Acta Herpetologica 4(1): 57-71, 2009

A comparative study of contractility of the heart ventricle in some ectothermic vertebrates

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Abstract. The purpose of this study was to analyze contractility of the heart ventricle in selected reptilian and amphibian species having the same ventricular excitation pattern. Systolic time intervals and indices of contractility of the heart ventricle were measured in anaesthetized frogs, snakes, and tortoises by use of polycardiography. The electromechanical delay was significantly shorter in tortoises compared with the other two species. The isovolumetric contraction time in frogs was approximately two-fold longer than in reptiles. The pre-ejection period was the longest in frogs and the shortest in tortoises, whereas snakes were intermediate. The ejection time was slightly longer in tortoises compared with the other two species. The greatest isovolumetric contraction index and the smallest myocardial tension index corresponded to the frog and tortoise heart ventricle, respectively. The intrasystolic index in tortoises was significantly greater than in frogs, whereas quite similar to that in snakes. The frog ventricle had lower contractility compared with the reptilian one. Although ventricular contractility tended to be lower in snakes compared with tortoises, this difference was not statistically significant. Possible causes for these differences are discussed. We suppose a large variety in ventricular contractility among amphibian and reptilian species having the same ventricular activation pattern. This variety may be conditioned by heart anatomy, intracardiac shunting, lifestyles, and habitats. It can only be hypothesized that on the average, ventricular contractility is higher in reptiles compared with amphibians and in chelonians compared with snakes.

Keywords. Heart function, cardiac performance, amphibians, reptiles, frogs, snakes, tortoises.

INTRODUCTION

The heart fulfils the pump function and pushes blood into the arterial system providing blood circulation. This is due to myocardium contraction that results from its electrical excitation. Four types of ventricular myocardial activation were developed as a result of the heart evolution in Vertebrates (Roshchevsky and Shmakov, 2003). However, the question, how different excitation patterns relate to cardiac performance, has not been addressed yet.

Amphibians and reptiles are characterized by the same ventricular activation pattern, which presents the successive character of excitation spreading from endocardium to epicardium and from the base to the apex of the heart ventricle (Shmakov and Roshchevsky, 1982; Shmakov and Abrosimova, 1989; Roshchevsky and Shmakov, 2003; Azarov et al., 2007). This pattern differs from ventricular excitation patterns in fishes and homoeothermic vertebrates (Roshchevsky and Shmakov, 2003). At the same time, it is not known whether contractility of the heart ventricle differs between vertebrate species. Little information is available concerning contractility of the heart ventricle in reptiles and amphibians (Furnival et al., 1973; Sham et al., 1989). Systolic time intervals of the heart ventricle have not been measured in ectothermic vertebrates, although some attempts have been done for frogs (Shelton and Jones, 1965a, b) and snakes (Johansen, 1959; Johansen and Holl, 1960).

The purpose of the study reported here was to analyze by measurements of systolic time intervals, how ectothermic vertebrates with the same ventricular excitation pattern differ in contractility of the heart ventricle. In addition, a comparison of ventricular contractility of three-chambered reptilian hearts was made. The frog *Rana temporaria* was chosen as a widely-distributed amphibian species, the snakes *Natrix natrix* and *N. tessellata* as typical ophidian species, and the Central Asian tortoise *Testudo horsfieldii* was selected as a representative of chelonians.

