,** 65–75 http://dx.doi.org/10.1161/01.RES.52.1.65

Kohlhaas M., Maack C. (2013): Calcium release microdomains and mitochondria. Cardiovasc. Res. **98,** 259–268 http://dx.doi.org/10.1093/cvr/cvt032

Kohlhardt M., Herdey A., Kubler M. (1973): Interchangeability of Ca ions and Sr ions as charge carriers of the slow inward current in mammalian myocardial fibres. Pflügers Arch. **344,** 149–158 http://dx.doi.org/10.1007/BF00586548

Kulveitová H. (2010): Chemie II. Chemie prvků (in Czech)

Kumar S., Ahmad E., Kumar S., Khan R. H., Gourinath S. (2012): Flexibility of EF-hand motifs: structural and thermodynamic studies of calcium binding protein-1 from Entamoeba histolytica with Pb2+, Ba2+, and Sr2+. BMC Biophys. **5,** 15 http://dx.doi.org/10.1186/2046-1682-5-15

Lehnerdt F. (1909): On the issue of the substitution of calcium in the bone system through strontium. Beitr. Pathol. Anat. **46,** 468–572

Lepsík M., Field M. J. (2007): Binding of calcium and other metal ions to the EF-hand loops of calmodulin studied by quantum chemical calculations and molecular dynamics simulations. J. Phys. Chem. B. **111,** 10012–10022 http://dx.doi.org/10.1021/jp0716583

Maack C., O'Rourke B. (2007): Excitation-contraction coupling and mitochondrial energetics. Basic Res. Cardiol. **102,** 369–392 http://dx.doi.org/10.1007/s00395-007-0666-z

McCormack J. G., Osbaldeston N. J. (1990): The use of the Ca2+-sensitive intramitochondrial dehydrogenases and entrapped fura-2 to study Sr2+ and Ba2+ transport across the inner membrane of mammalian mitochondria. Eur. J. Biochem. **192,** 239–244 http://dx.doi.org/10.1111/j.1432-1033.1990.tb19221.x

Mizuno J., Araki J., Mohri S., Minami H., Doi Y., Fujinaka W., Miyaji K., Kiyooka T., Oshima Y., Iribe G., Hirakawa M., Suga H. (2001): Frank-Starling mechanism retains recirculation fraction of myocardial Ca(2+) in the beating heart. Jpn. J. Physiol. **51,** 733–743 http://dx.doi.org/10.2170/jjphysiol.51.733

Morkin E. (2000): Control of cardiac myosin heavy chain gene expression. Microsc. Res. Tech. **50,** 522–531 http://dx.doi.org/10.1002/1097-0029(20000915)50:6<522::-AID-JEMT9>3.0.CO;2-U

Morohashi T., Sano T., Yamada S. (1994): Effects of strontium on calcium metabolism in rats. I. A distinction between the pharmacological and toxic doses. Jpn. J. Pharmacol. **64,** 155–162 http://dx.doi.org/10.1254/jjp.64.155

Nayler W. G. (1965): Calcium and other divalent ions in contraction of cardiac muscle. In: Muscle. p. **167,** Pergamon Press, London

Niggli E. (1989): Strontium-induced creep currents associated with tonic contractions in cardiac myocytes isolated from guinea-pigs. J. Physiol. **414,** 549–568 http://dx.doi.org/10.1113/jphysiol.1989.sp017703

Ondriaš K., Mojžišová A. (2002): Coupled gating between individual cardiac ryanodine calcium release channels. Gen. Physiol. Biophys. **21,** 73–84

Ono K., Iijima T. (2010): Cardiac T-type Ca2+ channels in the heart. J. Mol. Cell. Cardiol. **48,** 65–70 http://dx.doi.org/10.1016/j.yjmcc.2009.08.021

O'Rourke B., Blatter L. A. (2009): Mitochondrial Ca2+ uptake: Tortoise or hare? J. Mol. Cell. Cardiol. **46,** 767–774 http://dx.doi.org/10.1016/j.yjmcc.2008.12.011

Ošťádal B., Ošťádalová I., Kolář F., Charvátová Z., Netuka I. (2009): Ontogenetic development of cardiac tolerance to oxygen deprivation - possible mechanisms. Physiol. Res. **58,** S1–12

Pappano A. J., Sperelakis N. (1969): Spontaneous contractions of cultured heart cells in high K+ media. Exp. Cell Res. **54,** 58–68 http://dx.doi.org/10.1016/0014-4827(69)90293-6

Poršová M., Porš J., Kolombo I., Pabišta R., (2007): Actual medication of the bone metastases. Urologie pro Praxi **8,** 272–280 (in Czech)

Pors N. S. (2004): The biological role of strontium. Bone **35,** 583–588 http://dx.doi.org/10.1016/j.bone.2004.04.026

