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HYPOXIA AND ISCHAEMIA IN BUFFER-PERFUSED TOAD HEARTS

TOM MCKEAN*, ANNA SCHERZER AND HAN PARK

Department of Biological Sciences and WAMI Medical Program, University of Idaho, Moscow, ID 83844-3051, USA

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Summary

Previous studies on the effects of ischaemia or hypoxia in ectothermic vertebrate hearts have generally used preparations that were not performing at physiological levels of pressure and flow. The conclusions that ischaemia or hypoxia are not stressful to these organisms were examined in another species, *Bufo marinus*, in which a buffer-perfused heart was performing physiological levels of work. The *in situ* preparation demonstrated the Frank–Starling relationship and mechanical characteristics similar to the hearts of intact animals. The hearts recovered from 60 min of ischaemia and reperfusion with no reduction in pressure, flow or heart rate parameters. Hearts exposed to 30 min of hypoxia at physiological filling and diastolic afterload pressures ceased generating a continuous cardiac output during the hypoxia. In most cases, there was a gradual reduction of cardiac output to zero, but in 27 % of the hearts studied, intermittent beating was observed. During reoxygenation, the hearts recovered 50–90 % of their prehypoxic function and were damaged. Hearts exposed to hypoxia with reduced filling and diastolic afterload continued to develop a cardiac output throughout the hypoxia and demonstrated an overshoot phenomena with the onset of reoxygenation. If demand is in the normal range at the onset of hypoxia, the hearts intrinsically reduce demand either by reducing pressure development or by conversion to intermittent beating. Toad hearts appear not to be damaged by ischaemia, a condition in which demand is low.

Key words: heart, hypoxia, ischaemia, *Bufo marinus*, toad, cardiac output, heart rate.

Introduction

Studies describing the effects of ischaemia and hypoxia on the mammalian heart are numerous. Mammalian hearts generally have a high reliance on an uninterrupted supply of oxygen and substrates to provide energy for the adequate circulation of blood, including the coronary circulation that supplies the heart itself. Many ectothermic vertebrates either lack a coronary circulation or have one that supplies a limited volume of myocardium (Axelsson, 1995; Brady and Dubkin,1964; Shelton and Jones, 1965). Studies on the cardiac response to ischaemia and hypoxia in lower vertebrates have been focused on fish and turtles. Studies on ischaemia and hypoxia in the turtle heart have been performed in isolated buffer-perfused preparations and have concluded that these animals survive ischaemic and hypoxic insults with little or no damage to the heart (Wasser *et al.* 1990, 1992; Farrell *et al.* 1994). Scherzer (1995) examined the response of the toad *Bufo marinus* to ischaemia followed by reperfusion. Injury to the heart did not occur with ischaemia, or with ischaemia and substrate-free buffer perfusion, but did occur if oxidative metabolism had been eliminated by prior administration of 1 mmol l^{-1} amytol. A limitation of all of the above studies except the turtle work of Farrell *et al.* (1994) is that the buffer-perfused preparations were hypodynamic and were not doing physiological levels of work prior to the hypoxic or ischaemic insult.

In the present study, an *in situ* buffer-perfused heart preparation for *Bufo marinus* is described. The effects of changes in preload and afterload are described together with the response of the heart to hypoxia and ischaemia.

Materials and methods

Bufo marinus (L.) were obtained from commercial suppliers, who obtained the animals from Mexico. They were housed indoors in a large glass aquarium where they had access to fresh water and a heat lamp for basking. The toads were periodically fed live crickets or beef liver with a vitamin supplement. The aquarium was kept at room temperature (approximately 21 °C).

The animals were anaesthetized by placing them in a solution of MS-222 (Sigma 3-aminobenzoic acid ethyl ester, 25 mg ml^{-1}). They were kept in the bath solution of MS-222 until the loss of the corneal reflex. When the toads had been anaesthetized, they were given an intraperitoneal injection of

*e-mail: tmck@uidaho.edu

0.2 ml of heparin solution (Sigma, sodium heparin, 5 mg ml^{-1}). The toads were then laid on their backs on paper towels soaked in MS-222. To expose the heart, the ventral skin was folded away and the sternum was completely removed. The pericardium and any adhering tissues were carefully cut to expose fully the heart and major blood vessels.

Blood flow in intact anaesthetized animals

Once the heart and major blood vessels were fully exposed, a flow probe (Transonic Systems Inc. no. 2S567, Ithaca, NY, USA) was placed around the left and right aortas sequentially to measure the instantaneous flow in each. The factory-calibrated flow probe was connected to a T101 ultrasonic blood-flow meter (Transonic Systems Inc., Ithaca, NY, USA) on which the instantaneous and mean flows in the aorta were displayed. The flow probe was removed before introducing the perfusion.

