The Journal of Experimental Biology **199**, 693–697 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0159

COMPARISON OF THE RESPONSES TO HYPOXIA, ISCHAEMIA AND ISCHAEMIC PRECONDITIONING IN WILD MARMOT AND LABORATORY RABBIT HEARTS

TOM McKEAN AND WADE MENDENHALL

Department of Biological Sciences and WAMI Medical Program, University of Idaho, Moscow, ID 83843, USA

Accepted 2 November 1995

Summary

Marmots (*Marmota flaviventris*) are burrowing mammals that may be subjected to low levels of oxygen and high levels of carbon dioxide in their underground environment. Since marmots successfully deal with this physiological challenge, we hypothesized that the isolated perfused marmot heart would be damaged less and recover better from a bout of induced hypoxia or ischaemia than would the heart of a comparison animal, the New Zealand laboratory rabbit (*Oryctolagus cuniculus*).

Isolated marmot and rabbit hearts were made hypoxic by a 30 min perfusion with an oxygen-deficient buffer. The hearts were then perfused with an oxygen-replete buffer and measurements of heart rate, left ventricular pressure and lactate dehydrogenase (LDH) release (an indicator of cell damage) were made over 5 or 10 min intervals for 30 min of hypoxia and 30 min of recovery. There were no species differences in the responses, except that the heart rate in marmots was about 50 % of the rate in rabbits during the hypoxia part of the experiment. There was no evidence that the marmot hearts were damaged less or recovered better from hypoxia and reoxgenation than the rabbit hearts. Marmot and rabbit hearts were also subjected to 30 min of total ischaemia; measurements of heart rate, left ventricular pressure and LDH release were obtained during 30 min of reperfusion and compared with the pre-ischaemia values for these variables. There were no significant species differences. When the 30 min ischaemic period was preceded by a 5 min period of ischaemia and a 10 min reperfusion period (preconditioning), the rabbit hearts were protected by this brief ischaemic insult and recovered better than the hearts that had not been subjected to the preconditioning ischaemia. This was not true in the marmot hearts, however, as the preconditioning ischaemia did not promote a greater recovery over that in its absence. When preconditioned marmots hearts were compared with preconditioned rabbit hearts, there were no statistical differences in the responses.

The hypothesis that marmot hearts would be damaged less and recover better from hypoxia and ischaemia was not supported by the experimental data.

Key words: hypoxia, ischaemia, ischaemic preconditioning, heart, marmot, rabbit, *Marmota flaviventris*.

Introduction

Hearts of many mammalian species have mechanisms for self-protection under the pernicious conditions of hypoxia and ischaemia. These mechanisms are directed towards a restoration of the normal ratio of energy demand *versus* energy supply. In addition, the mammalian heart's ability to survive a metabolic insult is dependent on the prior history of exposure to a hypoxic or ischaemic insult. Murray *et al.* (1986) showed in dogs that damage to the myocardium by a 30 min interruption of coronary blood flow followed by reperfusion could be reduced if a short occlusion and reperfusion period preceded the more prolonged occlusion. This phenomenon was named ischaemic preconditioning (IP) because the short period of ischaemia preconditioned the heart to survive the more sustained ischaemic period and the reperfusion that followed.

Ischaemic preconditioning has been demonstrated in dogs (Auchampach *et al.* 1992), swine (Kimura *et al.* 1992), rabbits (Thornton *et al.* 1992) and rats (Liu and Downey, 1992). It has been shown to occur both in intact animals (Cohen *et al.* 1994) and in cardiac muscle systems *in vitro* (Thornton *et al.* 1993; Walker *et al.* 1994; Wall *et al.* 1994). The mechanisms responsible for IP are unknown and may be species-dependent. Likely candidates are A1-adenosine receptor activation (Thornton *et al.* 1992), activation of K_{ATP} channels (Auchampach *et al.* 1992) and the mobilization of protein kinase C from the cytosol (Liu *et al.* 1994).