Rossi A. E., Dirksen R. T. (2006): Sarcoplasmic reticulum: the dynamic calcium governor of muscle. Muscle Nerve **33,** 715–731

http://dx.doi.org/10.1002/mus.20512
Saris N. E., Åkerman K. E. O. (1980): Uptake and release of bivalent cations in mitochondria. In: Current Topics in Bioenergetics. (Ed. Sanadi D. R.), pp. 103–179, Academic Press, New York
Schulze D. H., Muqhal M., Lederer W. J., Ruknudin A. M. (2003): Sodium/calcium exchanger (NCX1) macromolecular complex. J. Biol. Chem. **278,** 28849–28855
http://dx.doi.org/10.1074/jbc.M300754200
Scriven D. R. L., Moore E. D. W. (2013): Ca2+ channel and Na+/Ca2+ exchange localization in cardiac myocytes. J. Mol. Cell. Cardiol. **58,** 22–31
http://dx.doi.org/10.1016/j.yjmcc.2012.11.022
Shannon T. R., Ginsburg K. S., Bers D. M. (2000): Potentiation of fractional sarcoplasmic reticulum calcium release by total and free intra-sarcoplasmic reticulum calcium concentration. Biophys. J. **78,** 334–343
http://dx.doi.org/10.1016/S0006-3495(00)76596-9
Shirokova N., Niggli E. (2008): Studies of RyR function in situ. Methods **46,** 183–193
http://dx.doi.org/10.1016/j.ymeth.2008.09.017
Simmerman H. K. B., Jones L. R. (1998): Phospholamban: Protein structure, mechanism of action, and role in cardiac function. Physiol. Rev. **78,** 921–947
Sipido K. R., Carmeliet E., Van De Werf F. (1998): T-type Ca2+ current as a trigger for Ca2+ release from the sarcoplasmic reticulum in guinea-pig ventricular myocytes. J. Physiol. **508,** 439–451
http://dx.doi.org/10.1111/j.1469-7793.1998.439bq.x
Solaro R. J., Rarick H. M. (1998): Troponin and tropomyosin: Proteins that switch on and tune in the activity of cardiac myofilaments. Circ. Res. **83,** 471–480
http://dx.doi.org/10.1161/01.RES.83.5.471
Spencer C. I., Berlin J. R. (1997): Calcium-induced release of strontium ions from the sarcoplasmic reticulum of rat cardiac ventricular myocytes. J. Physiol. **504,** 565–578
http://dx.doi.org/10.1111/j.1469-7793.1997.565bd.x
Standen N. B., Stanfield P. R. (1978): A potential- and time-dependent blockade of inward rectification in frog skeletal muscle fibres by barium and strontium ions. J. Physiol. **280,** 169–191
http://dx.doi.org/10.1113/jphysiol.1978.sp012379
Suchopar J., Kučera Z. (2005): Strontii ranelas. Remedia **15,** 323–329 (in Czech)
Sugihira N., Aoki Y., Suzuki K. T. (1992): ATP-dependent strontium uptake by basolateral membrane vesicles from rat renal cortex in the absence or presence of calcium. Biol. Trace Elem. Res. **34,** 45–54
http://dx.doi.org/10.1007/BF02783897
Švíglerová J., Kuncová J., Stengl M. (2014): Contractile functions of myocardium and their regulation. Cesk. Fysiol. **63,** 25–33
Syuu Y., Araki J., Lee S., Suzuki S., Mizuno J., Mohri S., Mikane T., Shimizu J., Takaki M., Suga H. (1998): Effects of Ca2+ and epinephrine on Ca2+ recirculation fraction and total Ca2+ handling in canine left ventricles. Jpn. J. Physiol. **48,** 123–132
http://dx.doi.org/10.2170/jjphysiol.48.123
Štengl M., Pučelík P. (1999): Effects of strontium ions on contraction and action potential in rabbit papillary muscles. A comparison with effects of tetraethylammonium ions. Gen. Physiol. Biophys. **18,** 19–33
Tada M., Katz A. M. (1982): Phosphorylation of the sarcoplasmic reticulum and sarcolemma. Annu. Rev. Physiol. **44,** 401–423
http://dx.doi.org/10.1146/annurev.ph.44.030182.002153
Tohse N., Kameyama M., Irisawa H. (1987): Intracellular Ca2+ and protein kinase C modulate K+ current in guinea pig heart cells. Am. J. Physiol. **253,** 1321–1324
Vornanen M. (1985): Effect of strontium on the contractile properties of postnatally developing rat heart ventricles. Can. J. Physiol. Pharmacol. **63,** 9–17
http://dx.doi.org/10.1139/y85-002
Weber K., Erben R. G., Rump A., Adamski J. (2001): Gene structure and regulation of the murine epithelial calcium channels ECaC1 and 2. Biochem. Biophys. Res. Commun. **289,** 1287–1294
http://dx.doi.org/10.1006/bbrc.2001.6121
Wood Ch. M., Farrell A. P., Brauner C. J. (2011): Homeostasis and Toxicology of Nonessential Metals. Academic Press, London
Zoghbi M. E., Copello J. A., Villalba-Galea C. A., Vélez P., Diaz Sylvester P. L., Bola-os P., Marcano A., Fill M., Escobar A. L. (2004): Differential Ca2+ and Sr2+ regulation of intracellular divalent cations release in ventricular myocytes. Cell Calcium **36,** 119–134
http://dx.doi.org/10.1016/j.ceca.2004.01.023

Received: February 19, 2015
Final version accepted: June 10, 2015
First published online: November 27, 2015