In situ *perfused heart preparation*

Prior to cannulation, 2-0 gauge silk thread was placed loosely around the two vena cavae, two pulmonary veins, two ventral aortas and the sinus venosus. To introduce perfusion, the lower end of the sinus venosus was tied off, and a small incision was made in the upper sinus venosus to expose the two atria and for the fitting of an input polyethylene cannula (o.d.=4 mm, i.d.=3 mm). Prior to the introduction of the input cannula, a cut was made in the interatrial septum so that both atria were perfused by the single cannula. This was done as quickly as possible to ensure cardiac perfusion with the physiological perfusate before the heart was completely empty of blood. The input cannula was tied firmly in place with 2-0 gauge silk thread and was connected to a reservoir of perfusate that was kept at a column height less than 3 cm above the heart. Once the perfusion was introduced, the left and right pulmonary veins and the left and right vena cavae were sequentially tied off using surgical silk.

The outflow from the heart was isolated by placing 15 gauge stainless-steel cannulae in the left and right ventral aortas. The cannulae were placed in the systemic channels of the aortas. The tips were advanced to the common aorta or into the distal conus arteriosus. The stainless-steel cannulae were tied firmly in place with 2-0 gauge silk thread. The ties also blocked the flow of perfusate in the pulmocutaneous channels of the aortas so that the aortic outflows were completely isolated to the systemic channel. The outflows from the two stainless-steel cannulae were made common by connecting the two stainless-steel cannulae outflows to a single polyethylene tube (o.d.=4 mm, i.d.=3 mm). The complete surgical procedure was usually completed in less than 20 min.

Physiological perfusate

Hearts were perfused with a phosphate-buffered amphibian Ringer's solution as suggested by Touraki and Lazo (1992). The buffer had the following composition (mmol l^{-1}): NaCl, 110; KCl, 1.88; $CaCl_2$, 1.8; $NaHCO_3$, 1.43; Na_2HPO_4, 0.07; glucose, 5.6. NaH_2PO_4 was added to lower the buffer pH to 7.4 for early experiments and to 7.8 for later experiments. The buffer was kept at room temperature (approximately 21 °C) prior to perfusion or stored in a refrigerator overnight and brought to room temperature before perfusion.

Experimental conditions for cardiac perfusion and instrumentation

The input polyethylene cannula in the sinus venosus (Fig. 1) was connected to a 50 ml plastic syringe barrel that was used as the perfusate reservoir. The height of the perfusate reservoir was controlled by adjusting the speed of the Cole-Palmer Masterflex pump (Barrington, IL, USA) that was utilized to pump perfusate from a beaker to the reservoir. The height of the perfusate column never exceeded 4 cm above the heart.

After the perfusion had been introduced and the output stainless-steel cannulae had been inserted, a flow probe (Transonic Systems Inc., no. 3S871, Ithaca, NY, USA) was placed around the common, larger-diameter outflow tube connecting the two output cannulae. The factory-calibrated