Certain mammalian species have hearts that are thought to be tolerant to hypoxia or ischaemia, and among them are animals that dive, such as muskrats (McKean and Landon, 1992) and seals (White *et al.* 1990), and those that burrow and/or hibernate. Among the fossorial animals are ground squirrels and marmots, which may encounter a hypoxic environment in the burrow. Burlington *et al.* (1970) have shown that the hearts of ground squirrels are adapted to hypoxia and, although marmot hearts have not been studied

directly, the whole animal exhibits cardiovascular adaptations to hypoxia (Burlington *et al.* 1971).

The purpose of this study was to compare the responses of the hearts of marmots and laboratory rabbits to hypoxic and ischaemic conditions. The rationale for these experiments was that, by virtue of its fossorial habit, the marmot might possess more effective cardioprotective mechanisms than the laboratory rabbit. Heart rate, left ventricular pressure development and lactate dehydrogenase release were examined in rabbit and marmot hearts during recovery from (1) hypoxic perfusion, (2) global ischaemia or (3) ischaemic preconditioning.

Materials and methods

Marmots were live-trapped in Idaho from several weeks to 2 months after emerging from hibernation. They were supplied with food and water *ad libitum*. Rabbits were obtained from a licensed animal dealer. Both species were sedated with xylazine (20 mg) and ketamine (100 mg) injected intramuscularly. After the animals had become sedated, sodium pentobarbital (150 mg) was injected into an ear vein in the rabbit and intraperitoneally in the marmot. When the animals had been completely anaesthetized, the hearts were removed and placed in ice-cold Krebs–Henseleit buffer, weighed and then mounted on a coronary perfusion apparatus (Streeby and McKean, 1994). Left ventricular pressure was recorded with a balloon catheter inserted through the left atrium into the ventricle, and heart rate was determined from the ventricular pressure trace. Lactate dehydrogenase activity (LDH) was determined in samples of the coronary effluent using the method of Wroblewski and LaDue (1955).

Hypoxia

In the hypoxia experiments, hearts were perfused for 30 min with buffer that had been bubbled with 95 % N_2:5 % CO_2. This was followed by perfusion with the oxygenated buffer (95 % O_2:5 % CO_2). Measurements of blood pressure and flow, heart rate and enzyme release were taken at 5 or 10 min intervals during reoxygenation and compared with values obtained at the end of a 30 min equilibration period that preceded the hypoxia.

Ischaemia

For the ischaemic control experiments, hearts were equilibrated for 45 min, baseline measurements taken and then the perfusion pump was turned off for 30 min and then turned back on for an additional 30 min. Measurements were taken at 5 min intervals during the 30 min reperfusion period.

Ischaemic preconditioning

For the IP experiments, baseline measurements were obtained following 30 min of equilibration. The perfusion pump was turned off for 5 min and then back on for 10 min. This was followed by the 30 min ischaemic period during which the pump was turned off. The pump was then turned back on for 30 min for the reperfusion period. Measurements were taken at 5 min intervals during reperfusion.

During the experiments involving ischaemia, the temperature in the right ventricle was maintained at 37±0.5 °C by immersing the heart in buffer heated by a water jacket. Temperature control becomes critical during the ischaemia part

