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The role of the sarcoplasmic reticulum in the generation of high heart rates and blood pressures in reptiles

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Summary

The functional significance of the sarcoplasmic reticulum (SR) in the generation of high heart rates and blood pressures was investigated in four species of reptile; the turtle, *Trachemys scripta*; the python, *Python regius*, the tegu lizard, *Tupinanvis merianae*, and the varanid lizard, *Varanus exanthematicus*. Force-frequency trials and imposed pauses were performed on ventricular and atrial tissue from each species with and without the SR inhibitor ryanodine, and in the absence and presence of adrenaline. In all species, an imposed pause of 1 or 5 min caused a post-rest decay of force, and a negative force-frequency response was observed in all species within their *in vivo* frequency range of heart rates. These relationships were not affected by either ryanodine or adrenaline. In ventricular strips from varanid lizards and pythons, ryanodine caused significant reductions in twitch force within their physiologically relevant frequency range. In atrial tissue from the tegu and varanid lizards, SR inhibition reduced twitch force across the whole of their physiological frequency range. In contrast, in the more sedentary species, the turtle and the python, SR inhibition only decreased twitch force at stimulation frequencies above maximal *in vivo* heart rates. Adrenaline caused an increase in twitch force in all species studied. In ventricular tissue, this positive inotropic effect was sufficient to overcome the negative effects of ryanodine. In atrial tissue however, adrenaline could only ameliorate the negative effects of ryanodine at the lower pacing frequencies. Our results indicate that reptiles recruit Ca^{2+} from the SR for force development in a frequency and tissue dependent manner. This is discussed in the context of the development of high reptilian heart rates and blood pressures.

Key words: snake, lizard, turtle, trabeculae, ryanodine, adrenaline.

Introduction

In the mammalian heart, the sarcoplasmic reticulum (SR) provides an intracellular store of Ca^{2+} that can be mobilised for contraction and re-sequestered for subsequent relaxation. The close proximity of the SR to the contractile proteins reduces the diffusion distance for Ca^{2+} and facilitates rapid regulation of contractility and high frequencies of contraction (Bers, 2002). Consequently, three-quarters of the Ca^{2+} contributing to myofillament activation is released from the SR in mammalian myocytes, while sarcolemmal Ca^{2+} transport is less important (Bers, 2001).

The contribution of the SR to cardiac function differs amongst species (Bers, 2001). Thus, the density and complexity of the SR network is poorly developed or even absent in most amphibians and fish (Bossen and Sommer, 1984; Lillywhite et al., 1999). These observations correlate with physiological data that demonstrate most ectothermic cardiac muscle to be insensitive to ryanodine (Driedzic and Gesser, 1988; Hove-Madsen and Gesser, 1989; Vornanen, 1989), a compound that inhibits the SR Ca^{2+} release channel (Rousseau et al., 1987). The absence of a functional SR in fish is partially compensated for by much longer and thinner cardiac myocytes. The large surface area relative to volume reduces the diffusional distance for Ca^{2+} movement, thereby increasing the impact of sarcolemmal Ca^{2+} flux (Vornanen et al., 2002). Thus, in most fish, extracellular Ca^{2+} cycling is sufficient to support myocyte contraction (Tibbits et al., 1991).

Comparisons between mammals and some ectothermic vertebrates have linked SR dependence and specialisations in excitation–contraction coupling processes with high resting and maximal heart rates. Highly active fish species, such as tuna, show enhanced SR dependence of contraction (Keen et al., 1992; Shiels et al., 1999; Shiels and Farrell, 2000), and fish relying extensively on the SR often generate higher aortic blood pressures than other fish (Farrell et al., 1998). These results suggest that SR dependence and development of Ca^{2+}

cycling may be involved in the evolution of higher resting and maximal heart rates, and possibly blood pressures, in non-mammalian vertebrates.

Reptiles represent an interesting phylogenetic group, with extant species exhibiting large and fundamental differences in cardiac anatomy and function. In most chelonians and squamates, the ventricle is anatomically and functionally undivided, and blood pressures are equal in the systemic and pulmonary circulations (e.g. Hicks, 1998). As a consequence, blood pressure is relatively low to avoid pulmonary oedema. As exceptions, varanid lizards and pythons have functionally divided circulations with high, mammalian-like, systemic blood pressures, while pressures remain low in the lungs (Burggren and Johansen, 1982; Wang et al., 2003). Extant reptiles also exhibit large interspecific variations in maximal heart rates. Maximal heart rates of turtles and pythons are approximately 50 min^{-1}, whereas heart rates in varanid (Wang et al., 1997) and tegu lizards (G.G. and T.W., personal observations) can exceed 100 min^{-1} at similar temperatures. Moreover, ultrastructural studies have demonstrated variation in the complexity of the SR in turtles, lizards and snakes (Leak, 1967; Okita, 1971; Forbes and Sperelakis, 1971; Forbes and Sperelakis, 1974). We have exploited this interspecific diversity within reptiles to investigate whether species with high heart rates and/or high blood pressures rely more on SR Ca^{2+} cycling to support cardiac contraction than more sedentary species.