MATERIALS AND METHODS

Animals, anesthesia, and surgical procedures

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). Experiments were carried out on anuran amphibians *R. temporaria* (n = 6), grass snakes *N natrix* and dice snakes *N. tessellata* (n = 4), and tortoises *T.* (*Agrionemys*) *horsfieldii* (n = 6). The frogs were collected in summer in the central regions of the European part of Russia and stored at 0-4 °C till the use in experiments. The reptiles were caught in the Moscow region (snakes) of Russia and in Kazakhstan (tortoises) a few weeks before the experiments began. All animals were obtained from commercial suppliers. After transportation to our laboratory (a few days before the experiments), animals were kept in a terrarium at 20-25 °C and supplied with food and tap water. The experiments were performed from January to May (frogs), in July (snakes, tortoises) and November (snakes). Frogs and tortoises varied in weight from 96 to 116 g (mean ± SD: 102 ± 10 g) and from 80 to 138 g (108 ± 20 g), respectively. Snakes varied in length from 62 to 83 cm (70 ± 9 cm) and in weight from 38 to 102 g (62 ± 28 g, $P < 0.05$ compared with the other two species). The experiments were performed at a room temperature (18-22°C). Frogs were anaesthetized by immersion in a 40% ethanol solution. Snakes and tortoises were anaesthetized by an intra-abdominal injection of xylazine (40 μg/g) and sodium thiopental (50 μg/g). In case of need, ether was used for additional anesthesia. Each frog was placed ventral side up, the heart was exposed by cutting of clavicles and coracoids and removing of the sternum. Each reptile was placed ventral side up, tracheotomized, and mechanically ventilated. Mechanical lung ventilation was performed to avoid hypoxia, resulted from the depressant action of sodium thiopental on respiration. The heart was exposed by a longitudinal midline incision of the ventral side of the body in snakes and by removing of the plastron in tortoises.

At the end of the experiment, each animal was euthanized. Frogs were euthanized by decapitation followed by pithing. Reptiles were euthanized with an overdose of sodium thiopental (100

μg/g, intra-abdominally), which caused cardiac arrest (diagnosed from an ECG). After euthanasia, the heart was removed and the ventricle was weighed in each animals examined.

Electrocardiographic and apexcardiographic recordings

In each anaesthetized animal, polycardiographic tracings were obtained by means of a computer system (Poly-Spectrum-EPS, Neurosoft, Russia) that had a speed of 50 mm/s and an accuracy of 24 bits (Fig. 1). The polycardiogram consisted of a standard bipolar lead ECG and apexcardiogram that were recorded synchronously during 30 to 60 s. The electrocardiographic channel of the polygraph had a bandwidth and sampling rate of 0.5 to 75 Hz and 1000 Hz, respectively.

To obtain a standard bipolar lead ECG, needle electrodes were attached intramuscularly in a modified Einthoven lead system. In frogs and tortoises, the red and yellow electrodes were attached to the proximal aspect of the right and left forelimb, respectively; the green electrode was attached to the proximal aspect of the left hind limb; the ground electrode was placed on the right hind limb. In snakes, the placement of electrodes relatively to the position of the heart was the same as in frogs. The position of the heart in snakes was defined by inspection and palpation of the heart beat. The three standard bipolar leads were I, II, and III as derived from Einthoven's triangle: for the lead I, the negative component was the red electrode and the positive component was the yellow electrode; for the lead II, the negative component was the red electrode and the positive component was the green electrode; for the lead III, the negative component was the yellow electrode and the positive component was the green electrode. The ECG was standardized so that 20 mm was equivalent to 1 mV.

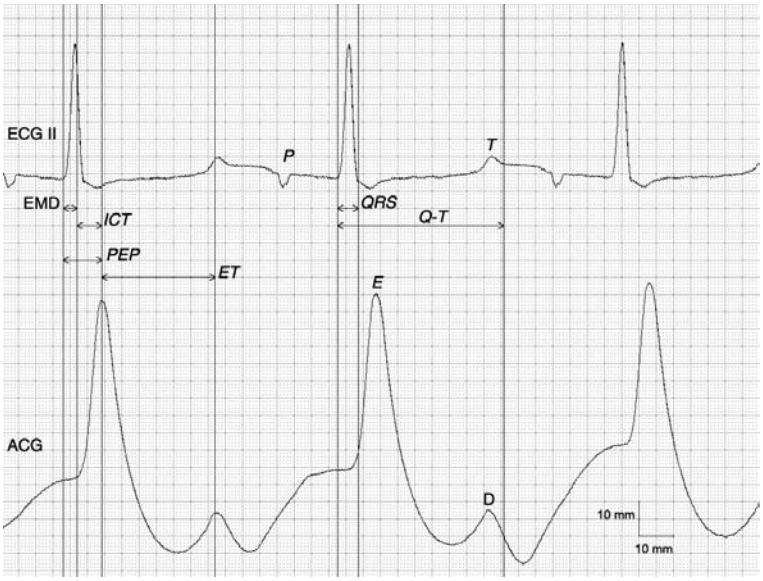

Fig. 1. Representative polycardiographic tracing obtained from a snake, indicating the simultaneous recording of a standard bipolar lead II ECG ("red electrode – green electrode") and apexcardiogram (ACG). Electrocardiographic intervals are marked in the ECG: the P wave (P), QRS complex (QRS), T wave (T), and Q-T interval (Q-T). The ejection peak (E) and the D point are indicated on the ACG. Systolic time intervals are marked: the electromechanical delay (Q-S_1), isovolumetric contraction time (ICT), pre-ejection period (PEP), and ejection time (ET). Paper speed = 50 mm/s; 2 cm = 1 mV.