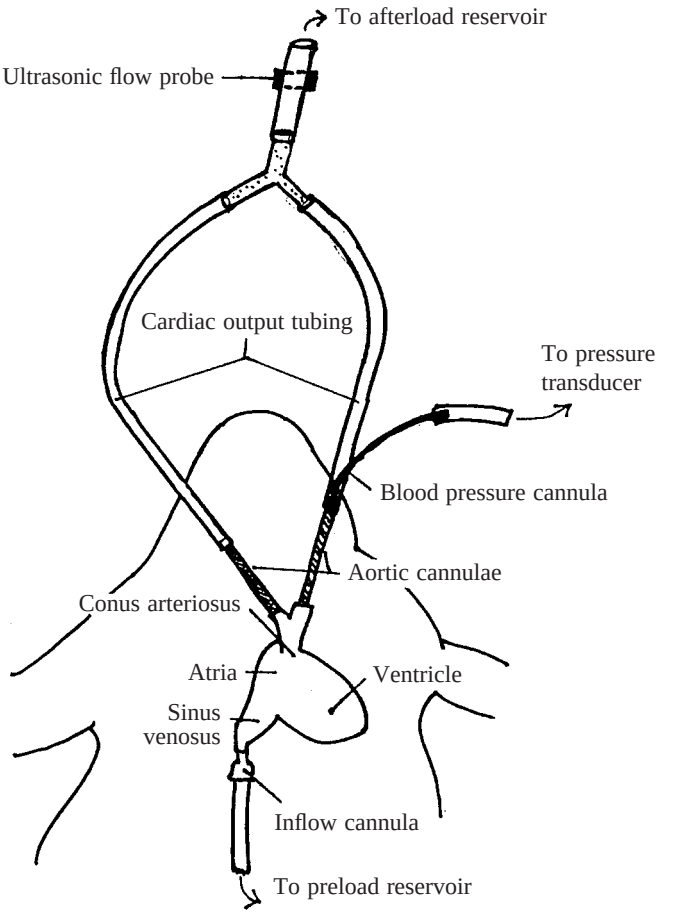

Fig. 1. Schematic diagram of the *in situ Bufo marinus* heart and cannula placement. The cardiac output tubing from the two aortas is joined with a Y-connector, and a flow probe is placed around the tubing leaving it. The height of the cardiac output reservoir could be raised or lowered to change the afterload, and the height of the sinus venosus inflow reservoir could be raised or lowered to change the preload.

flow probe was connected to the T101 ultrasonic blood-flow meter on which the instantaneous and mean flow of the cardiac outflow were displayed.

The output pressure was measured continuously at the tips of the left aortic cannula. This was carried out by inserting a piece of 23 gauge stainless-steel tubing into the left aortic cannula and extending it along the inside wall (within the catheter) of the 15 gauge stainless-steel cannula. One end of the 23 gauge tubing was opened to the tip of the 15 gauge cannula, and the other end was connected to a pressure transducer (World Precision Instruments, Inc., model BLPR, Sarasota, FL, USA). The pressure transducer was filled with buffer and was free of air. The pressure transducer was kept at the level of the heart and zeroed to the atmosphere; it was connected to a transbridge amplifier (World Precision Instruments, Inc., model TBM4-F, Sarasota, FL, USA), regularly checked for drift and calibrated using a static water column.

The pressure signals from the transbridge amplifier and the flow signals from the flowmeter were directed to a microcomputer using a DASH-8 A/D board (MetraByte Corporation, Stoughton, MA, USA). The digital information was sampled and displayed on the computer screen using Labtech Notebookpro v.8.1 (Laboratory Technologies Corp., Wilmington, MA, USA). Both pressure and flow signals were sampled at 20 Hz and were saved for later analysis. Mean output pressure, systolic pressure, diastolic pressure and mean flow rate as well as the instantaneous flow rate and pressure values were displayed on the computer screen in real time during the experiments.

Experimental protocol for oxygenated condition

The intrinsic mechanical properties of the *Bufo marinus* heart due to filling pressures and diastolic afterload pressures were examined at room temperature. The output pressure was set at 2 kPa (1 cmH_2O=0.098 kPa=0.736 mmHg) by placing the outflow tubing 20 cm above the level of the heart. A pressure of 2 kPa (20 cmH_2O) is the approximate value recorded in the intact toad (West and Smits, 1994; Wahlqvist and Campbell, 1988). The filling pressure was then varied by moving the reservoir 2–4 cm in increments of 1 cm. The cardiac outputs were allowed to stabilize for at least 5 min before the filling pressure was incremented. At each increment, data was sampled and saved for analysis. The output pressure was then varied by increasing the height of the output tubing reservoir from 20 cm to failure in increments of 10 cm. It was important to keep the preload height at 3 cm with each increment of afterload because the hearts could not maintain the same flows at higher afterloads. A preload height of 3 cm was selected because the heart performed at near physiological conditions at this preload, and this was the approximate sinus venosus pressure measured in intact animals. Height of failure was considered to be when the afterload height was too great for positive flow of perfusate to occur.

Experimental protocol for hypoxic conditions

Toads were perfused with buffer at a preload of 0.3 kPa (3.0 cmH_2O) and an afterload of 2 kPa for approximately 30 min. During this time, a sample of buffer was vigorously bubbled with 100 % N_2 and, following the equilibration period, hearts were perfused with the deoxygenated buffer. The P_{O_2} of the buffer was not measured in each individual experiment, however, measurement of P_{O_2} in several buffer samples using an oxygen electrode gave an upper limit of 2.5 kPa, and this was sufficiently low to produce a marked physiological effect. A tent was placed over the heart and N_2 gas was infused under the tent so that the external surface of the heart was also O_2-deprived. A temperature probe was placed under the tent next to the heart and, if necessary, a heat lamp was used to keep the temperature between 19.5 and 20.5 °C. The heart was perfused for 30 min with the hypoxic buffer, after which the tent and external N_2 were removed and oxygenated buffer perfusion was restored for another 30 min. Hypoxia in the heart was also evaluated during reduced workload by reducing the atrial filling pressure to 0.2 kPa and diastolic afterload to 1 kPa.

