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THE EFFECT OF ATP-SENSITIVE POTASSIUM CHANNEL MODULATION ON HEART RATE IN ISOLATED MUSKRAT AND GUINEA PIG HEARTS

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Summary

Muskrats (*Ondontra zibethicus*) are common freshwater diving mammals exhibiting a bradycardia with both forced and voluntary diving. This bradycardia is mediated by vagal innervation; however, if hypoxia is present there may be local factors that also decrease heart rate. Some of these local factors may include ATP-sensitive potassium channel activation and extracellular accumulation of potassium ions, hydrogen ions and lactate. The purpose of this study was to investigate the role of these factors in the isolated perfused hearts of muskrats and of a non-diving mammal, the guinea pig. Although lactate and proton administration reduced heart rate in isolated muskrat and guinea pig hearts, there was no difference in the response to lactate and proton infusion between the two species. Muskrat hearts were more sensitive to the heart-rate-lowering effects of exogenously applied potassium than were guinea pig hearts. Early increases in extracellular potassium concentration during hypoxia are thought to be mediated by the ATP-sensitive potassium channel. Activation of these channels under normoxic conditions had a mildly negative chronotropic effect in both species; however, activation of these channels with Lemakalim under hypoxic conditions caused the guinea pig heart to respond with an augmented bradycardia similar to that seen in the hypoxic muskrat heart in the absence of drugs. Inhibition of these channels by glibenclamide during hypoxia was partially successful in blocking the bradycardia in guinea pig hearts, but inhibition of the same channels in hypoxic muskrat hearts had a damaging effect as two of five hearts went into contracture during the hypoxia. Thus, although ATP-sensitive potassium channels appear to have a major role in the bradycardia of hypoxia in guinea pigs, the failure to prevent the bradycardia by inhibition of these channels in muskrat hearts suggests that multiple factors are involved in the hypoxia-induced bradycardia in this species.

Introduction

Muskrats (*Ondatra zibethicus*) are common freshwater diving mammals with a well-developed diving response that occurs with either forced or voluntary diving (Jones *et al.* 1982; MacArthur and Karpan, 1989). This diving response consists of a decline in heart

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rate and cardiac output, and a redistribution of blood flow to the brain, heart and adrenal glands while the rest of the body is hypoperfused.

During forced dives and long natural dives, diving mammals become hypoxic (Clausen and Ersland, 1970/71; Ferrante and Opdyke, 1969; Kooyman *et al.* 1980), which may be damaging to the heart. McKean and Landon (1982) and McKean (1984) established that the isolated perfused muskrat heart is able to withstand hypoxic conditions better than the heart of the terrestrial guinea pig. When unpaced hearts of the two species were subjected to hypoxia, guinea pig heart rate decreased to about 60 % of the control rate, while muskrat heart rate decreased to almost zero (McKean and Landon, 1982). By reducing heart rate during hypoxia, the rate of energy expenditure of the heart, and thus the damage done, would also be reduced.

Adenosine was originally examined as a mediator of the hypoxia-induced bradycardia observed in the guinea pig and muskrat heart. In these studies, it was found that muskrat hearts release larger amounts of adenosine during hypoxia than do guinea pig hearts and that muskrat hearts are more sensitive to the negative chronotropic effects of exogenously administered adenosine. However, experiments that examined the effects of adenosine receptor blockers during hypoxia showed that the hypoxia-induced bradycardia is not exclusively mediated by adenosine in the isolated muskrat heart, as it is in the guinea pig (McKean *et al.* 1993*b*).

Other possible candidates for hypoxia-induced bradycardia in the isolated heart are hydrogen ions, lactate and potassium ions. These substances increase in concentration during hypoxia and produce a decrease in heart rate (Kjekshus *et al.* 1982; McKean 1982; Scholander, 1940; Weiss *et al.* 1989; Zhou *et al.* 1991). The early increases in extracellular potassium concentration that occur during hypoxia are thought to be mediated by the ATP-sensitive potassium channel. These channels open and allow potassium to pass under conditions when cellular energy demand exceeds energy supply, for example during ischemia or hypoxia in the heart (Wilde *et al.* 1990). Upon opening of these channels, action potential duration is shortened and cells initially hyperpolarize, which may limit calcium entry into myocytes and reduce heart rate and energy expenditure.

Since ATP-sensitive potassium channels, hydrogen ions, lactate and potassium ions may play a role in the isolated heart bradycardia, the purpose of this study was to compare the responses of isolated muskrat and guinea pig hearts to ATP-channel agonists and antagonists as well as to exogenously administered hydrogen, lactate and potassium ions to evaluate their importance in animals adapted to diving.

Materials and methods

Muskrats weighing between 402 and 1444 g were live-trapped in Idaho and Washington and were used within 2 days of capture. Permits for live trapping were obtained from the wildlife divisions of the respective states. Guinea pigs weighing between 373 and 1010 g were obtained from a licensed animal dealer. Animals of both sexes were used in the study. All procedures involving animals had prior approval of the institutional animal care and use committee. Animals were anesthetized with diethyl