The four species of reptiles studied were chosen because they represent the four possible combinations of pressures and maximal heart rates: the turtle (*Trachemys scripta*) has low heart rates and blood pressures; the tegu lizard (*Tupinambis merinae*) has high heart rates and low blood pressures; the python (*Python regius*) has low heart rates and high blood pressures; and finally the savannah monitor lizard (*Varanus exanthematicus*) has both high heart rates and high blood pressures.

Materials and methods

Experimental animals

Turtles (*Trachemys scripta* Gray, *N*=7) were obtained from Lemberger Inc. (Oshkosh, WI, USA), and transported to Aarhus University where they were maintained in 1 m×1 m fiberglass tanks containing water at 28°C (40 cm depth) and dry basking platforms with heating lamps to allow for behavioral thermoregulation. Pythons (*Python regius* Shaw, *N*=7) and varanid lizards (*Varanus exanthematicus* Bosc, *N*=8) were obtained from a local animal supplier and transported to Aarhus University where they were kept in vivaria (150 cm×60 cm×60 cm) under a daily photoperiod of 12 h:12 h light:darkness. The vivaria contained a heating lamp which generated temperatures between 25 and 35°C. The tegu lizards (*Tupinambis merianae* Duméril and Bribon, *N*=6) were obtained from UNESP, Rio Claro, Brazil, then transported to Aarhus University and maintained in vivaria as described above.

Experimental preparations

Each animal was decapitated and the heart quickly removed and transferred to ice-cold oxygenated physiological saline in a Petri dish, for further dissection. The constituents of the physiological saline were the same for each experimental species: NaCl, 115 mmol l^{-1}; KCl, 2.5 mmol l^{-1}; $MgSO_4$, 1 mmol l^{-1}; NaH_2PO_4, 1 mmol l^{-1}; glucose, 5 mmol l^{-1}; $CaCl_2$, 2 mmol l^{-1}; $NaHCO_3$, 25 mmol l^{-1} with a pH of 7.45 when equilibrated to 2.0 kPa CO_2 and 99.3 kPa O_2 at 30°C. Four longitudinal myocardial strips were prepared from each heart, two from the left atrium and two from the cavum arteriosum. For each tissue, one strip served as the experimental strip and the other served as a control. To account for the slow deterioration of the preparation over the duration of the experiment, control strips were exposed to the same conditions of stimulation frequency and strength as the experimental strips but were not subjected to SR inhibition. Thus, changes in tension due to deterioration during the experiment were accounted for by subtracting changes in tension in controls (relative to the initial contractility) from the experimental results (see Shiels et al., 1999; Shiels and Farrell, 2000). Preparations that deteriorated substantially (>25%) were discarded; however, due to a limited number of tegu lizards available all data were used to calculate mean values, including two atrial and one ventricle preparations that declined by 30–40%.

The heart strips were mounted vertically using 3-0 surgical silk; one end was attached to a thin glass rod and the other end to one of the two platinum stimulation electrodes. The second stimulation electrode was positioned close to the upper end of the preparation. Both stimulation electrodes were connected to Grass SD 9 stimulators (Quincy, MA, USA). The glass rod was connected to a force transducer (Statham UC 2, Oxnard, CA, USA). The cardiac strips were electrically paced at 12 min^{-1} (0.2 Hz), with pulses of 5 ms and a voltage twice that eliciting maximal response. The preparations were allowed to stabilise for 30 min before they were stretched by adjusting the length of the preparation with a micrometer screw to provide maximum induced force of contraction on electrical stimulation. The preparations were then left to stabilise for a period of at least 30 min before experimentation. Signals from the force transducers were recorded by an AcqKnowledge (version 3.7.1) MP100 data-acquisition system at 200 Hz. After each experiment, length and wet mass of each cardiac strip were measured and force (mN) relative to cross-sectional area (mm^2) was estimated assuming a density of 1.0 mg mm^{-3} and uniform thickness of the strips.

Contractile performance of the cardiac tissue

The experimental protocol was designed to determine the relative contribution of the SR to force production at various stimulation frequencies. In addition, since adrenaline is an important modulator of contractility and SR function, we deemed it necessary to investigate the effect of adrenergic stimulation on force production in the absence and presence of SR blockade. SR dependence was assessed using ryanodine, a

specific blocker of the SR Ca^{2+} release channel. Ryanodine was applied at a concentration (10 μmol l^{-1}) where it locks the SR Ca^{2+} release channel in a closed state, rendering the SR ineffective in Ca^{2+} cycling (Rousseau et al., 1987). This dose of ryanodine also provides maximal effect on twitch force development of trout heart preparations (Hove-Madsen, 1992).