Experimental protocol for ischaemia

The ischaemia experiments were similar to the hypoxia experiments except that ischaemia was initiated by uncoupling the sinus venosus reservoir from the sinus cannula. This deprived the heart of perfusion buffer. A tent and N_2 gas were supplied as before and the hearts were subjected to 60 min of ischaemia. Reperfusion was initiated by reconnecting the cannula, and perfusion continued for 30 min. During hypoxia and ischaemia, it was necessary to decrease the afterload to heart level otherwise the outflow tubing emptied retrogradely into the conus arteriosus and ventricle. During recovery from hypoxia and ischaemia, it was necessary to increase the preload and afterload gradually, otherwise the heart became distended with buffer and did not contract. A contraction under these circumstances could be initiated by the application of several mechanical stimuli to the engorged atria.

Statistics

Statistical differences were determined using the *t*-test or analysis of variance (ANOVA) with a *P* value less than 0.05. Data are expressed as means ± S.D. unless stated otherwise.

Results

Intact animals

The heart rate in five anaesthetized animals was 50.6±3.6 beats min^{-1} (mean ± S.D.). Mean blood flow in the left aorta exceeded that in the right aorta (34±16 ml min^{-1} *versus* 14±6 ml min^{-1}). The mean cardiac output for these animals with a mean body mass of 409±141 g was 102±56 ml min^{-1} kg^{-1}.

Buffer-perfused hearts

Fig. 2 shows a recording of aortic flow rate and conus arterious pressure in a perfused *Bufo marinus* heart. Afterload was set at 2 kPa while preload was 0.3 kPa. Note the prominent atrial pressure wave preceding the much larger ventricular

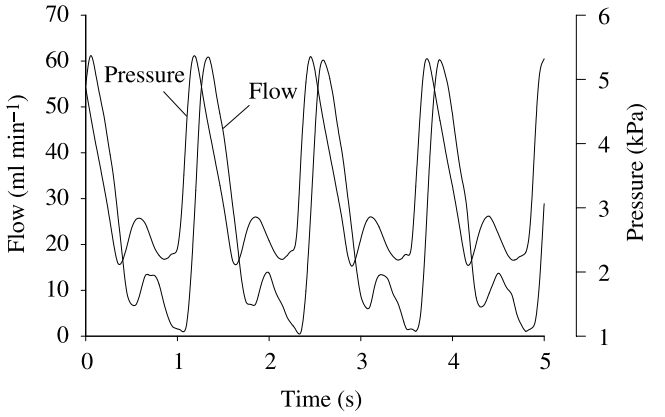

Fig. 2. Recording of conus arteriosus pressure (upper trace) and aortic flow (lower trace) in a buffer-perfused toad heart. Diastolic afterload was 1.96 kPa and sinus venosus filling pressure was 0.3 kPa. Buffer pH was 7.4. Sampling rate for data acquisition was 20 s^{-1}.

pressure wave. Note also that there is a corresponding flow rate increase during the atrial contraction. The atrial pressure and flow waves were not as prominent in other animals investigated, but they were present to some degree in all animals studied.

The *in situ* perfused *Bufo marinus* heart shows a Frank–Starling relationship (Fig. 3A). Increases in filling pressure result in significantly increased conus arteriosus pressure and aortic flow rate, but no significant change in heart rate. Diastolic afterload was maintained at 2 kPa. The cardiac output at 0.3 kPa filling pressure was 72±13 ml min^{-1} kg^{-1} (mean ± S.D.).

Increases in afterload (diastolic pressure) also influence the performance of the *in situ* perfused heart. These experiments were performed at a preload of 0.3 kPa.

Diastolic afterload has an effect on aortic flow rate (Fig. 3B). Note that there is a tendency for aortic flow to decrease as afterload increases. When aortic flow ceases, failure occurs, and this was at a pressure of 4.8±0.9 kPa. The cardiac output at 2 kPa afterload was 65±15 ml min^{-1} kg^{-1}. These experiments were performed using a buffer of pH 7.4. When buffer pH was increased to a more physiological value of 7.8 (West and Smits, 1994), cardiac performance was improved as shown in Table 1.