ether and their hearts were removed, placed in ice-cold buffer, weighed and then mounted on a coronary perfusion apparatus. Oxygenated buffer (95 % O_2, 5 % CO_2) was heated to 37 ˚C and was then forced in a retrograde direction from the aorta into the coronary circulation at a pressure of 8 kPa (Langendorff preparation). The perfusate fluid, Krebs–Henseleit buffer (KH), had the following composition (in mmol l^{-1}): KCl, 4.75; KH_2PO_4, 1.19; $CaCl_2$, 2.54; NaCl, 118; $NaHCO_3$, 25.00; and glucose, 5.56. Insulin, at 12.5 i.u. l^{-1}, was also present. Flow through the coronary circulation was produced by a variable-speed roller pump and was quantified by collection of the coronary effluent into a graduated cylinder. Left ventricular pressure was recorded with a balloon catheter inserted through the left atrium into the ventricle. The balloon was inflated with fluid so the difference between systolic and diastolic pressures was maximized. Heart rate was determined from the ventricular pressure trace. The pH of the coronary effluent was measured by holding a calomel pH electrode under the apex of the heart and letting the effluent flow over the electrode. The electrode did not interfere with the cardiac contraction. KCl, 100 mmol l^{-1}; HCl, 0.1 mol l^{-1}; pinacidil (an ATP-sensitive potassium channel agonist), 2.1 mmol l^{-1}; lactate, 250 mmol l^{-1} and at pH 7.0; or Lemakalim (an ATP-sensitive potassium channel agonist), 0.35 mmol l^{-1}, were each infused separately into the perfusion line with an infusion pump at one of seven different pump speeds to generate a range of concentrations that produced a change in heart rate and left ventricular pressure. The various concentrations were presented in random order. Sufficient time was given between trials for recovery as judged by the return of heart rate and pressure to control values. A trial was initiated by turning on the infusion pump. After waiting 15 s for an effect to occur, left ventricular pressure and aortic pressure were continuously monitored and data were collected during the next 15 s. The infusion pump was then turned off and recovery ensued. These periods were sufficiently long for a stable response to occur but not so long as to damage the myocardium or to produce a recovery period that exceeded 5 min. The concentration of the infused compound was estimated by multiplying the stock concentration by the dilution factor (flow from the infusion pump)/(flow from the infusion pump + flow from the perfusate pump). Actual concentrations were not determined analytically.

Hypoxia was produced in the isolated hearts by perfusing the hearts at a constant flow rate with Krebs–Henseleit buffer equilibrated with a gas mixture of 95 % N_2 and 5 % CO_2. The same flow rate was used in both hypoxic and normoxic perfusion. The ATP-sensitive potassium channel antagonist glibenclamide, 0.77–1.2 μmol l^{-1}, was infused continuously into the perfusion line in order to block the function of these potassium channels. In other experiments, the ATP-sensitive potassium channel agonist Lemakalim, 6.2–9.7 μmol l^{-1}, was infused in order to stimulate opening of these same channels. Lactate dehydrogenase (LDH) release was also quantified spectrophotometrically by the method of Wroblewski and LaDue (1955) during the 30 min of hypoxia followed by 30 min of reoxygenation.

Data are presented as the mean ± 1 standard deviation unless stated otherwise. *N* refers to the number of animals in each group. Statistical analyses were carried out using Sigma Stat (Jandel Scientific, Corte Madera, CA). The principle of conditional error (Netter and Wasserman, 1974) was used to compare statistically the regressions between muskrats

and guinea pigs. In other cases, means were compared using Student's *t*-test, analysis of variance (ANOVA) or by two-way analysis of variance. Significance was achieved if the *P* value was less than 0.05.

All chemicals were purchased from Sigma Chemical Company (St Louis, MO), except Lemakalim (BRL 38227) and pinacidil, which were gifts from SmithKline Beecham Pharmaceuticals (Betchworth, England) and Eli Lilly and Co. (Indianapolis, IN) respectively.

Results

Mean body mass of the muskrats (952±1259 g) and guinea pigs (606±1131 g) differed significantly and muskrat hearts (3.49±10.85 g) were larger than guinea pig hearts (2.61±10.44 g). The coronary flow produced by an aortic perfusion pressure of 8 kPa was greater ($P<0.05$) in muskrats (24.46±17.49 ml min^{-1}) than in guinea pigs (19.0±14.46 ml min^{-1}). Control isolated heart rates were significantly higher in muskrat hearts (240.24±161.15 min^{-1}) than in guinea pig hearts (198.88±124.04 min^{-1}). This heart rate difference between species has been noted previously (McKean, 1986) but has not been explained. *In vivo* heart rates are similar between the two animals (McKean, 1986).

Infusion of 0.1 mol l^{-1} hydrochloric acid into the coronary circulation decreased heart rate and left ventricular pressure in both muskrats and guinea pigs (Fig. 1A,B). Although the muskrat heart was less affected by acid infusion than the guinea pig heart, the difference was not statistically significant for heart rate ($P=0.086$) or for left ventricular pressure ($P=0.059$).

Lactate infusion produced a 20 % decrease in heart rate and a 60 % decrease in left ventricular pressure at the highest lactate concentrations of 80 mmol l^{-1} (Fig. 2A,B). There was no statistically significant difference ($P>0.05$) between the responses of muskrat and guinea pig hearts to the infused lactate.

In contrast, the muskrat heart was more sensitive to potassium ion infusion than the guinea pig heart (Fig. 3A,B). The difference in response between the two species was statistically significant for both heart rate ($P=0.0017$) and ventricular pressure ($P=0.008$). The effect of K^+ infusion on heart rate was not a gradual transition, but was a rapid, step-like decrease in both species.

Lemakalim infusion (10^{-4} to 10^{-6} mol l^{-1}) caused significant changes in heart rate, as shown in Fig. 4A. The heart rate response to Lemakalim showed considerable variability. This is reflected in the r^2 values of the linear regressions, which were 0.253 for the muskrat and 0.330 for the guinea pig. Heart rate in both muskrat and guinea pigs decreased with increasing concentrations of Lemakalim ($P=0.02$ and $P=0.001$, respectively), with a greater effect seen in the muskrat. There was a significant difference between the species ($P<0.05$), but heart rate was only reduced to approximately 65 % in the muskrat and 75 % in the guinea pig at the highest concentrations. One muskrat heart responded dramatically to Lemakalim infusion as heart rate values for the five highest infusion concentrations were all around 50 % of control. With respect to ventricular pressure (Fig. 4B), there was a statistically significant decreasing trend with increasing

concentrations of Lemakalim (P=0.005 for muskrat and P=0.001 for guinea pig). There was no statistical difference between species in the ventricular pressure response. Ventricular pressure values above control values were observed at lower concentrations in some animals; one guinea pig heart showed this trend at the higher concentrations of