Following stabilisation of force, each preparation was subjected to a 1 min and a 5 min pause (post-rest experiment) without stimulation. Stimulation was then resumed, and once force had stabilised, a force-frequency (F-F) trial was performed, where stimulation frequency was increased in 0.3 Hz steps from 0.2 Hz. Each frequency was maintained for 20 s, allowing force to become stable. The F-F trials were terminated once preparations exhibited irregular contractions. Stimulation frequency was subsequently returned to 0.2 Hz and preparations were allowed to recover. The experimental strips were then exposed to 10 μmol l^{-1} ryanodine and all preparations were left for 40 min before the previous protocol was repeated. Next, 10 μmol l^{-1} of adrenaline was added to both control and experimental strips and when force had stabilised, usually within 10 min, the protocol was repeated for a third time.

Calculations and statistical analysis

To quantify the effect of the experimental manipulations on contraction of the cardiac strips, the following parameters were measured: twitch force, resting tension, rate of rise of contraction and rate of 50% relaxation. Twitch force was calculated in absolute terms, and standardised for cross-sectional area as described earlier. In the force-frequency trials, parameters were recorded at 0.2 Hz immediately before the trial and at each test frequency. In the case of the varanid lizards, however, atrial preparations contracted spontaneously, which made it difficult to analyse data at the control frequency of 0.2 Hz; therefore, the control frequency for this species was taken at 0.8 Hz, where spontaneous contractions ceased. In post-rest experiments, twitch force was measured at 0.2 Hz prior to the pause, and the first contraction following the pause, and a relative change was calculated. Twitch force was measured as the difference between peak and resting force after electrical stimulation. The rate of rise of contraction (R_{peak}) was calculated by dividing twitch force with the time taken to reach peak force (mN mm^{-2} s^{-1}). The rate at which force fell from peak force to 50% relaxation of force ($R_{peak\rightarrow 50\%\ rel}$) was also calculated (mN mm^{-2} s^{-1}). Significant (P=<0.05) reductions in force due to ryanodine and increases after adrenaline were assessed using a one-way repeated measure analysis of variance (one-way RM-ANOVA). Statistical tests were only performed when N was >5.

Results

Force-frequency effects

Untreated ventricular and atrial muscle from each species exhibited a negative F-F relationship, with twitch force declining as frequency was increased (Fig. 1). There was a tendency for a small positive F-F relationship to occur at low stimulation frequencies, creating a biphasic response; however, this was not statistically resolvable. In most species resting tension (RT) remained relatively constant at low stimulation frequencies, and significantly increased at the higher pacing frequencies (Fig. 1). RT of ventricular and atrial tissue was unaffected by ryanodine or adrenaline at all stimulation frequencies, therefore only changes in RT in untreated tissue have been included in Fig. 1. In atrial tissue from the turtle, RT tended to oscillate spontaneously in an unpredictable manner. These oscillations were particularly pronounced during the post-rest experiments (Fig. 3), and were abolished with adrenaline.

In all four species, atrial muscle achieved higher maximal frequencies of contraction than ventricular muscle (Fig. 1). The turtle achieved the highest frequencies of contraction, with individual ventricular strips reaching frequencies of 3.2 Hz and some atrial strips reaching 4.1 Hz. However, while turtles could achieve the highest rates of contraction, this effect was also accompanied by a large increase in RT, and absolute twitch force was lower than the other species (Fig. 1).

Effects of ryanodine

Ryanodine did not significantly alter the shape of the F-F relationships in any of the four species. In general, the effects of ryanodine were more pronounced on atrial tissue compared with ventricular tissue. In ventricular muscle, significant effects of ryanodine on twitch force were only observed in pythons and varanid lizards, where twitch force was significantly reduced within and beyond their physiologically relevant frequency range (Fig. 1B–D). In atrial tissue, ryanodine reduced twitch force in the tegu and varanid lizards across their *in vivo* frequency range (Fig. 1G,H). In particular, at the maximal achievable *in vivo* heart rates of varanid lizards, 90–100 min^{-1} (1.5 Hz), ryanodine reduced atrial twitch force by more than 45% (Fig. 1H). Furthermore, in two of the six animals, atrial contractions were completely abolished by ryanodine. In contrast, SR dependence in atrial tissue from turtles and pythons was only apparent at supra-physiological frequencies (Fig. 1E,F).