To obtain apexcardiographic recordings, a tensometric pulse wave transducer (Neurosoft, Russia) (frequency range, 0.3 to 200 Hz; sensitivity, 100 mV/Pa; and time constant, 0.6 s) was fixed near the heart ventricle. The position of the transducer was adjusted to the apex of the heart ventricle and was modified to improve unsatisfactory tracings, if any. The sampling rate and sensitivity of the apexcardiographic channel of the polygraph were 1000 Hz and 0.3 to 1.5 86 mV/mm, respectively.

Data analysis, calculations and statistics

The ventricle index was calculated as ventricle weight / body weight. Heart rate was determined from measurement of the R-R interval in the limb lead II ECG. The durations of the QRS complex and Q-T interval were measured. The electromechanical delay (EMD) was measured from the onset of the QRS complex in the limb lead II ECG to the onset of the upstroke in the apexcardiographic tracing (Fig. 1). The isovolumetric contraction time (ICT) was measured from the onset of the upstroke to the ejection peak in the apexcardiogram. The pre-ejection period (PEP) was calculated as EMD plus ICT. The ejection time (ET) was measured from the ejection peak to the D point indicated on the apexcardiogram. The duration of mechanical systole (S_m) was computed as ICT plus ET. The duration of electromechanical systole (S_o) was calculated as EMD plus S_m. The duration of diastole was calculated by subtracting S_o from the R-R interval.

On the basis of the measurements, indices of ventricular contractility were defined. The myocardial tension index was defined as PEP/S_o. This index reflects the time a heart needs to prepare for blood ejection (i.e., unproductive expenditure of contraction time). The myocardial tension index decreases when systolic function improves. The PEP-to-ET ratio, which is a commonly used index of ventricular contractility and performance, was determined. The PEP-to-ET ratio decreases when systolic function improves, whereas reduced ventricular contractility is reflected by an increase in the PEP-to-ET ratio. The intrasystolic index was calculated as ET/S_m. This index reflects the time a heart needs to eject blood (i.e., productive expenditure of contraction time). The intrasystolic index increases when systolic function improves. The isovolumetric contraction index was computed as ICT/PEP. Reduced ventricular contractility is reflected by an increase in ICT/PEP. The duration of cardiac output ejection was defined as a product of ET and heart rate.

All values for each animal were averaged on the basis of measurements from 5 consecutive cardiac cycles. Statistical analysis was performed using descriptive statistics. Data are presented as mean ± SD. Differences were considered to be significant at $P < 0.05$.

RESULTS

Heart ventricle weight and electrocardiographic parameters

The mean values of heart ventricle weight were found to be similar in frogs (216 ± 53 mg), snakes (271 ± 126 mg), and tortoises (231 ± 46 mg). The ventricle index in snakes (4.39 ± 0.52 mg/g) was twofold greater ($P < 0.001$) than in frogs (2.10 ± 0.32 mg/g) and tortoises (2.12 ± 0.12 mg/g).

The durations of the QRS complex, R-R interval, and Q-T interval were summarized (Table 1). There were no significant differences between the three species in the R-R interval, which was slightly longer in frogs and shorter in snakes. The duration of the QRS complex was more than 1.5-fold greater in snakes compared with the other two species. In comparison with frogs and tortoises, snakes had intermediate values of the Q-T interval.

Table 1. Electrocardiographic intervals and heart rate for the species studied. Values are mean ± SD. Ranges of values are given in round brackets. * $P < 0.05$ *vs.* snakes.