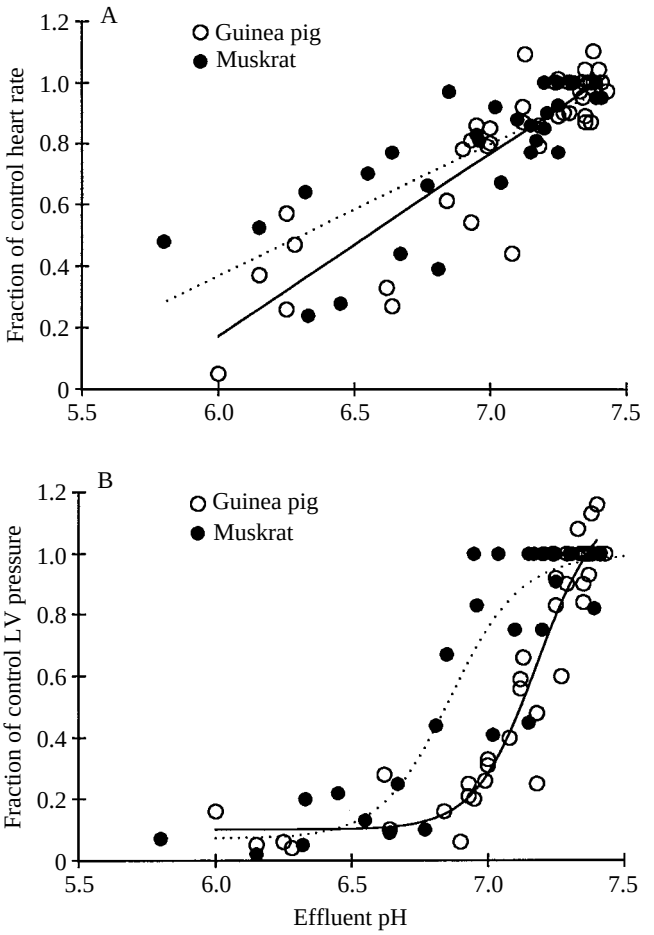

Fig. 1. Heart rate and left ventricular (LV) pressure change in muskrat and guinea pig hearts in response to acid infusion. (A) Heart rate. Dotted line is for muskrat and solid line is for guinea pig. The lines are determined by linear regression analysis and the slopes do not differ (P=0.086). N=6 for guinea pigs and N=5 for muskrats. (B) Left ventricular pressure change during acid infusion. Dotted line is for muskrat and solid line for guinea pig. The lines were drawn by SigmaPlot (Corda Madera, CA). The pooled data were fitted by the logistic equation, $y=(a-d)/[1+(x/c)^b]+d$, where y is heart rate, x is pH and the remaining terms are constants with a as maximum value, b as slope parameter, c as value at inflexion point and d as minimum value. The responses of individual hearts were fitted with the logistic equation using SigmaPlot and the coefficients (a–d) determined. The coefficients obtained in muskrat hearts were then compared with those obtained in guinea pig hearts using Student's t-test or the Mann–Whitney test. The pressure response to acid infusion did not differ between species (P=0.059). N=6 for guinea pig, N=5 for muskrats.

Lemakalim as well. Aortic pressure (Fig. 4C) decreased significantly with increasing concentrations of Lemakalim (P<0.001) in the guinea pig heart but there was no significant relationship in the muskrat (P=0.08). The vehicle, 1 part dimethylsulfoxide (DMSO):50 parts KH buffer, had no effect on the measured variables when infused for a 30 s trial before beginning the experiment.

Pinacidil infusion (approximately 10^{-3} to 10^{-6} mol l^{-1}) caused a decrease (80 % of control at highest concentrations; P<0.05) in heart rate in muskrats with increasing concentrations, but this was not seen with the guinea pig (Fig. 5A). Comparison of the two regression lines showed no statistical difference. Ventricular pressure decreased with increasing concentrations of pinacidil in both the muskrat and the guinea pig (P<0.001 for both), and again there was no statistical difference between the species (Fig. 5B). Aortic pressure also showed the same trend in both guinea pigs and muskrats (P=0.002 and P=0.001 respectively), with no difference in response between the species (Fig. 5C).

Glibenclamide infusion (approximately 1 μmol l^{-1}) during 30 min of hypoxia was not

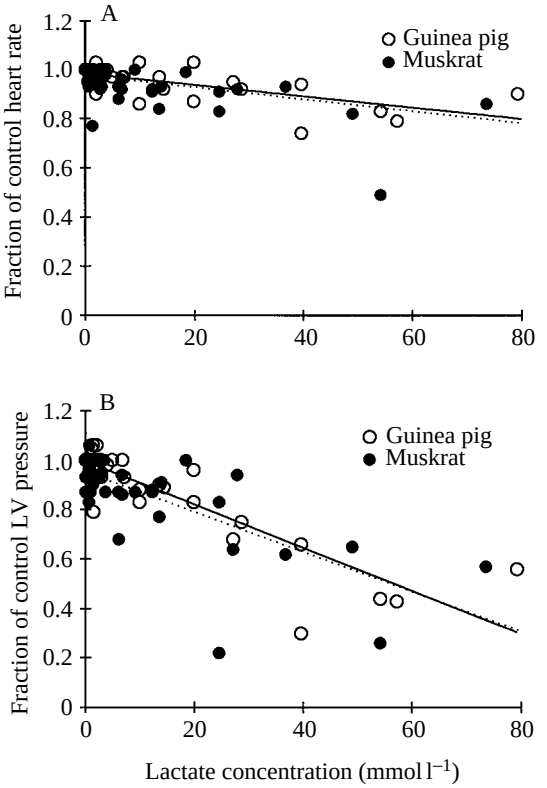

Fig. 2. Change in heart rate and left ventricular (LV) pressure for muskrats and guinea pigs in response to lactate infusion. (A) Heart rate. Dotted line is for muskrats and solid line is for guinea pigs. The lines are generated by linear regression analysis. The slopes are the same (P>0.05); N=5 for both species. (B) Change in left ventricular pressure. Dotted line is for muskrats and solid line is for guinea pigs. The slopes are the same (P>0.05); N=5 for both species.

successful in blocking bradycardia in the muskrat or the guinea pig (P<0.001 and P=0.016 respectively). However, guinea pig heart rate only decreased to 74.5±16 % of the non-hypoxic heart rate (Fig. 6A). There was a significant difference in the species response (P<0.001). Muskrat hearts were somewhat intolerant to glibenclamide infusion during hypoxia as two of five hearts went into contracture after 10 min of hypoxia and failed to recover. Ventricular pressure response during the glibenclamide infusion (Fig. 6B) showed no significant change in either species (P=0.196 for guinea pigs and P=0.601 for muskrats), and there was no species difference in ventricular pressure response. Aortic pressure (Fig. 6C) changed significantly during the 30 min hypoxic period in the presence of glibenclamide, with values rising above control values in both species (P=0.02 for guinea pigs and P=0.03 for muskrats). The vehicle, 1 part DMSO:9 parts KH buffer, was given for 5 min before the experiment and had no effect on the heart.