Effects of adrenaline

Adrenergic stimulation of the control strips significantly increased twitch force at 0.2 Hz in the turtle (ventricular tissue, 6.0±1.4 to 8.3±1.8 mN mm^{-2}; atrial tissue, 3.3±1.5 to 5.2±2.1 mN mm^{-2}), the python (ventricular tissue, 5.8±0.8 to 8.8±2.2 mN mm^{-2}; atrial tissue, 4.6±1.0 to 12.8± 2.7 mN mm^{-2}), the tegu lizard (ventricular tissue, 7.1±1 to 11.7±1.9 mN mm^{-2}; atrial tissue, 3.4±0.6 to 7.3± 2.4 mN mm^{-2}), and the varanid lizard (ventricular tissue, 6.9±0.7 to 9.3±1.6 mN mm^{-2}; atrial tissue, 3.5±0.7 to 7.6±1.5 mN mm^{-2}). Furthermore, adrenaline significantly increased twitch force in all species and tissue types at almost all frequencies tested, shifting the F-F relationship upwards (data not shown). Exposing tissue pre-treated with ryanodine to adrenaline caused an immediate and pronounced rise in

twitch force in both ventricular and atrial strips from all species (Fig. 1). However, the shape of the F-F relationship was not affected. In ventricular muscle pre-treated with ryanodine, adrenaline increased twitch force at almost all frequencies tested and could counteract any negative effects of ryanodine (Fig. 1A–D). However, in atrial tissue, adrenaline could only ameliorate the negative effects of ryanodine at the lower pacing frequencies (Fig. 1E–H).