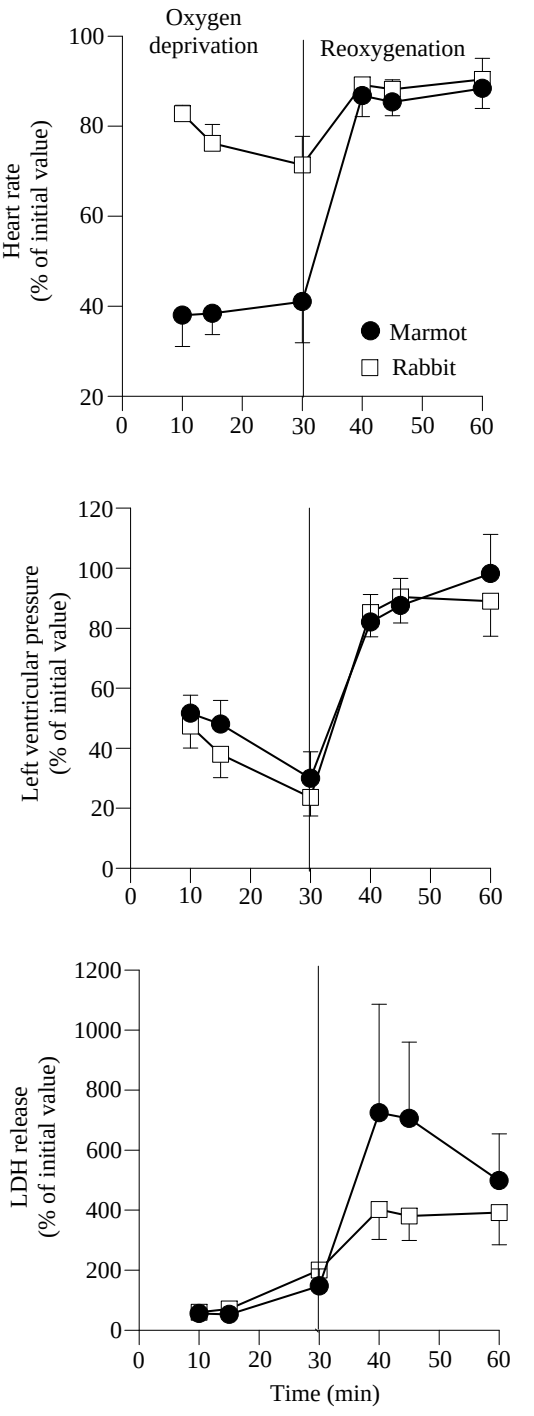

Fig. 1. Responses of marmot and rabbit hearts to hypoxia followed by reoxygenation. (A) Heart rate, (B) left ventricular pressure and (C) release of lactate dehydrogenase (LDH). *N*=5 for both marmots and rabbits. The vertical lines at 30 min delineate the transition between hypoxic perfusion and reoxygenation. Statistical differences occurred only between marmot and rabbit heart rates during hypoxic perfusion. Values are means ± S.E.M.

of the experiment when heated buffer is not flowing through the coronary circulation.

Data are presented as mean ±1 S.E.M. *N* refers to the number of animals per group and was generally 5 or 6. Statistics were performed using PC-SAS. Percentage baseline data were compared using general linear models procedure with repeated-measures analysis of variance. An arcsin transform of the normalized data was not performed since values during reperfusion frequently exceeded the baseline values and the arcsin transform is not possible for values greater than 1. Differences were considered significant at $P<0.05$. All chemicals were purchased from Sigma Chemical Company, St Louis, MO, USA.

Results and discussion

The working hypothesis of the study was that the marmot heart was adapted to hypoxia and would survive hypoxic and ischaemic insults better than the rabbit heart. This hypothesis was not borne out by the results of the study. There are a number of factors that may have influenced the results.