Results for experiments involving muskrat and guinea pig hearts, where Lemakalim or

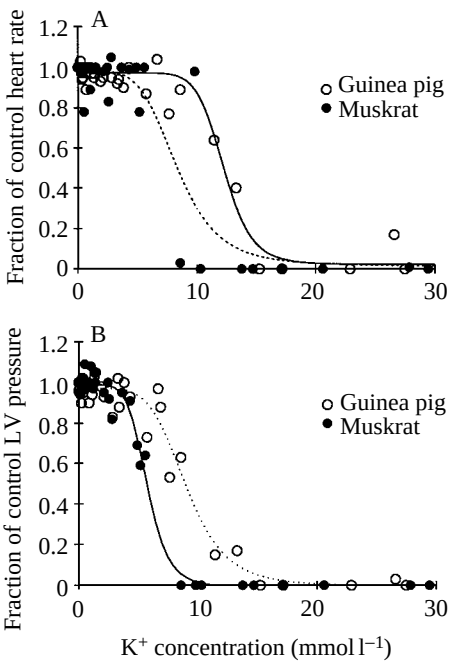

Fig. 3. Change in heart rate and left ventricular (LV) pressure of muskrat and guinea pig hearts in response to potassium ion infusion. (A) Heart rate. Dotted line is for muskrat and solid line is for guinea pig. For statistical purposes the pooled data were fitted by the logistic equation equation, $y=(a-d)/[1+(x/c)^b]+d$, where y is heart rate, x is K^+ concentration and the remaining terms are constants with a as maximum value, b as slope parameter, c as value at inflexion point and d as minimum value. The c value for muskrat hearts is significantly different from the c value for guinea pig hearts (P=0.017). N=5 for guinea pigs and N=4 for muskrats. The responses of individual hearts were fitted with the logistic equation using Sigma Plot and the coefficients ($a-d$) determined. The coefficients obtained from muskrat hearts were then compared with those obtained from guinea pig hearts using Student's t-test or the Mann–Whitney test. (B) Change in left ventricular pressure. The c values differ between species (P=0.008); N=5 for both species.

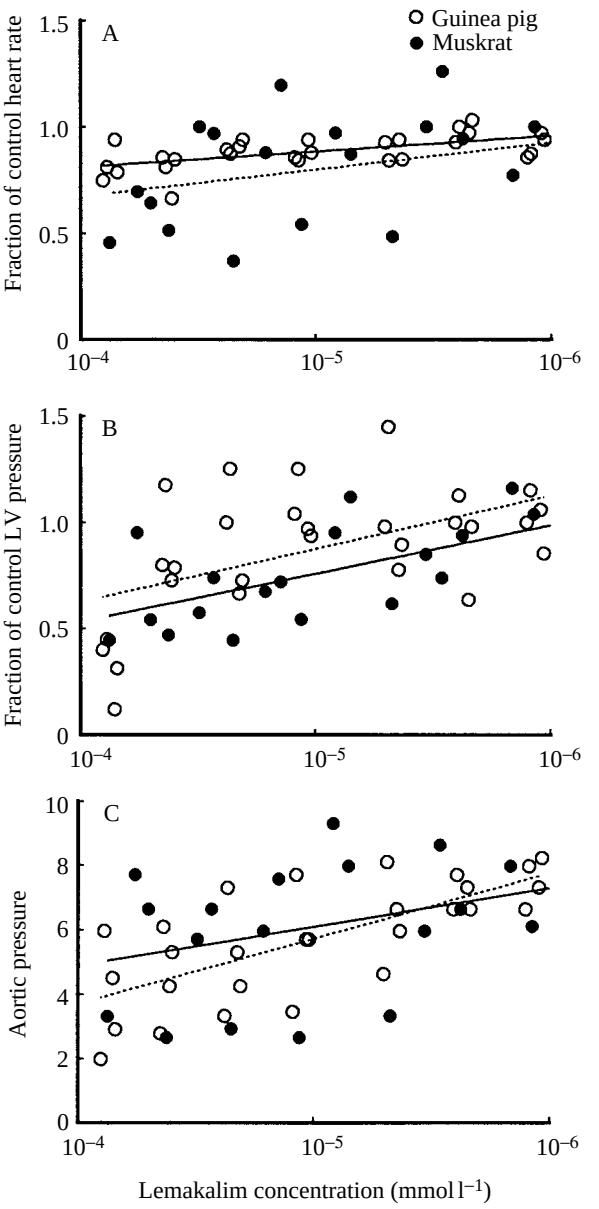

Fig. 4. Change in heart rate, left ventricular (LV) pressure and aortic pressure of muskrat and guinea pig hearts in response to infused Lemakalim. (A) Heart rate. Dotted line is for guinea pig and solid line is for muskrat. The lines are generated by linear regression analysis. Slopes of the lines differ significantly (P<0.05); N=4 for both species. (B) Change in left ventricular pressure. Dotted line is for guinea pig and solid line is for muskrat. The lines are generated by linear regression analysis. Slopes of the lines do not differ (P>0.05); N=4 for both species. (C) Change in aortic pressure. Dotted line is for guinea pig and solid line is for muskrat. The lines are generated by linear regression analysis. No statistically linear relationship was found in the muskrat (P=0.089); N=4 for both species.