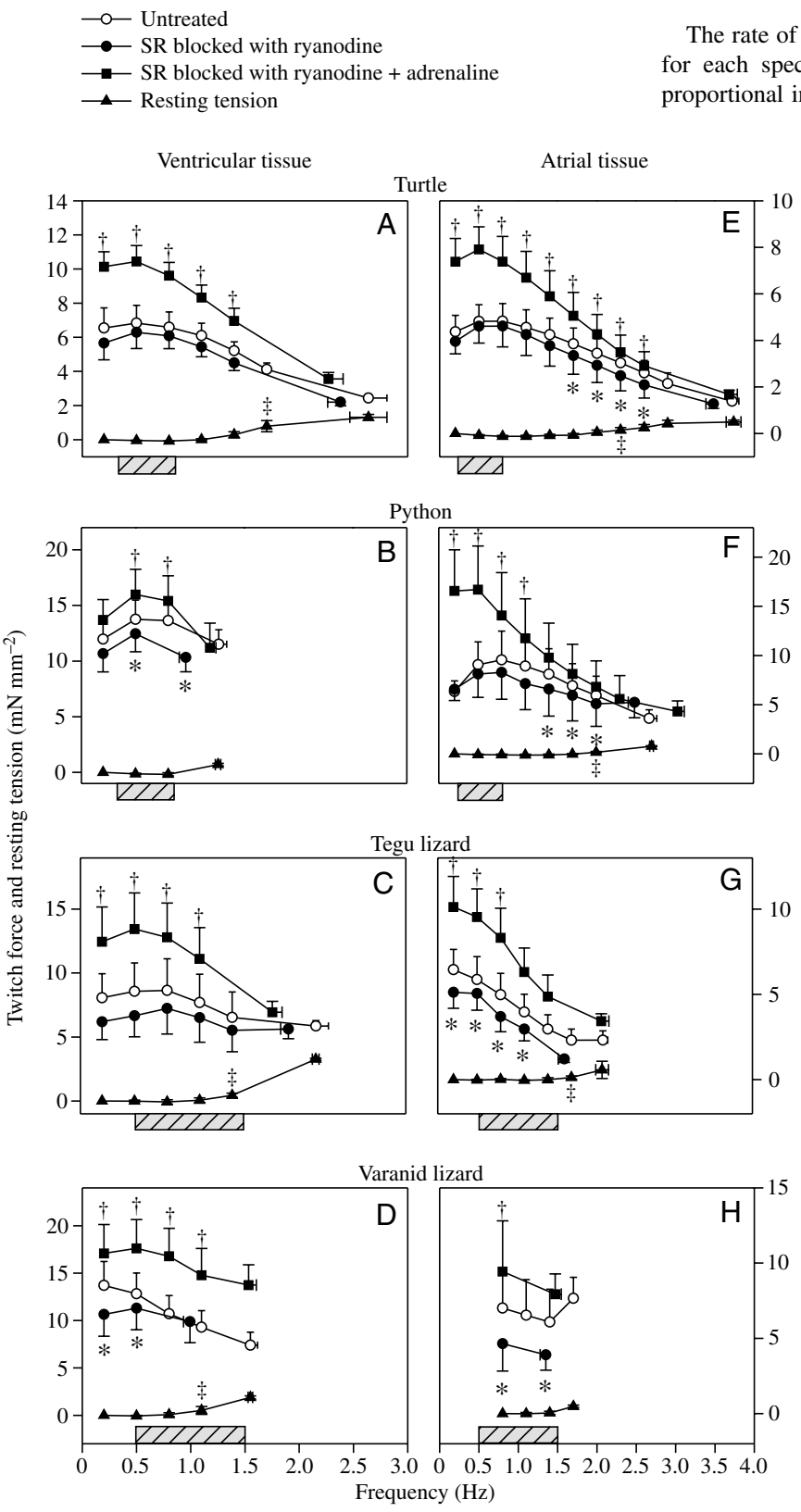

Fig. 1. Force-frequency relationships and resting tension after treatment with different drugs (see key) in ventricular (A–D) and atrial (E–H) muscle from each species. (A,E) Turtle, *Trachemys scripta* (*N*=7); (B,F) python, *Python regius* (*N*=7); (C,G) tegu lizard, *Tupinambis merianae* (*N*=6); (D,H) varanid lizard, *Varanus exanthematicus* (*N*=8). The resting tension data is for untreated tissue only. Horizontal hatched grey bars indicate the species range of *in vivo* heart rate for each species. The last point on each curve displays an *x*-error, as the *N*-value is less than that stated above. Values are mean ± s.e.m. *Twitch force is significantly reduced following pre-treatment with ryanodine, †twitch force is significantly increased following treatment with adrenaline, and ‡the frequency at which resting tension significantly increased ($P<0.05$).

Contraction kinetics

The rate of rise of contraction and 50% relaxation at 0.2Hz for each species and tissue type is given in Table 1. The proportional increase in rate of rise of contraction and rate of 50% relaxation from 0.2 Hz to the maximal achievable frequency for each species is given in brackets beside each value. Although contraction tended to be faster in atrial *vs* ventricular tissue, this difference was not statistically significant. In all species and tissue types, contractions were faster with increased frequency (Table 1). In the turtle, python and tegu lizard, the relative magnitude of this response was unchanged following treatment with ryanodine. In the varanid lizard, ryanodine significantly slowed the rate of rise of contraction in both ventricular (from 26.5±5.1 to 18.2± 3.9 mN mm^{-2} s^{-1} at 0.2 Hz) and atrial tissue (from 31.1±11.1 to 21.3±9.3 mN mm^{-2} s^{-1} at 0.8 Hz). Ryanodine also slowed the rate of 50% relaxation in atrial tissue (from 43.2±13.5 to 28.1±8.8 mN mm^{-2} s^{-1} at 0.8 Hz).

Post-rest effects

An imposed pause of 1 min between contractions induced a post-rest decay of force (Fig. 2A) in all species studied, which was more pronounced after 5 min (Fig. 2B). The relative magnitude of the post-rest decay was not significantly altered by treatment with either ryanodine or adrenaline, though

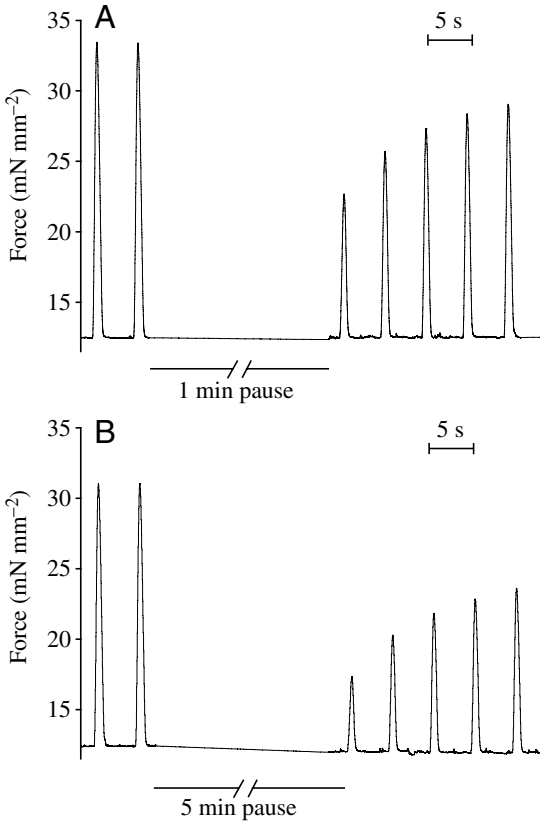

Fig. 2. Original traces from post-rest experiments. Representative trace of a 1 min (A) and 5 min (B) pause in ventricular muscle from a varanid lizard, *Varanus exanthematicus*. Stimulation ceased for a period of 1 min (A) and 5 min (B), and the following 5 contractions were recorded.

there was a tendency for greater post-rest decay after ryanodine treatment (data not shown).