Hypoxia

There were two types of responses to hypoxia at higher workloads. The type 1 response was a cessation of beating within 6 min of the onset of hypoxia (Fig. 4A). In these animals, the atria were still beating but there was no ventricular filling or pressure development. This behaviour was demonstrated by 73 % of the toad hearts. In a minority of animals (27 %), a cessation of beating did not occur; instead, periodic beating was recorded (Fig. 4B). The flow rate trace was similar to the pressure trace shown.

Complete recovery from hypoxia did occur in the hearts perfused with the buffer at pH 7.4. At the end of the 30 min hypoxia, the hearts were perfused with oxygenated buffer and the gas environment surrounding the heart was changed to room air. It was necessary to increase preload and afterload gradually as described above. During the early recovery process, the initial reoxygenation pressures and flows were greater than the prehypoxia pressures and flows. Later in the recovery process, these values returned towards normal.

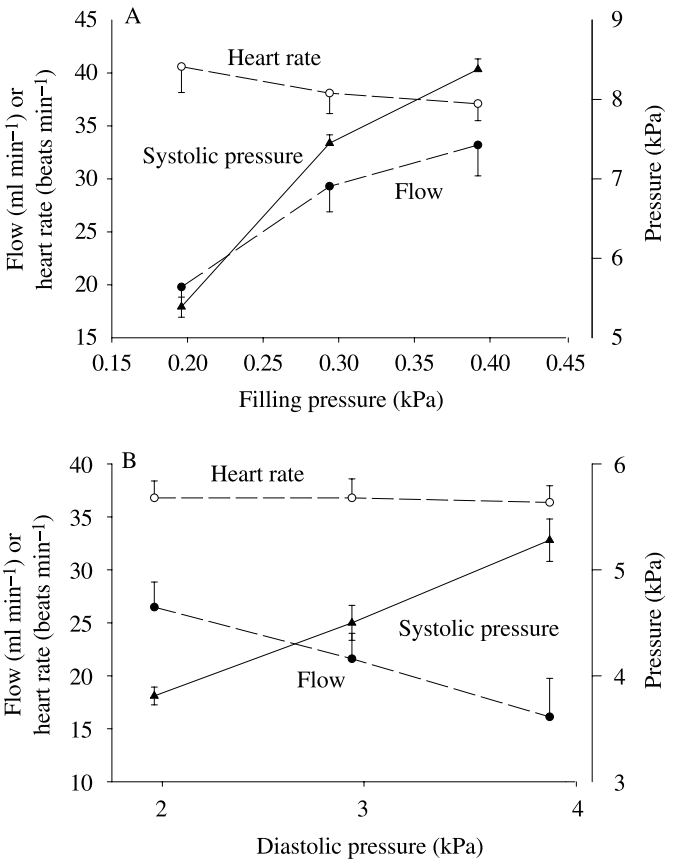

Fig. 3. Frank–Starling relationship in buffer-perfused toad hearts. (A) Sinus venosus filling pressure was varied at a constant diastolic afterload of 2.0 kPa. Buffer pH was 7.4. *N*=5 hearts. One-way ANOVA indicates that filling pressure affects aortic flow (*P*=0.013) and conus arteriosus (systolic) pressure (*P*=0.0002) but not heart rate (*P*=0.49). Values plotted are the means ± S.E.M. (B) Effect of diastolic afterload on flow rate, heart rate and systolic pressure. *N*=7 hearts. Diastolic afterload significantly affects systolic pressure (*P*=0.00002), its effect flow on flow is nearly significant (*P*=0.059), and there is no significant effect on heart rate (*P*=0.98). Buffer pH was 7.4. Values plotted are the means ± S.E.M.

Table 1. *Normal values for buffer-perfused hearts in buffer pH of 7.8*

Body mass (g)	Heart mass (g)	Cardiac output (ml min^{-1} kg^{-1})	Systolic pressure (kPa)	Heart rate (beats min^{-1})
179.6±26.9	0.97±0.14	84.5±30.6	6.36±1.10	35.4±6.9

Values are means ± S.D. (*N*=5).