Parameters	Frog n = 6	Snake n = 4	Tortoise n = 6
R-R interval, ms	2138 ± 782 (1386-3638)	1776 ± 161 (1591-1974)	1934 ± 420 (1573-2678)
Heart rate, min^{-1}	30 ± 9 (16-44)	34 ± 2 (30-38)	32 ± 6 (22-38)
QRS complex, ms	100 ± 22 * (80-140)	169 ± 16 (150-190)	107 ± 32 * (55-155)
Q-T interval, ms	1016 ± 169 (805-1225)	1112 ± 34 (1068-1148)	1195 ± 154 (980-1450)

Table 2. Systolic time intervals, their proportions in the cardiac cycle, and indices of ventricular contractility for the species studied. Values are mean ± SD. Proportions of systolic time intervals of the cardiac cycle are given in round brackets. R-R, the R-R interval of ECG; EMD, the electromechanical delay; ICT, the isovolumetric contraction time; PEP, the pre-ejection period; ET, the ejection time; S_m, mechanical systole; S_o, electromechanical systole; D_o, diastole; MTI, the myocardial tension index; ISI, the intrasystolic index; ICI, the isovolumetric contraction index; PEP/ET, the PEP-to-ET ratio; t_{CO}, the duration of cardiac output ejection. * $P < 0.05$ *vs.* tortoises. † $P < 0.05$ *vs.* frogs.

Parameters	Frog n = 6	Snake n = 4	Tortoise n = 6
R-R, ms (%)	2138 ± 782 (100)	1776 ± 161 (100)	1934 ± 420 (100)
EMD, ms (%)	117 ± 31 * (5.9 ± 0.8)	143 ± 29 * (8.0 ± 0.9 * †)	90 ± 19 (4.8 ± 0.4)
ICT, ms (%)	209 ± 103 (10.1 ± 2.0 *)	111 ± 10 † (6.3 ± 0.5 *)	91 ± 39 † (4.7 ± 0.7)
PEP, ms (%)	327 ± 131 * (16.0 ± 2.7 *)	254 ± 33 * (14.3 ± 1.1 *)	181 ± 55 (9.5 ± 1.0)
ET, ms (%)	777 ± 170 (39.4 ± 5.7)	762 ± 207 (42.5 ± 5.6)	829 ± 277 (43.1 ± 4.4)
S_m, ms (%)	982 ± 150 (49.3 ± 5.4)	875 ± 201 (48.9 ± 5.1)	919 ± 261 (47.8 ± 4.0)
S_o, ms (%)	1100 ± 163 (55.3 ± 5.9)	1018 ± 216 (56.9 ± 5.3)	1010 ± 257 (52.6 ± 3.8)
D_o, ms (%)	1038 ± 749 (44.7 ± 6.0)	758 ± 100 (46.3 ± 3.1)	925 ± 294 (47.4 ± 3.8)
MTI	0.296 ± 0.115	0.255 ± 0.045	0.191 ± 0.081 †
ISI	0.791 ± 0.112	0.865 ± 0.031	0.892 ± 0.066 †
ICI	0.616 ± 0.082	0.440 ± 0.052 †	0.490 ± 0.072 †
PEP/ET	0.45 ± 0.26	0.35 ± 0.08	0.24 ± 0.14
t_{CO}, s	24 ± 8	25 ± 5	26 ± 7

Systolic time intervals and indices of ventricular contractility

The values of systolic time intervals were summarized (Table 2). The differences in the duration of the R-R interval were accounted for to a greater extent by the duration of diastole and to a lesser extent by the duration of systole. The ejection time was quite similar in the studied animals, although being slightly longer in tortoises compared with the other two species. The differences in the pre-ejection period and the duration of its constituent parts were found to be significant between the animals examined. The electromechanical delay in tortoises was shorter than in the other two species. The isovolumetric contraction time in frogs was approximately twofold longer than in both reptiles. As a consequence, the longest pre-ejection period was in frogs, whereas the shortest one was in tortoises.

The significant differences in the electromechanical delay, isovolumetric contraction time, and pre-ejection period were also found to be between the animals, when assessing proportions of various phases of the cardiac cycle (Table 2). The proportion of the isovolumetric contraction time was significantly greater in frogs compared with tortoises, whereas a less difference was found to be between snakes and tortoises. The proportion of the electromechanical delay was significantly greater in snakes compared with the other two species. As a result, the proportion of the pre-ejection period was found to be quite similar in snakes and frogs but significantly higher in these species compared with tortoises. Proportions of the ejection time, mechanical systole, electromechanical systole, as well as diastole, did not differ between the species examined.