Discussion

This is the first study to assess the functional significance of the SR in reptiles. An obvious drawback to using ryanodine is that other Ca^{2+} transport mechanisms may compensate for the loss of SR function. Our study, therefore, is likely to underestimate the actual contribution from the SR to E–C coupling. Our results indicate that contraction and relaxation of cardiac muscle in reptiles may involve Ca^{2+} cycling through the SR. Furthermore, as with other ectothermic vertebrates, the relative contribution of the SR varied with stimulation frequency, tissue-type, and appeared to correlate with maximal *in vivo* heart rates and blood pressures.

Effects of SR inhibition

Ventricular strips from the four species were only marginally affected by ryanodine. However, ryanodine reduced ventricular twitch force by 10–20% within *in vivo* frequencies in python, and reduced twitch force at the low range of the *in vivo* heart rates of varanid lizards. Pythons and

Table 1. *Rate of rise of contraction and of 50% relaxation at 0.2 Hz in untreated ventricle and atrial muscle from each species*

	R_{peak} (mN mm^{-1} s^{-1})	$R_{peak\rightarrow 50\%\ rel}$ (mN mm^{-1} s^{-1})
Turtle		
Ventricle	12.7±1.7 (1.3±0.1)	25.3±4.1 (1.3±0.1)
Atria	13.8±2.3 (1.4±0.3)	24.3±3.8 (1.2±0.2)
Python		
Ventricle	17.1±2.3 (1.6±0.2)	34.2±4.1 (1.3±0.1)
Atria	16.4±2.4 (1.9±0.4)	41.7±7.4 (1.2±0.2)
Tegu lizard		
Ventricle	16.9±4.3 (1.3±0.2)	26.1±6.8 (1.3±0.1)
Atria	18.3±3.1 (0.7±0.1)	38.3±8.4 (0.5±0.1)
Varanid lizard		
Ventricle	26.5±5.1 (1.2±0.1)	32.9±5.8 (1.2±0.1)
Atria (0.8 Hz)	31.1±11.1 (1.1±0.1)	43.2±13.5 (1.1±0.1)

R_{peak}, rate of rise of contraction; $R_{peak\rightarrow 50\%\ rel}$, rate of 50% relaxation.

Turtle, *Trachemys scripta* (*N*=7); python, *Python regius* (*N*=7); tegu lizard, *Tupinanvis merianae* (*N*=6); varanid lizard, *Varanus exanthematicus* (*N*=8).

Values are means ± s.e.m. Values in parentheses denote fold change in rate from 0.2 Hz to maximal achievable frequency for each species and tissue type.

No significant differences in contraction kinetics were observed between atrial and ventricular tissue.

varanid lizards are unique amongst reptiles by having a functionally divided ventricle and high systemic blood pressures, so it is tempting to speculate that high blood pressures require SR Ca^{2+} utilisation. In support of this contention, SR-dependent fish, such as tuna, generate higher arterial blood pressures than other fish (Farrell, 1996; Vornanen et al., 2002). Increased force of contraction and systemic pressures are primarily accomplished by thickening the ventricular wall (Webb et al., 1971; Farrell et al., 1998), and if this is achieved *via* myocyte hypertrophy, the diffusional distance for Ca^{2+} movement will be increased which may require more efficient Ca^{2+} cycling, possibly *via* the SR.

As with fish and mammals, reptilian atrial tissue was generally more sensitive to SR inhibition than ventricle strips (Aho and Vornanen, 1999; Keen et al., 1992; Shiels et al., 1999; Bers, 2001; Mercier et al., 2002). These findings correlate with ultrastructural and biochemical studies showing higher SR densities and SR associated proteins in atrial *vs* ventricular muscle (Minajeva et al., 1997; Bossen et al., 1981; Luss et al., 1999). In mammals and fish, the duration of atrial contraction is shorter than in the ventricle, and the rates of contraction and relaxation are faster (Aho and Vornanen, 1999; Luss et al., 1999). Aside from a shorter action potential, the faster atrial contraction has been associated with a greater SR dependence (Minajeva et al., 1997; Luss et al., 1999). In accordance with this, the atria contracted and relaxed faster

than the ventricles in all four species of reptiles; however, this relationship was not statistically resolvable.

Ryanodine sensitivity of the atria in the four species of reptiles correlated with *in vivo* heart rates. In turtles and pythons, which have low maximal heart rates *in vivo*, the effect of SR inhibition only became apparent at supra-physiological frequencies, whereas ryanodine caused significant reductions in twitch force across all physiologically relevant rates of contraction in the more active tegu and varanid lizards with higher *in vivo* heart rates. The effect was most evident in the varanid lizard, with more than a 45% reduction in twitch force at their maximal *in vivo* heart rate. Ryanodine also slowed the rate at which maximal twitch force was achieved, and the rate of relaxation, suggesting that SR Ca^{2+} cycling is important for E–C coupling.