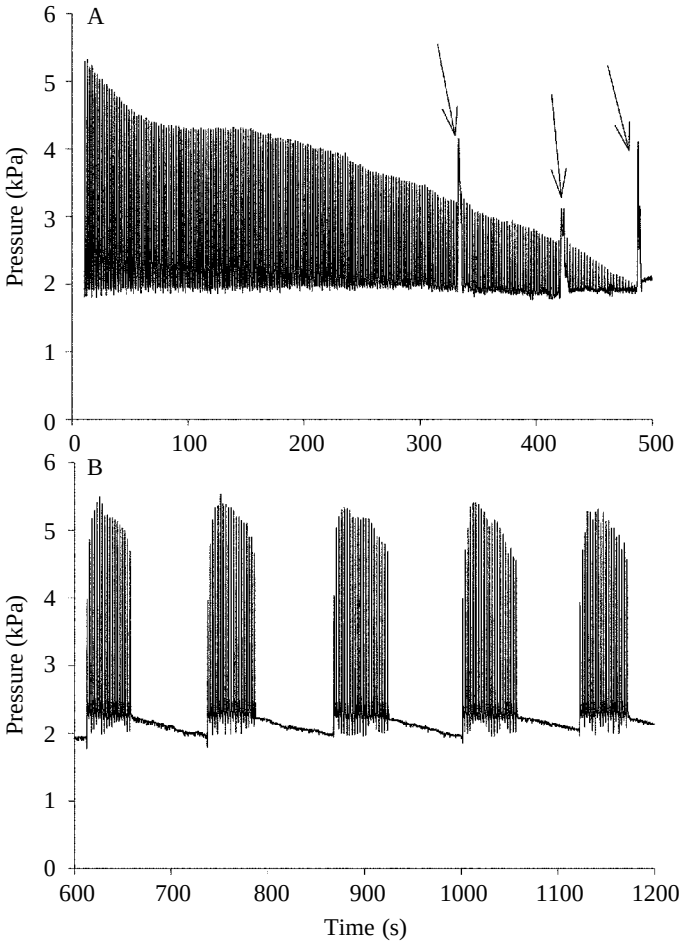

Fig. 4. Effect of hypoxia on toad heart conus arteriosus pressure development. (A) Gradual decline in beating. Preload was 0.3 kPa and afterload was 2.0 kPa. Buffer pH was 7.4. Data-acquisition sampling rate was 5 s^{-1} and recording was started approximately 10 s after the onset of hypoxia. The large peaks are augmented contractions following 'missed' beats (arrows). This was the most common response type. (B) Intermittent type response. Preload and afterload are 0.3 and 2.0 kPa, respectively. Buffer pH was 7.4. Sampling rate was 5 s^{-1} and recording began 10 min after the onset of hypoxia.

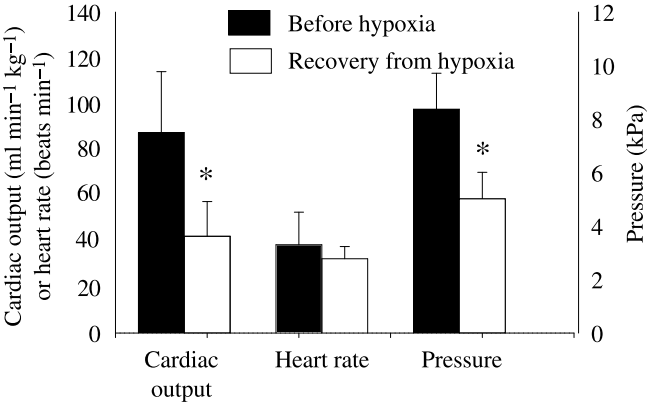

Fig. 5. Recovery of toad hearts following 30 min of hypoxia. *N*=5 hearts. The solid bars are the prehypoxia values and the open bars are the recovery values. There were significant differences (*) between prehypoxia and recovery values for cardiac output (*P*=0.0105) and systolic pressure (*P*=0.0122) (Student's *t*-test). Heart rate values did not change significantly (*P*=0.395). Buffer pH was 7.8. Values plotted are the mean + S.E.M.

Fig. 5 shows pre- and post-30 min hypoxia values hearts of cardiac output, heart rate and peak conus arteriosus pressure in five toad hearts. There were significant differences between pre- and post-hypoxia values in cardiac output and pressure, but not heart rate. In a group of five toads subjected to an identical hypoxic protocol but using a buffer pH of 7.4 instead of pH 7.8, there were no significant differences between pre- and post-hypoxia values (data not shown). In the group of seven animals in which preload and afterload were reduced to 0.20 kPa and 1 kPa, respectively, (pH 7.8) during the hypoxia experiments, the hearts continued to produce a cardiac output of 33.4±16.2 ml min^{-1} kg^{-1} compared with a pre-hypoxia cardiac output of 84.5±30.6 ml min^{-1} kg^{-1}. These hearts recovered completely during reoxygenation to give a cardiac output of 72.9± 14.1 ml min^{-1} kg^{-1}, and there was also complete recovery of heart rate (35.4±6.9 *versus* 32.0±9.1 beats min^{-1}) and peak systolic pressure (6.4±1.1 *versus* 7.4±2.0 kPa).