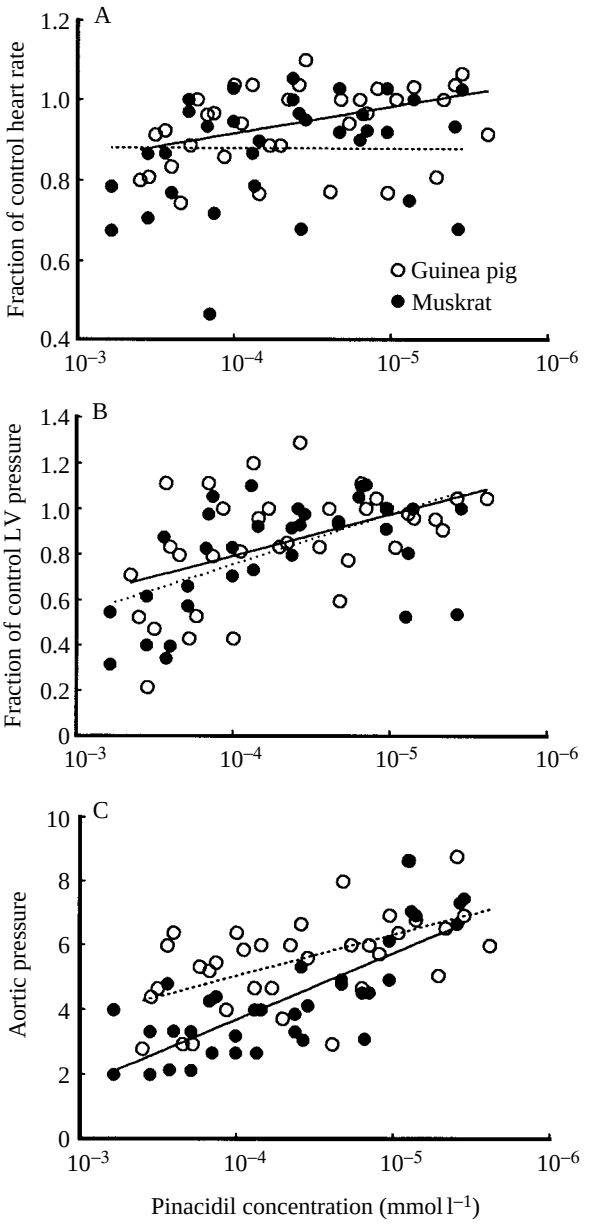

Fig. 5. Change in heart rate, left ventricular (LV) pressure and aortic pressure of muskrat and guinea pig in response to infused pinacidil. (A) Heart rate. Dotted line is for guinea pig and solid line is for muskrat. The lines are generated by linear regression analysis. Slopes of the two lines do not differ (P>0.05); N=5 for both species. (B) Change in left ventricular pressure. Solid line is for guinea pig and dotted line is for muskrat. The lines are generated by linear regression analysis. Slopes of the two lines do not differ (P>0.05); N=5 for both species. (C) Change in aortic pressure. Solid line is for guinea pig and dotted line is for muskrat. The lines are generated by linear regression analysis. Slopes of the two lines do not differ (P>0.05); N=5 for both species.

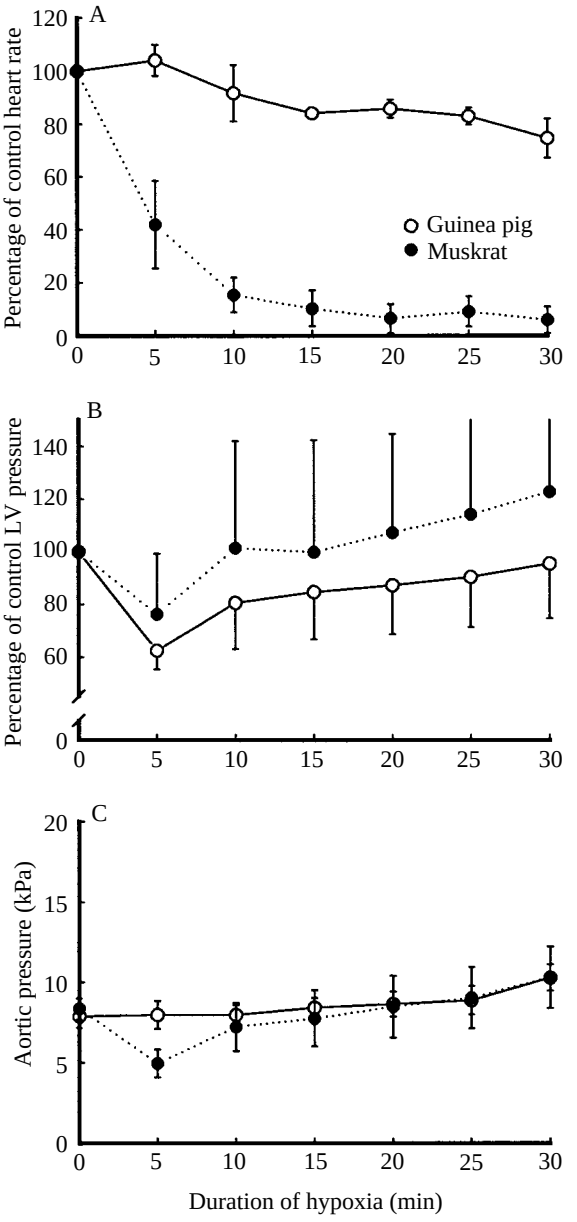

Fig. 6. Changes in heart rate, left ventricular (LV) pressure and aortic pressure for muskrats (●) and guinea pigs (○) during hypoxia with infusion of glibenclamide. Note that heart rate and left ventricular pressure have been normalized so that control values are 100 %. Error bars show S.D. (A) Heart rate decreased during hypoxia in both species (P<0.001 for muskrat and P=0.016 for guinea pig). Analysis of variance indicated that there was a species difference over the 30 min (P<0.001); N=5 for both species. (B) No significant change in ventricular pressure was evident in either species (P=0.196 for guinea pigs and P=0.601 for muskrats); N=5 for both species. (C) Aortic pressure increased significantly for both species (P=0.02 for guinea pigs and P=0.03 for muskrats); N=5 for both species.

ethanol (vehicle) was infused during 30 min of hypoxia, followed by a 30 min recovery period without the drug, are shown in Fig. 7 (muskrat) and Fig. 8 (guinea pig). LDH release, an indicator of heart tissue damage, rose significantly in control and Lemakalim-infused hearts of both species, with greatest values being seen during the recovery period. There were no statistically significant differences between control hearts and Lemakalim-