Mean heart mass of the marmots (8.5±0.7 g) and rabbits (7.6±0.6 g) did not differ. Coronary flows in the two species were also similar: the value for rabbits was 38.0±1.3 ml min^{-1} and that for marmots 35.9±0.5 ml min^{-1}. Heart rate following the equilibration period was higher in the marmots (198±12 beats min^{-1}) than in the rabbits (155±5 beats min^{-1}). This heart rate recorded in the buffer-perfused marmot hearts approximates the heart rate recorded for intact marmots (*Marmota monax*) in a laboratory environment (Burlington *et al.* 1971). The heart rate recorded for the rabbit in this study was about 60 % of the heart rate of *in situ* studies of preconditioning in rabbit hearts and lower than that of buffer-perfused isolated rabbit hearts paced at 200 beats min^{-1} (Thornton *et al.* 1992). Since heart rate was not a controlled variable in this study, conclusions from comparisons of the marmot heart with the rabbit heart need to take into account that the marmot heart was beating 28 % faster at the beginning of the experiments and thus may be beginning from a higher level of energy expenditure. This would tend to bias the data towards greater damage done to the marmot hearts as a result of the hypoxia and ischaemia compared with that done to the rabbit hearts. Left ventricular pressures (measured by the balloon catheter) did not differ between the two species. The values were 8.7±0.6 kPa in the marmot and 8.41±0.7 kPa in the rabbit. Left ventricular pressures are commonly used as indicators of ventricular contraction, but do not necessarily reflect systolic pressure in the intact animal. LDH release into the coronary effluent at the start of the experiment also did not differ between the two species. The values were 23.3±5.4 i.u. min^{-1} mg^{-1} wet heart tissue in the rabbit and 17.6±3.5 i.u. min^{-1} mg^{-1} in the marmot.

Hypoxia

The response to 30 min of hypoxia followed by 30 min of reoxygenation consisted of a reduction in heart rate and left ventricular pressure during hypoxia followed by a return towards baseline values in both species. The pattern of LDH release was one of a gradual increase during hypoxia, with a more accelerated release followed by a plateau or decline during reoxygenation. The changes in left ventricular pressure and LDH release during hypoxia and reperfusion were similar between the two species (Fig. 1). There were differences in the heart rate responses, however. When the marmot heart was

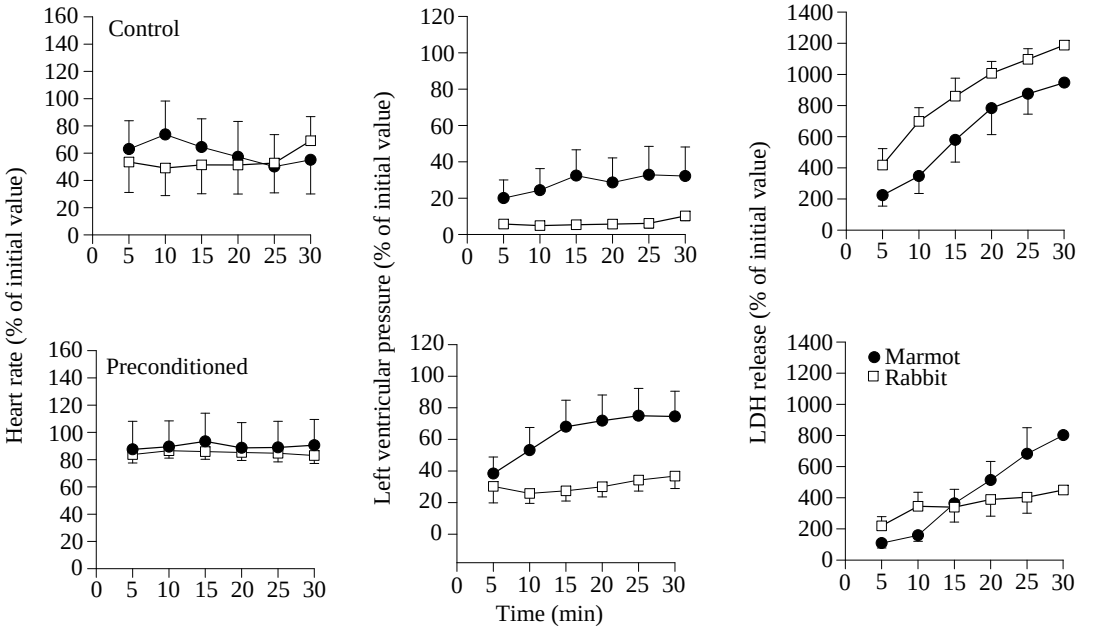

Fig. 2. Responses of marmot and rabbit hearts to reperfusion following 30 min of ischaemia in the absence (upper three graphs) and presence (lower three graphs) of a 5 min preconditioning stimulus. Values are means ± S.E.M., N=5 for rabbits and N=6 for marmots. Statistical differences occurred only for left ventricular pressure and for LDH release between preconditioned rabbit hearts and rabbit hearts not subjected to preconditioning.