Ischaemia

Six toads were subjected to 60 min of ischaemia followed by reperfusion. All animals recovered from ischaemia where recovery was taken as the ability to produce a statistically unchanged cardiac output against an afterload of 2 kPa. The longest time for recovery was 45 min and the shortest was 5 min. Mean time to recovery was 18 min. The recovery cardiac output was 79±19 % of the pre-ischaemic cardiac output. The recovery values for heart rate were 84±14 % and for systolic pressure were 96±35 % of pre-ischaemia values. These were not statistically significant changes.

Discussion

The *in situ* preparation described in the present study, under certain circumstances, has similar cardiac output, heart rate, preload and afterload values to those measured in the unanaesthetized, intact animal. Cardiac output in the present study with a pH 7.4 buffer was approximately 70 ml min^{-1} kg^{-1}. When the pH of the buffer was increased to a more physiological pH of 7.8, cardiac output increased to approximately 85 ml min^{-1} kg^{-1}. Cardiac output in anaesthetized unperfused animals was 102 ml min^{-1} kg^{-1}. West and Smits (1994) recorded cardiac outputs of 57 ml min^{-1} kg^{-1} in resting unanaesthetized *Bufo marinus*. K. Gamperl and T. Wang (personal communication) recorded cardiac outputs in unanaesthetized *Bufo marinus* of 50 ml min^{-1} kg^{-1} at 15 °C and 103 ml min^{-1} kg^{-1} at 25 °C.

Heart rates in the anaesthetized unperfused animals in the present study were approximately 50 beats min^{-1}, while the *in situ* heart rate of buffer-perfused hearts was 30–40 beats min^{-1}.

West and Smits (1994) recorded heart rates of approximately 30 beats min^{-1}. Very low heart rates of 13 and 23 beats min^{-1} have been recorded in unanaesthetized animals (Dumsday, 1990; Wahlquist and Campbell, 1988). MS-222 anaesthesia is known to activate the sympathetic nervous system (Smith, 1974), causing an increase in heart rate and probably also in cardiac output.

The peak systolic pressure of 6.36 kPa recorded in the *in situ* preparation in this study is higher than the 4.9 kPa recorded previously in intact animals (West and Smits, 1994). The catheters used in the present study were very short and the tips were situated in the aortic root or conus arteriosus, thus favouring an undamped recording of pressure close to the site of pressure generation.

The prominent atrial pulse wave seen in the flow record of Fig. 2 was also evident in the flow records of West and Smits (1994) and in the flow records from intact unperfused animals in the present study. In this study, the pericardium had been removed and, as a result, the atria may have filled to a greater extent than with an intact pericardium as was used in the study by West and Smits (1994). It would seem likely for the atrial wave of aortic flow to be caused by mechanical coupling between the atria and the conus arteriosus rather than atrial propulsion of fluid through the ventricle and into the conus and arterial system.

Cardiac output measured in this study compares favourably with values recorded from the phylogenetically more advanced turtle's heart. Power is calculated as $[(P\mathrm{a}-P\mathrm{v})\times R_\mathrm{f}\times M_\mathrm{v}^{-1}]$, where Pa is mean arterial pressure, Pv is mean venous pressure, R_f is flow rate and M_v is ventricular mass. The *in situ* toad heart produced approximately 0.82 mW g^{-1} heart compared with 1.5 mW g^{-1} in the turtle (*Chrysemys scripta*) heart (Farrell *et al.* 1994). The values used to calculate power for the toad heart were Pa=3.43 kPa, calculated from $[P\mathrm{d}+(P\mathrm{s}-P\mathrm{d})/3]$, where Ps is systolic pressure and Pd is diastolic pressure, Pv=0.3 kPa, R_f=0.25 ml s^{-1} and M_v=0.97 g. However, these measurements were made under different conditions in the two species. The temperature was 20 °C compared with 15 °C for the turtle experiments, which would favour a lower power generation in the turtle. However, the power expressed for the turtles was expressed per gram of ventricle, whereas the value for the toad was expressed per gram of heart (including the atria and conus arteriosus), and 1 nmol l^{-1} adrenaline was included in the buffer used in the turtle experiments, both of which would favour a higher power values in the turtle. In the turtle, an increase in filling pressure of 0.2 kPa resulted in an increase in cardiac output of approximately 10 ml min^{-1} kg^{-1}. A similar change in filling pressure in the toad heart produced a similar increase of 10–12 ml min^{-1} kg^{-1} in cardiac output. Thus, the toad heart has a Frank–Starling relationship that is quantitatively similar to that found in the turtle heart. Although failure was not specifically measured in the *in situ* turtle heart perfusion, extrapolation of the cardiac output *versus* afterload curves from Farrell *et al.* (1994) indicates that the turtle hearts would have failed at an afterload of 4 kPa, only slightly lower than the 4.9 kPa value determined in this study. The lower value in the turtle heart might be explained, at least partially, by the 5 °C difference in temperature between the two preparations.