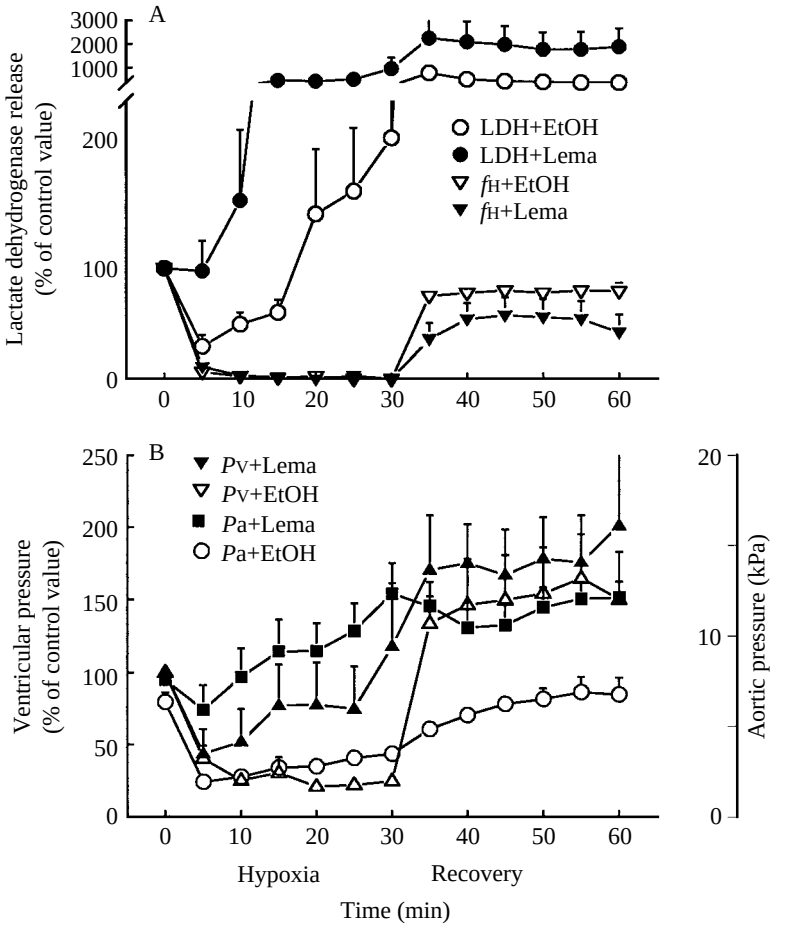

Fig. 7. Change in lactate dehydrogenase (LDH) release and heart rate (f_H) (A) and ventricular (Pv) and aortic pressures (Pa) (B) for muskrats during hypoxia (the first 30 min) and recovery (the second 30 min), with and without ethanol (EtOH) or the infusion of Lemakalim (Lema). Note that all values except aortic pressure have been normalized so that control values are 100 %. Error bars show S.D. (A) LDH release increased significantly for both treatments (P<0.001 for both). Heart rate decreased significantly for both treatments (P<0.001 for both) and returned to 50 % of the control value for Lemakalim and 80 % of the control value for ethanol. N=4 for ethanol and N=7 for Lemakalim. (B) Ventricular pressure increased significantly for both treatments (P<0.001 for both). Aortic pressure changed significantly for both treatments (P<0.001 for both). Aortic pressure differed significantly between treatments (P=0.004). N=5 for both treatments.

infused hearts in either species, nor were there interspecies differences in LDH release (P>0.05 for both).

Heart rate also changed significantly in all of the experiments. Muskrat heart rate dropped to near zero during hypoxia in both control and Lemakalim-infused animals, returning to approximately 50 % of control in the Lemakalim experiments and 80 % of control in ethanol (EtOH) experiments (Fig. 7A). However, there were no significant