The values of indices of ventricular contractility were summarized (Table 2). The greatest isovolumetric contraction index was found to be in frogs. The smallest myocardial tension index was observed in tortoises. The value of the intrasystolic index in tortoises was significantly greater than in frogs and quite similar to that in snakes. The variety in PEP-to-ET ratios among the species was considerable but not statistically significant.

DISCUSSION

Ventricle index

Ventricle weights were comparable in all species studied. At the same time, the ventricle index was similar in tortoises and frogs, whereas it was significantly greater in snakes compared with the other two species. These differences are probably conditioned by lifestyle, with active species having a greater ventricle index. The heart index varies in a wide range among anuran amphibians, chelonians, and snakes (Crile and Quiring, 1940; Wilber, 1962; Wang et al., 2002; Vinogradov and Anatskaya, 2006). Although the heart index on the average is comparable between these ectotherms, it is higher in snakes. This is in consistent agreement with our data. However, there is some disagreement between our data and other findings (Vinogradov and Anatskaya, 2006). In *Natrix* snakes, the ventricle index in the present study is 2.2-fold higher than the heart index reported for the other study (Vinogradov and Anatskaya, 2006). This disagreement is likely attributable to the significant difference between snakes from the two studies in body weight.

Electrocardiographic data

The values of electrocardiographic intervals in frogs reported in our study are approximately twofold higher than those of other studies, which were performed at a similar temperature (Shmakov and Abrosimova, 1989; Cakir and Strauch, 2005). On the other hand, our data are in consistent agreement with findings in toads (Chapovetsky and Katz, 2003),

The mean duration of the QRS complex reported in our study for tortoises is similar to that in turtles (Wilber, 1962). On the other hand, our data differ from other findings in tortoises of the same species (Shmakov and Roshchevsky, 1982; Roshchevsky and Shmakov, 2003). The difference between study results is likely attributable to differing temperatures. The duration of the QRS complex reported in the present study is two- to threefold greater than the duration of the ventricular excitation process evaluated by use of intracardiac electrography at a higher temperature (Shmakov and Roshchevsky, 1982; Roshchevsky and Shmakov, 2003). In another study, the duration of depolarization of the ventricular epicardial surface in chelonians (*Pseudemys* and *Testudo*) during lung ventilation (190-210 ms) (Burggren, 1978) is greater than the duration of the QRS complex in tortoises in our study. The difference between study results is likely attributable to differing ventricle sizes. Heart sizes in tortoises in our study were approximately twofold less than those in the other study (Burggren, 1978).

The mean duration of the QRS complex reported in our study for snakes is 2.4-fold greater than that reported in the other study for ophidian species (Mullen, 1967). The other study used a protocol that involved a wide temperature range (Mullen, 1967). The values of the duration of the Q-T interval in reptiles in our study are within a range of values reported by other researches for chelonian and ophidian species (Wilber, 1962; Mullen, 1967).

The differences between study results in electrocardiographic data may be attributable to differing experimental conditions, such as anesthesia, temperature, and techniques, as well as interspecies differences in cardiovascular parameters, temperature and seasonal changes in cardiac activity in ectothermic vertebrates (Wilber, 1962; Risher and Claussen, 1987; Rocha and Branco, 1997, 1998; Chapovetsky and Katz, 2003). Ventricular function in amphibians and reptiles is known to be influenced by lung ventilation (Burggren, 1978; Segura et al., 1981). Probably, age and sex have an influence on electrical activity of the heart and, therefore, on electrocardiographic parameters in ectothermic animals. It should be mentioned that the duration of electrical (excitation and recovery) and thereof mechanical (contraction and relaxation) processes in the hearts of ectothermic vertebrates depends on heart size. This also contributes to both intra- and inter-observer variability of results.