SR dependence in fish hearts has also been linked to heightened cardiac performance and activity levels (Keen et al., 1992; Shiels et al., 1999; Shiels and Farrell, 2000). It seems that SR Ca^{2+} cycling in the less active species of teleosts, such as the rainbow trout, is limited to certain experimental test conditions (e.g. temperature and level of adrenaline) and particular stimulation frequencies (Hove-Madsen, 1992; Keen et al., 1994; Gesser, 1996; Shiels and Farrell, 1997; Shiels and Farrell, 1997), while more active species, such as the pacific mackerel and tuna, show sensitivity to ryanodine across a wider range of contraction frequencies (Keen et al., 1992; Shiels et al., 1999; Shiels and Farrell, 2000). Thus, it seems that in fish and reptiles, the involvement of SR Ca^{2+} cycling in E–C coupling may be involved in the generation of higher resting and maximal heart rates.

Force-frequency relationship

Ventricular and atrial tissue from the four reptiles exhibited a negative F-F relationship, similar to other ectotherms (see Shiels et al., 2002). The reduction in twitch force with increased frequency may reflect a decline in intracellular Ca^{2+} concentration, as demonstrated in ventricular and atrial myocytes from trout, where the Ca^{2+} transient is reduced by 30% when stimulation is increased from 0.6 to 1 Hz (Harwood et al., 2000). This reduction could be caused by reduced Ca^{2+} release from the SR; however, the shape of the F-F relationship was unaffected by ryanodine in the reptiles studied here, indicating the SR plays a minor role in shaping the F-F response. Alternatively, the negative F-F response could be explained by the relative refractoriness of the Ca^{2+} transport processes. During relaxation a finite period of time is required for the transport processes responsible for contraction to recover from inactivation before they can produce another contraction of similar amplitude, a process termed incomplete mechanical restitution. As frequency increases, and the duration between contractions decreases, incomplete mechanical restitution may occur, which could account for the reduction in twitch force associated with a negative F-F relationship. This is consistent with the rise in RT that accompanied the negative F-F response.

The turtle could achieve the highest rates of contraction amongst the four species and some muscle strips reached frequencies as high as 3–4 Hz. This is surprising given that *in vivo* heart rates of these turtles normally vary between 20–50 min^{-1} (0.3-0.9 Hz) and suggests that turtle myocytes can cycle Ca^{2+} rapidly, but as our study points to a small contribution from the SR, other mechanical or anatomical specialisations must account for the rapid E–C coupling. Recently, we have shown that ventricular and atrial myocytes from turtles are long and thin (approx. 160 μm in length, 5.5 μm wide and 4.5 μm deep). The surface area relative to volume is 17-fold, which is substantially larger than that of mammals (rabbit, 4.58; rat, 6.76) (Satoh et al., 1996), but similar to that of fish (rainbow trout, 18.2) (Vornanen, 1998) and crucian carp, 19.2 (Vornanen, 1998). This large surface area reduces the diffusional distance for Ca^{2+} between the sarcolemma and contractile apparatus, possibly increasing the impact of sarcolemmal Ca^{2+} influx. Thus, Ca^{2+} cycling across the sarcolemma alone is probably sufficient to initiate contraction. Furthermore, in the absence of a functional SR, the sarcolemmal Na^{+}–Ca^{2+} exchanger (NCX) will become the primary transport mechanism for Ca^{2+} efflux, and may also contribute to contractile Ca^{2+} entry as in fish (Vornanen, 1996; Vornanen, 1999). Therefore, in turtle myocytes the NCX may be more important for Ca^{2+} transport than the SR.

Resting tension

RT remained relatively constant within the *in vivo* frequencies for each species. However, at stimulation frequency above maximal *in vivo* heart rates, RT increased in most species, indicating rising levels of cytosolic Ca^{2+}. Increases in diastolic Ca^{2+} could occur following incomplete mechanical restitution. As the time between contractions is reduced with increasing frequency, extrusion of Ca^{2+} by the SR Ca^{2+} ATPase and the NCX may not be sufficient to match Ca^{2+} influx to efflux, and Ca^{2+} would accumulate in the cytosol (Bailey and Driedzic, 1990; Aho and Vornanen, 1999; Bers, 2002; Shiels et al., 2002). Since SR blockade had no significant effect on changes in RT, it is likely the NCX is unable to remove Ca^{2+} sufficiently at higher frequencies of stimulation.