Exposure of the anuran heart at physiological pressures and flow rates to hypoxia and ischaemia resulted in profound negative inotropy with cessation of pressure development. During ischaemia, the decrease in pressure generation was immediate since there was no fluid to pump. During anoxia, there were two types of responses observed: either a cessation of pressure generation that occurred 4–18 min after the onset of hypoxic perfusion or repeated cycles of beating followed by asystole that then led into a new cycle of beating. There was recovery of mechanical function during reoxygenation or reperfusion if the preparation had a reduced energy requirement because of a reduced preload and afterload or if the buffer pH was mildly acidic relative to a normal pH of 7.8. Koop and Piper (1992) have shown in rat cardiac myocytes that mild acidosis (pH 7.0 *versus* normal pH 7.4) has an energy-conserving and protective effect on myocytes exposed to hypoxia. During early recovery from hypoxia, there was a short period of enhanced mechanical function. Such an enhancement has been reported during recovery from hypoxia in mammalian hearts (McKean and Landon, 1982). The mechanism for this enhancement is unknown, but may involve an overshoot in phosphocreatine levels and perhaps Ca^{2+} concentrations (Kaplin *et al.* 1995; Yoshikawa *et al.* 1993).

During ischaemia and hypoxia, small contractions sometimes occurred. These contractions did not usually result in any meaningful pressure development except in the intermittent beating that was seen in some hearts during hypoxia. These observations indicate that the inotropic and chronotropic states of the heart are differentially affected by hypoxia and ischaemia.

Pörtner *et al.* (1991) subjected *Bufo marinus* to environmental hypoxia and found that the heart experienced decreases in high-energy phosphate levels and increases in lactate concentration. Wasser *et al.* (1990, 1992), using ^{31}P nuclear magnetic resonance methodology, showed that buffer-perfused turtle hearts (*Chrysemys picta bellii*) showed a decrease in ATP and phosphocreatine concentrations and an increase in proton levels, inorganic phosphate levels and lactate concentration during both ischaemia and anoxia, while both contractions and pressure development continued. Both protons and inorganic phosphate have been shown to inhibit the contraction of heart muscle and to cause a right shift in the force–pCa^{2+} relationship (Rëugg, 1987). Farrell *et al.* (1994), in an *in situ* turtle heart preparation that was performing physiological but low levels of work prior to hypoxia, found that hypoxia reduced the heart rate only slightly but that cardiac output and power generation were reduced by up to 50 %. In contrast to both of the turtle heart studies, the results of the present study revealed either a complete cessation or intermittent pumping during hypoxia at normal preload and afterload. If these variables were reduced as in the study of Farrell *et al.* (1994), the hearts continued to beat and to develop a cardiac output. Farrell *et al.* (1994) justify their use of lower

atrial filling and diastolic pressures during hypoxia because these determinants of cardiac function actually do decrease when the intact turtle is exposed to hypoxia. During mild hypoxia (breathing 5 % O_2, 95 % N_2), there is a slight increase in cardiac output in unanaesthetized toads (K. Gamperl and T. Wang, personal communication). The turtle hearts used by Farrell *et al.* (1994) were perfused with buffer equilibrated with 3 % O_2. Measurements of cardiac output in toads breathing less than 5 % O_2 have not been obtained, but it is likely that, as the concentration of oxygen decreases much below this value, cardiac contractions will begin to fail. If there were a concomitant decrease in filling pressure, the heart could continue to beat and generate a reduced cardiac output throughout the hypoxic bout.

In summary, an *in situ* preparation was developed for *Bufo marinus* hearts in which buffer was pumped at physiological heart rates, pressures and flow rates. The preparation demonstrated a Frank–Starling mechanism and sensitivity to afterload. Reversible cardiac failure occurred during ischaemia or hypoxia with normal filling and output pressures. During hypoxia with reduced metabolic demand, the heart continued to pump.

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