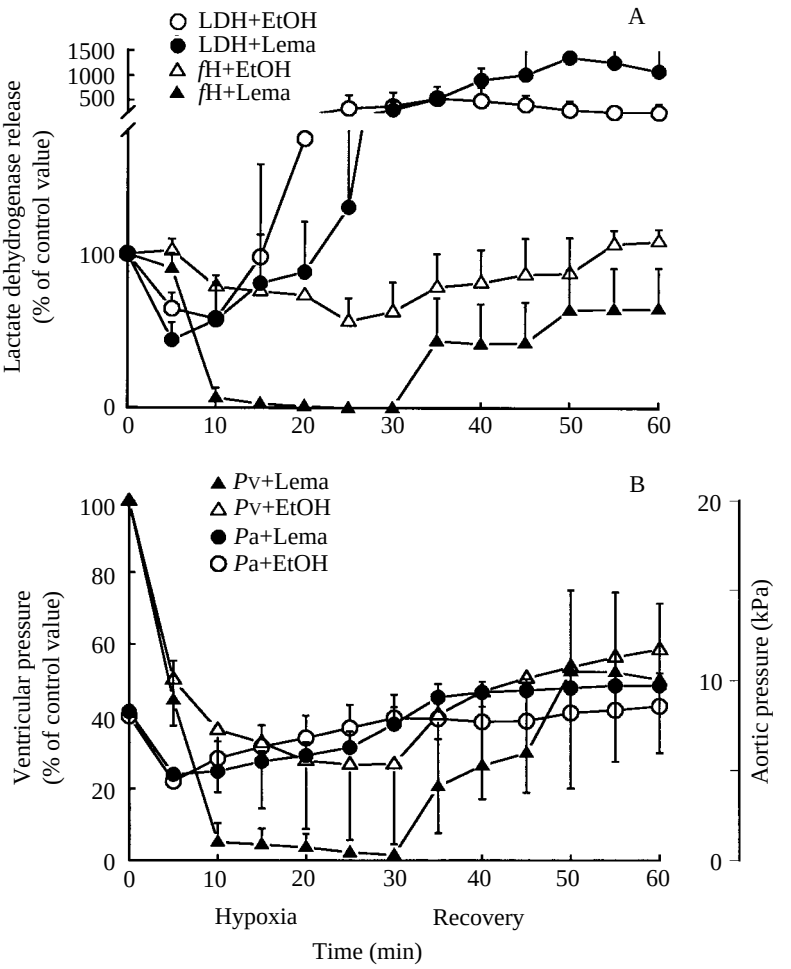

Fig. 8. Changes in lactate dehydrogenase (LDH) release and heart rate (f_H) (A) and left venticular (Pv) and aortic pressure (Pa) (B) for guinea pigs during hypoxia (the first 30 min) and recovery (the second 30 min), with and without ethanol (EtOH) or the infusion of Lemakalim (Lema). Note that all values except aortic pressure have been normalized so that control values are 100 %. Error bars show S.D. (A) LDH release increased significantly for both treatments (P=0.006 for EtOH and P=0.025 for Lemakalim). Heart rate also changed significantly for both treatments (P=0.013 for EtOH and P<0.001 for Lemakalim). Heart rate differed significantly between treatments (P=0.028). N=5 for both treatments. (B) Ventricular pressure decreased significantly for both treatments (P<0.001 for both). Aortic pressure changed significantly for both treatments (P<0.001 for both). N=5 for both treatments.

differences between control and Lemakalim experiments involving muskrats (P=0.211). Guinea pig heart rate dropped to approximately 60 % of the control value in the EtOH experiments and to near zero in the Lemakalim experiments during hypoxia (Fig. 8A). This difference was statistically significant (P=0.028). Full recovery in heart rate was achieved in the EtOH experiments compared with only 64 % recovery in experiments involving Lemakalim, with some animals never recovering from the near-zero heart rates. Comparing heart rate values between muskrats and guinea pigs revealed that the only difference seen in the Lemakalim experiments was at 5 min of hypoxia, when muskrats showed a much lower value than guinea pigs; all other values did not differ. In the EtOH experiments there was a significant difference (P=0.018) between species, since guinea pig heart rate during hypoxia did not decrease as much that in muskrats.

Aortic pressure in control and Lemakalim experiments changed significantly for both species. Muskrat aortic pressure with EtOH dropped by 66 % during hypoxia and returned to control values during recovery whereas, when Lemakalim was administered, aortic pressure rose steadily from 8 to 12 kPa over the course of the experiment (Fig. 7B). This difference between the control and Lemakalim experiment was statistically significant (P=0.004). In guinea pigs, the aortic pressure showed an initial drop during the hypoxic period and returned to control levels later in hypoxia and during recovery in both EtOH and Lemakalim experiments (Fig. 8B). Comparisons between species show that there was a statistical difference when EtOH was infused (P=0.006) but no difference when Lemakalim was infused.

Ventricular pressure also changed significantly during hypoxia and reoxygenation in all animals. In muskrats, when Lemakalim or when the vehicle was infused, ventricular pressure dropped during hypoxia and then rose above control values during the recovery period (Fig. 7B). There was no statistical difference between the two treatments with respect to ventricular pressure. Guinea pig ventricular pressure in both treatments responded similarly to the muskrat, but there was a significant (P<0.001) interspecies difference for both treatments, as ventricular pressure did not return to control values in the guinea pig hearts (Fig. 8B).

Discussion

Isolated muskrat and guinea pig hearts responded to the infusion of protons, lactate and potassium ions with a decreasing heart rate and left ventricular pressure. Species differences were observed in some of the measured variables. A low pH affected guinea pig hearts more than muskrat hearts, and this was most evident in the changes observed in left ventricular pressure at a pH of 6.9, which in guinea pig was only about 10 % of the control level but was 40 % of the control level in muskrats. Differences in heart rate were much less pronounced, although muskrat heart rate was less affected by increasing proton concentration than was guinea pig heart rate. Changes in pH are known to affect the calcium sensitivity of the cardiac muscle contraction (Ruegg, 1987) as well as the kinetics of many biological processes, including energy metabolism (Hochachka, 1980). The differences seen between species in response to changes in pH may be due to differences in one or more of these factors.

The changes in heart rate and left ventricular pressure in response to infused lactate recorded in this study did not differ between muskrat and guinea pig hearts. Furthermore, the response to lactate over the physiological range of up to 18 mmol l^{-1} was relatively modest, with only a 15–20 % decrease in both pressure and heart rate.

The effects of potassium on the hearts of the two species in this study differed; the concentration of potassium needed to produce a 50 % inhibition of heart rate and left ventricular pressure (IC_{50}) was almost 50 % lower in muskrat hearts than in guinea pig hearts. The concentrations of potassium in the perfusate are within the range of the interstitial concentration of potassium encountered in ischemic hearts, but are greater than those encountered in hypoxic hearts (Weiss and Shine, 1982). The interstitial concentration of potassium during potassium infusion in this study is unknown, but is likely to be less than the concentration in the perfusate. The reason for this is that the capillary endothelium represents an incomplete barrier to potassium movement and there is not likely to be complete equilibrium within the 15 s infusion time. The dose–response relationship between heart rate and potassium ion concentration is very steep, especially for the muskrat. This means that small increases in extracellular potassium concentration would produce large changes in heart rate, even to the point of asystole.

Increases in extracellular potassium concentration during metabolic stress are thought to be mediated primarily by the ATP-sensitive potassium channel. Anion-coupled potassium efflux, i.e. with lactate or phosphate, also occurs but is thought to be less important (Weiss *et al.* 1992). The experiments in which the ATP-sensitive potassium channel agonists were infused are somewhat difficult to interpret. ATP-sensitive potassium channels were activated first using pinacidil. Infusion of pinacidil into hearts showed there were no species differences in the effects it had on heart rate, ventricular pressure and aortic pressure, and that even at the highest concentrations of agonist (100 μmol l^{-1}) less than a 20 % inhibition of heart rate was seen. A different agonist, Lemakalim, administered to another group of animals, gave rather different results. Heart rate decreased in both species in response to Lemakalim infusion but the decrease was significantly greater in the muskrat than the guinea pig. Lemakalim was more effective than pinacidil in the inhibition of heart rate for both muskrats and guinea pigs, with almost 40 % inhibition being seen at the highest concentrations (near the 10^{-4} mol l^{-1} level) of agonist tested. Less than 20 % inhibition of heart rate was achieved by this same concentration of pinacidil. One muskrat heart responded with a greater decline in heart rate at the five highest concentrations of infused Lemakalim, but the reason for this augmented response in this animal is not known. Guinea pigs responded similarly and consistently to both ATP-sensitive potassium channel agonists with respect to heart rate but, as can be seen by the data points on Figs 4 and 5, there was considerable scatter in the muskrat heart rate data. This scatter was seen within animals. The reason for inconsistent effects on heart rate between the two drugs is not known. Considerable scatter was also observed in the measurement of ventricular pressure in both species, with some values being observed above control values at lower concentrations of Lemakalim. Again, the reason for this scatter and for the rise in ventricular pressure in response to Lemakalim infusion is unknown.