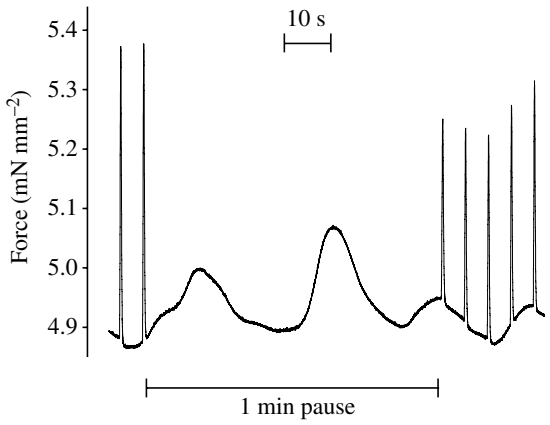

Fig. 3. Representative trace of a 1 min pause in atrial tissue from a turtle, *Trachemys scripta*. Stimulation ceased for a period of 1 min, and the following 5 contractions were recorded.

RT oscillated in a spontaneous and unpredictable manner in turtle atrial strips (Fig. 3). These slow cyclic 'tonus waves' were independent of electrical stimulation, but the amplitude of the wave seemed to be potentiated during a pause of stimulation. Atrial tonus waves have been observed previously in tortoise and turtle atrial tissue (Gannon et al., 2003), and have been attributed to the presence of smooth muscle in the atria (reviewed in Meek, 1927). Histological studies have demonstrated the presence of endocardial smooth muscle in turtle atria, and more sparsely in the ventricle (Shaner, 1923; Gannon et al., 2003). When specific smooth muscle tissue poisons were applied to atrial tissue from the turtle, the 'tonus waves' were abolished (Snyder and Andrus, 1919). The physiological function of smooth muscle in atrial tissue may be related to the regulation of ventricular blood volume. It has been suggested that changes in auricular tone may have a regulating effect on ventricular filling, and thus stroke volume (see Meek, 1927).

Effects of adrenergic stimulation

Consistent with previous studies on reptiles, adrenaline increased twitch force in atrial and ventricular tissue in all species and at almost all frequencies (Meester et al., 1965; Kirby and Burnstock, 1969; Van Harn et al., 1973; Paz de la Vega, 1983; Ojewole and Akinwande, 1984; Chiu and Sham, 1985). This classic positive inotropic effect can be attributed to a rise in cytosolic Ca^{2+} concentration. Adrenaline phosphorylates L-type Ca^{2+} channels, increasing their open probability, thus allowing greater trans-sarcolemmal Ca^{2+} influx (Reuter, 1983).

The positive inotropic influence of adrenaline overwhelmed the negative effects of ryanodine in python and varanid ventricular tissue, suggesting that in physiological situations where sympathetic tone is high, such as exercise (Wang et al., 2001), the relative contribution of the SR is overshadowed by an adrenergically stimulated increase in sarcolemmal Ca^{2+} influx, which is sufficient to allow adequate Ca^{2+} cycling. In atrial tissue pre-treated with ryanodine, the positive inotropic effect of adrenaline was generally limited to the lower pacing frequencies. A similar situation exists in ventricular muscle from the pacific mackerel, tuna and rainbow trout (Keen et al., 1992; Shiels et al., 1999; Shiels and Farrell, 1997; Shiels and Farrell, 2000). Thus, adrenergic stimulation is insufficient to combat the negative effects of higher frequencies of contraction, and at this point SR Ca^{2+} cycling becomes more important. Supporting this idea, in turtle and python atrial tissue, ryanodine decreases twitch force significantly only at frequencies at which adrenaline is unable to produce a positive inotropic effect.

Post-rest effects

An imposed pause between electrical stimulations for 1 or 5 min caused post-rest decay of twitch force in all species, indicating a positive F-F relationship at low frequencies. In mammals, this response is attributed to Ca^{2+} leak from the SR during rest and is associated with the relative efficiencies of the NCX and the SR Ca^{2+}ATPase pump in removing leaked Ca^{2+} from the cytosol (Bers, 2001). Thus, the post-rest decay in reptiles may be attributed to a relatively leaky and inefficient SR, and active Ca^{2+} extrusion from the myocyte *via* the NCX.

Perspectives

The inter-specific variability in heart rate, and large fundamental differences in cardiac structure and function, make reptiles an interesting phylogenetic group to investigate evolutionary questions regarding cardiovascular function. We have shown that SR dependence in reptiles is frequency, tissue type and species dependent, and suggest it may also be correlated with activity level. However, in situations involving adrenergic stimulation of the myocardium such as exercise, circulating catecholamines will probably be sufficient to increase the output of the heart.

Thus, in conjunction with sarcolemmal transport, the SR functions as an additional source of activator Ca^{2+} to produce larger and faster Ca^{2+} transients in active species of reptiles. Accordingly, the SR becomes important as contraction frequencies increase, and when stronger force development is necessary. In the more sedentary species, it seems that the NCX may play a more important role in Ca^{2+} cycling than the SR. It is plausible therefore, that in addition to beat-to-beat changes in response to metabolic demands, the presence of a functional SR may be a contributing factor in the evolution of higher resting and maximal reptilian heart rates and possibly increased blood pressures, particularly in a functionally divided circulation.

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