Systolic time intervals

Little information is available concerning the phase structure of the cardiac cycle in amphibians (Shelton and Jones, 1965a, b) and reptiles (Johansen, 1959; Johansen and Holl, 1960). In the present study, an attempt was made to obtain information on systolic time intervals and indices of contractility of the heart ventricle in frogs *Rana temporaria*, snakes *Natrix*, and tortoises *Testudo horsfieldii*.

The values of the duration of ventricular systole reported in our study for snakes are significantly greater than those of another study (Johansen, 1959). The values of the dura-

tion of the ejection period reported by other researchers for snakes (*N. natrix* and *Vipera berus*, Johansen and Holl, 1960) are less than those of our study. One might assume that the differences between study results are most likely attributable to differing heart rates, because the ejection time and duration of ventricular systole are correlated with heart rate in endothermic vertebrates (Goch, 1981; Kharin and Shmakov, 2006) with various patterns of ventricular excitation. However, an additional investigation is required to confirm this assumption for ectothermic vertebrates. The values of the isovolumetric contraction time reported in our study for snakes are in consistent agreement with other findings in snakes *N. natrix* (Johansen, 1959). On the other hand, our data differ from values reported for the duration of isovolumetric contraction in snakes *N. natrix* and *Vipera berus*) in another study, in which the isovolumetric contraction was defined by use of cineradiography (Johansen and Holl, 1960).

The value of the isovolumetric contraction time in frogs reported in our study is 2.5-fold higher than that reported by other researches (Shelton and Jones, 1965a). This difference between study results may be attributable to interspecies differences, as well as differing experimental conditions (anesthesia and methods) and heart sizes. The isovolumetric contraction time should be less in a small heart ventricle compared with a large one. Frogs used in our study and another one (Shelton and Jones, 1965a) differed significantly in body weights; therefore, one might assume that heart sizes also differed. It should also be noted that in ectothermic vertebrates, the isovolumetric contraction time is possibly correlated with heart rate. If that's the case, this relation may contribute to the difference between study results in isovolumetric contraction times. Our data differ significantly from values reported for heart rate in frogs and snakes in other studies (Shelton and Jones, 1965a; Johansen, 1959; Johansen and Holl, 1960). However, an additional investigation is required to confirm this assumption, because there is a disagreement among data on correlation between the isovolumetric contraction time and heart rate reported for endothermic vertebrates (Goch, 1981; Kharin and Shmakov, 2006) with different patterns of ventricular excitation.

We found the significant difference between the species in the electromechanical delay, isovolumetric contraction time, and pre-ejection period. The similarity of other systolic time intervals (i.e., the ejection time, mechanical systole, and electromechanical systole), as well as diastole, in the three species may be attributable to the similar length of the cardiac cycle.

The electromechanical delay and QRS complex were found to be longer in snakes compared with the other two species. The proportion of the cardiac cycle that was comprised of the electromechanical delay was also greater in snakes. These findings are quite appropriate to the greater ventricle index in snakes in our study, because a large heart needs more time to be excited.

During the isovolumetric contraction of a heart ventricle, intraventricular pressure rises to the pressure level in the aortic arches. We found the isovolumetric contraction time to be significantly less in both reptiles compared with frogs. Taking into account this fact, one might assume that the frog ventricle developed higher pressure than the reptilian one, whereas a similar pressure level was developed by the heart ventricle in snakes and tortoises. However, systemic blood pressure in frogs *R. temporaria* (Shelton and Jones, 1965a, 1968) is approximately twofold lower than in snakes *Natrix* (Johansen, 1959; Kharin, S.N., unpubl.). In comparison with the frogs and snakes, tortoises *T. graeca* (Shelton and Burggren, 1976),

closely related to tortoises *T. horsfieldii*, have intermediate values for systemic blood pressure. Thus, it is most likely, that among the species studied, the frog ventricle developed the lowest pressure for the longest period. Snakes and tortoises in our study had comparable values for the isovolumetric contraction time, although there was a significant difference between these reptilian species in the proportion of the cardiac cycle that was comprised of the isovolumetric contraction time. This difference was probably related to the greater ventricle index in snakes compared with tortoises. Thus, one might assume that in comparison with the tortoise heart ventricle, the snake one developed higher pressure for the longer period. This assumption is in consistent agreement with an influence of gravity on cardiovascular parameters in snakes (Seymour, 1987; Young et al., 1994; Seymour and Arndt, 2004).