exposed to hypoxia by perfusing it with a buffer bubbled with 95 % N_2:5 % CO_2, there was a dramatic decrease in heart rate to less than 40 % of the prehypoxic rate. Heart rate declined by only 25 % in the rabbit during hypoxia. Although the hearts of the two species were developing approximately the same ventricular pressures during hypoxia, the marmot heart was contracting about 60 % as often as the rabbit heart. Therefore, all other factors being equal, the energy demand of the marmot heart should have been less that of the rabbit heart. This putative reduction in energy demand in the marmot heart would have spared ATP that would be available to maintain ion pumping and other required membrane functions that are necessary for the preservation of cell viability. Release of LDH, an indicator of cellular damage, was the same in the two species, so any reduction in energy demand that occurred in the marmot heart was not manifest in augmented preservation of the myocardium during hypoxia and reoxygenation. In a previous study using muskrat and guinea pig hearts, McKean and Landon (1982) showed similar heart rate and left ventricular pressure responses to those in this study for marmots and rabbits. The LDH responses differed between the two species, however, with the hypoxia-adapted muskrat heart showing a much smaller LDH release compared with the guinea pig heart. The reason for the increased LDH release in the face of an apparent decrease in energy demand is unknown.

Ischaemia and ischaemic preconditioning

Heart rate and left ventricular pressure were depressed following 30 min of ischaemia and showed varying degrees of recovery towards baseline values during the 30 min of reperfusion. LDH release increased above the baseline value during the 30 min reperfusion period. These trends were seen both in marmots and in rabbits and during ischaemia with and without preconditioning. A comparison of the responses to ischaemia in the rabbit and marmot without preconditioning is shown in the upper part of Fig. 2. There were no significant differences between species.

When ischaemia is preceded by an ischaemic preconditioning period of 5 min, a different response results and this is shown in the lower part of Fig. 2. The same trend of a depression followed by varying degrees of recovery for left ventricular pressure and heart rate during reperfusion was seen as in hearts that had not been preconditioned. As during ischaemia without preconditioning, the LDH release values increased during reperfusion. No statistically significant differences were found between the marmot and the rabbit.

The protective effect of ischaemic preconditioning was evident in rabbit hearts: the left ventricular pressure and LDH release of IP animals and control animals were statistically different. There was no difference in heart rates between IP and control animals, however. The ischaemic preconditioning demonstrated in the rabbit in this study confirms the results of several other studies. There was an increase in heart rate of approximately 35 %, a 500 % increase in left ventricular pressure and a 50 % reduction in LDH release in the preconditioned compared with the control rabbit hearts. Therefore, the effect of preconditioning is highly significant in this species. In rabbits, where the necrotic areas of the myocardium are mapped and then quantified following ischaemia or ischaemia with preconditioning, preconditioning may reduce the infarcted area from 40 to 9 % of the area at risk (Thornton *et al.* 1993). These studies also indicate that the magnitude of IP is considerable and may provide a benefit to the organism.

Although the values for heart rate and left ventricular pressure in preconditioned marmots appeared to be uniformly above the values for control marmots for all time periods, the differences were not statistically significant. Likewise, LDH release values appeared to be uniformly smaller in the preconditioned marmots but the differences were not statistically significant. The result of this study do not support the hypothesis that preconditioning also occurs in the marmot heart.

In summary, the heart of a fossorial rodent, the marmot, was compared with that of the laboratory rabbit with regard to intrinsic cardioprotective mechanisms. The marmot heart was shown to be as vulnerable as the rabbit heart to hypoxia and ischaemia and was not preconditioned by a 5 min ischaemic preconditioning stimulus, whereas the rabbit heart demonstrated the cardioprotective mechanism of preconditioning.