Intraventricular and systemic blood pressures are quite comparable both among anuran amphibians (Vogt, 1941; Shelton and Jones, 1965a, 1965b, 1968; Furnival et al., 1973; Segura et al., 1981; Sham et al., 1989; Burggren et al., 1992; Michalicek and Campbell, 1993; West and Smits, 1994; West et al., 1998; Rocha and Branco, 1997, 1998; Andersen et al., 2003) and chelonians (Steggerda and Essex, 1957; Woolley, 1959; Wilber, 1962; White and Ross, 1966; Shelton and Burggren, 1976; Stephens et al., 1983; Comeau and Hicks, 1994; Hicks and Comeau, 1994; Hicks et al., 1996; Crossley et al., 1998; Hicks and Farrell, 2000; Overgaard et al., 2002). At the same time, arterial blood pressure can differ sevenfold in various ophidian species (Johansen, 1959; Lillywhite and Seymour, 1978; Lillywhite and Pough, 1983; Seymour, 1987; Stinner and Ely, 1993; Wang et al., 2001, 2003; Seymour and Arndt, 2004). In snakes, there is also a large intraspecies variety in arterial blood pressures (Johansen, 1959; Lillywhite and Seymour, 1978; Stinner and Ely, 1993), which is probably conditioned by a wide diversity of habitats (Lillywhite and Pough, 1983; Seymour and Arndt, 2004) and activity levels (Stinner and Ely, 1993; Wang et al., 2001), as well as a dependence of blood pressure on body weight (Seymour, 1987), temperature (Lillywhite and Seymour, 1978; Stinner, 1987), and stress (Stinner, 1987; Stinner and Ely, 1993). On the average, blood pressure in turtles (Steggerda and Essex, 1957; Woolley, 1959; Wilber, 1962; White and Ross, 1966; Shelton and Burggren, 1976; Stephens et al., 1983; Comeau and Hicks, 1994; Hicks and Comeau, 1994; Hicks et al., 1996; Crossley et al., 1998; Hicks and Farrell, 2000; Overgaard et al., 2002) is quite comparable with that in aquatic snakes (Lillywhite and Pough, 1983; Seymour and Arndt, 2004) and anuran amphibians, whereas blood pressure in tortoises (Shelton and Burggren, 1976) seems to be close to terrestrial snakes (Johansen, 1959; Lillywhite and Seymour, 1978; Wang et al., 2000, 2001, 2002, 2003; Seymour and Arndt, 2004; Galli et al., 2005; Skals et al., 2005; Zaar et al., 2007). Thus, regarding blood pressure levels, one may assume that the species used in our study are typical representatives of anuran amphibians, tortoises, and terrestrial snakes.

The isovolumetric contraction phase is followed by a period, during which blood is ejected from the heart ventricle into the arterial system. The duration of the ejection period is characterized by the ejection time. The ejection time has been found in the present study not to differ significantly between the species examined that is likely attributable to a similarity of stroke volumes. It might be supposed, because ventricle weights are comparable among the three species. There is a large variety in stroke volumes and stroke indices (the ratio stroke volume / body weight) among anurans (Shelton and Jones, 1965a; West and Smits, 1994; Andersen et al., 2003), chelonians (Shelton and Burggren, 1976; Comeau

and Hicks, 1994; Hicks et al., 1996; Crossley et al., 1998; Overgaard et al., 2002) and snakes (Stinner, 1987; Secor et al., 2000; Wang et al., 2000, 2002; Galli et al., 2005; Skals et al., 2005). These differences are apparently conditioned by body size, lifestyle, and habitat. On the average, however, the stroke index in chelonians is comparable with that in frogs that is in consistent agreement with our findings regarding the ventricle index, which is similar in tortoises and frogs but slightly less in snakes. The minor difference between the two reptilian species in the ejection time was observed in our study. The mean value of the ejection time in snakes was slightly less than that in tortoises. This finding is in consistent agreement with data of other researches, which found differences among reptilian species in the rate of rise of contraction in cardiac tissue (Galli et al., 2006). In this study, the rate of rise of contraction in ventricular tissue in snakes was higher than in turtles.

Indices of ventricular contractility

In the present study, minor differences between snakes and tortoises in ventricular contractility were determined, when assessing indices of ventricular contractility. This finding is in accordance with the fact that various reptilian species are characterized by the same pattern of ventricular activation (Shmakov and Roshchevsky, 1982; Roshchevsky and Shmakov, 2003). Nevertheless, ventricular contractility tended to be lower in snakes compared with tortoises. First, anesthesia, which was used in our study, might contribute to the difference between snakes and tortoises in ventricular contractility. Second, the mentioned tendency might be attributable to the longer pre-ejection period of the snake heart ventricle in combination with the greater ventricle index and arterial pressure, which may be due to an influence of the activity level (Stinner and Ely, 1993; Secor et al., 2000; Wang et al., 2001) and gravity (Seymour, 1987; Young et al., 1994; Seymour and Arndt, 2004) on cardiovascular physiology in snakes. It should be mentioned that an ophidian heart taken as a whole does not conform well the Principle of Laplace (Seymour, 1987), although force production of a part of the snake heart ventricle depends to a greater degree on thickness of the ventricular wall rather than on intrinsic properties of cardiac tissue (Zaar et al., 2007). In general, peculiarities of heart anatomy and intracardiac blood shunting (Johansen and Holl, 1960; Robb, 1967; Snyder et al., 1999; Victor et al., 1999; Sklansky et al., 2001; Hicks, 2002; Chetboul et al., 2004; Schilliger et al., 2006), as well as lifestyle, may cause differences among reptilian species in ventricular contractility.

In the study reported here, systolic time intervals and ventricular contractility were assessed in anaesthetized animals. Anesthesia influences cardiac activity. Barbiturates have the cardiac depressant action (List et al., 1972; Manders and Vatner, 1976; Komai and Rusy, 1984). In spite of the fact that we used sodium thiopental for reptile anesthesia, we found ventricular contractility to be higher in both reptiles compared with frogs. In comparison with tortoises and frogs, snakes had intermediate values of indices of ventricular contractility. The large difference between snakes and frogs in ventricular contractility was generally not statistically significant. The larger difference between frogs and tortoises in ventricular contractility was statistically significant. These differences in ventricular contractility cannot be explained by ventricular excitation patterns or heart rates, because reptiles (Shmakov and Roshchevsky, 1982; Roshchevsky and Shmakov, 2003) and amphibians (Shmakov and Abrosimova, 1989; Roshchevsky and Shmakov, 2003) have the same pat-

tern of ventricular excitation, and heart rates have been observed in the present study to be similar in the animals examined. More possible explanations of higher ventricular contractility in both reptiles compared with frogs are peculiarities of heart anatomy and intracardiac blood shunting (Johansen and Holl, 1960; Robb, 1967; Snyder et al., 1999; Victor et al., 1999; Sklansky et al., 2001; Hicks, 2002; Chetboul et al., 2004; Schilliger et al., 2006).

In summary, contractility of the heart ventricle was higher in tortoises *T. horsfieldii* compared with anuran amphibians *R. temporaria*, whereas snakes *Natrix* were intermediate. These differences were likely conditioned by heart anatomy, intracardiac blood shunting, and lifestyle; anesthesia might also cause some differences in systolic time intervals and ventricular contractility. It should also be noted that our study included a small number of animals, as well as nonstandard methods for the cardiac evaluation of ectothermic vertebrates; therefore, our data are inappropriate to be used as reference values. However, we attempted to perform a comparative evaluation of cardiac performance in three species of ectothermic vertebrates with attention to the similarity between their ventricular excitation patterns. Taking into account a large variety among amphibian and reptilian species in lifestyles, habitats, intracardiac blood shunting, and heart anatomy, we suppose large differences in ventricular contractility among ectothermic vertebrates having the same ventricular excitation pattern; therefore transferring the results of our study to all amphibians and reptiles should be undertaken with caution. However, it can only be hypothesized that on the average, ventricular contractility is higher in reptiles compared with amphibians and in chelonians compared with snakes.

ACKNOWLEDGEMENTS

The present study was supported by the Russian Science Support Foundation and the Ural Branch of the Russian Academy of Sciences (the program of support for basic research performed in the Ural Branch of RAS in association with the Far Eastern Branch of RAS).

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