This study was supported by grants-in-aid from the American Heart Associations of Idaho and Montana and PHS grant R15HL46506.

References

AUCHAMPACH, J. A., GROVER, G. J. AND GROSS, G. J. (1992). Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc. Res.* **26**, 1054–1062.

BURLINGTON, R. F., VOGEL, J. A., BURTON, T. M. AND SALKOVITZ, I. A. (1971). Cardiac output and regional blood flow in hypoxic woodchucks. *Am. J. Physiol.* **220**, 1565–1568.

BURLINGTON, R. F., WHITTEN, B. K., SIDEL, C. M., POSOVIATA, M. A. AND SALKOVITZ, I. A. (1970). Effect of hypoxia on glycolysis in perfused hearts from rats and ground squirrels (*Citellus lateralis*). *Comp. Biochem. Physiol.* **35**, 403–414.

COHEN, M. V., YANG, X. AND DOWNEY, J. M. (1994). Conscious rabbits become tolerant to multiple episodes of ischaemic preconditioning. *Circulation Res.* **74**, 998–1004.

KIMURA, Y., IYENGAR, J., SUBRAMANIAN, R., CORDIS, G. A. AND DAS, D. K. (1992). Preconditioning of the heart by repeated stunning: attenuation of post-ischaemic dysfunction. *Basic Res. Cardiol.* **87**, 128–138.

LIU, Y. AND DOWNEY, J. (1992). Ischaemic preconditioning protects against infarction in the rat heart. *Am. J. Physiol.* **263**, H1107–H1112.

LIU, Y., YTREHUS, K. AND DOWNEY, J. M. (1994). Evidence that translocation of protein kinase C is a key event during ischaemic preconditioning of rabbit myocardium. *J. molec. cell. Cardiol.* **26**, 661–668.

MCKEAN, T. AND LANDON, R. (1982). Comparison of the response of

muskrat, rabbit and guinea pig heart muscle to hypoxia. *Am. J. Physiol.* **243**, R245–R250.

MURRAY, C. E., JENNINGS, R. B. AND REIMER, K. A. (1986). Preconditioning with ischaemia: a delay of lethal cell injury in ischaemic myocardium. *Circulation* **74**, 1124–1136.

STREEBY, R. R. AND MCKEAN, T. A. (1994). The effect of ATP-sensitive potassium channel modulation on heart rate in isolated muskrat and guinea pig hearts. *J. exp. Biol.* **197**, 101–118.

THORNTON, J., LIU, G. S., OLSSON, R. A. AND DOWNEY, J. M. (1992). Intravenous pretreatment with A1-selective adenosine analogues protects the heart against infarction. *Circulation* **85**, 659–665.

THORNTON, J., THORNTON, C., STERLING, D. L. AND DOWNEY, J. M. (1993). Blockade of ATP-sensitive potassium channels increases infarct size but does not prevent preconditioning in rabbit hearts. *Circulation Res.* **78**, 44–49.

WALKER, D. M., MARBER, M. S., WALKER, J. M. AND YELLO, D. M. (1994). Preconditioning in isolated superperfused rabbit papillary muscle. *Am. J. Physiol.* **266**, H1534–H1540.

WALL, T., LINSEMAN, D., SHEBUSKI, R. AND HARTMAN, J. (1994). Preconditioning myocytes before metabolic inhibition. *FASEB J.* **8**, A855.

WHITE, F. C., ELSNER, R., WILLFORD, D., HILL, E. AND MERHOFF, E. (1990). Response of harbor seal and pig heart to progressive and acute hypoxia. *Am. J. Physiol.* **259**, R849–R856.

WROBLEWSKI, F. AND LADUE, I. S. (1955). Lactate dehydrogenase activity in blood. *Proc. Soc. exp. Biol. Med.* **90**, 210–213.