Inhibiting the action of ATP-sensitive potassium channels was partially successful in

blocking the bradycardia of hypoxia in guinea pigs. When the isolated guinea pig heart is exposed to hypoxia over 30 min, the heart rate decreases to about 60 % of the control level (McKean, 1982). In the presence of glibenclamide, a bradycardia was still present but the decrease was only to 80 % of control heart rate. A rise in aortic pressure was also present in the guinea pig during glibenclamide infusion in hypoxia. Aortic pressure typically drops during hypoxia (McKean, 1982) and these results suggest that the hypoxia-induced vasodilation that is normally seen may be partially mediated by the ATP-sensitive potassium channel. The isolated muskrat heart was quite intolerant to glibenclamide infusion during hypoxia as two of five hearts went into contracture. Bradycardia was seen within the first 5 min of hypoxia, with contracture occurring at about 10 min. This contracture contributed to the rise in left ventricular pressure and aortic pressure that was seen in the muskrat during glibenclamide infusion.

The effect of ATP-sensitive potassium channel agonist administration during hypoxia was to augment the existing response, with only one exception. This was the steady increase in aortic pressure observed during Lemakalim infusion and hypoxia. This result opposes the hypothesis that ATP-sensitive potassium channels may play a role in the vasodilation occurring during hypoxia. This hypothesis resulted from the observation that a rise in aortic pressure was seen in the guinea pig when ATP-sensitive potassium channels were inhibited during hypoxia. If ATP-sensitive potassium channel opening were mediating the response, an increased vasodilation upon activation of these channels would be expected; however, such a result was not seen.

During reoxygenation, left ventricular pressure in muskrat hearts exceeded control values with ethanol and Lemakalim infusion, whereas left ventricular function never recovered in guinea pig hearts. This same phenomenon was observed in an earlier study in which the tension developed by muskrat papillary muscles during recovery exceeded control tension, whereas tension development in rabbit papillary muscle never recovered to the pre-hypoxia value (McKean and Landon, 1982). A possible explanation for these species differences might involve the Na^+/Ca^{2+} exchange mechanism. If, following hypoxia, there was an increase in intracellular sodium ion concentration followed by Na^+/Ca^{2+} exchange, subsequent cardiac contractions would be augmented until such time as intracellular calcium ion levels were restored to control levels.

Although no statistical differences were found, it was also surprising that when Lemakalim was applied, LDH values were higher and heart rate did not recover as much as in hearts treated with ethanol infusion. This suggests that Lemakalim, under these conditions, had a damaging effect on the heart. Other studies have shown a beneficial effect of ATP-sensitive potassium channel agonist administration during ischemia or hypoxia (Auchmpach *et al.* 1991; D'Alonzo *et al.* 1992; Grover *et al.* 1990). Heart rate in isolated guinea pig heart responded dramatically to Lemakalim during hypoxia, as the heart rate dropped to zero or near zero in all animals in the presence of Lemakalim. These results were identical to those from a hypoxic isolated muskrat heart with no drug treatment. The cardiac effects of Lemakalim were more potent during hypoxic perfusion than during normoxic perfusion. This may be explained by an interaction between ATP binding sites and potassium-channel-opener binding sites on the ATP-sensitive potassium channel. The ATP-sensitive potassium channel agonists pinacidil and RP

49356 shifted the half-maximal ATP concentration for ATP-sensitive potassium channel inhibition to higher values (Findlay *et al.* 1989; Thuringer and Escande, 1989). In contrast, intracellular ATP increases the half-maximal concentration of RP 49356 for channel activation (Thuringer and Escande, 1989). These results indicate an interaction between ATP concentrations and ATP-sensitive potassium-channel-opener binding sites and may explain why the dramatic chronotropic effect was only seen during hypoxia, a condition of metabolic stress.

Previous work (McKean *et al.* 1993*b*) examined adenosine as a potential mediator of the hypoxia-induced bradycardia seen in the isolated muskrat heart. In those studies, the hypoxia-induced bradycardia was blocked by adenosine deaminase and 8-sulfophenyl theophylline in the guinea pig, while in the muskrat the bradycardia was only slightly reduced or not affected. Activation of ATP-sensitive potassium channels may be linked to the stimulation of the adenosine A_1 receptor. Activation of this channel, known to be dependent upon a G-protein (Kirsch *et al.* 1990), was accelerated by application of adenosine in whole cell studies. In the present study, hypoxia-induced bradycardia was partially blocked in the guinea pig by infusion of the ATP-sensitive potassium channel antagonist glibenclamide, a result which could be explained by this G-protein coupling between the A_1 receptor and ATP-sensitive potassium channels.

In conclusion, proton and lactate infusion caused no species differences in heart rate. There are, however differences between muskrat and guinea pig hearts in response to infusion of potassium ions, supporting the hypothesis that potassium ions play an important role in isolated heart bradycardia in the muskrat. Although species differences were seen both upon activation of ATP-sensitive potassium channels under normal conditions and during inhibition of their function under hypoxic conditions, these results did not support the hypothesis that bradycardia in the hypoxic isolated muskrat heart is due to an ATP-sensitive potassium channel mechanism. The evidence for this is based primarily on the failure of glibenclamide to block the hypoxic bradycardia in the muskrat. These potassium channels do, however, appear to play an important role in the bradycardia of hypoxia in guinea pigs. Channel blockade by glibenclamide nearly abolishes the hypoxic bradycardia, and channel activation by Lemakalim converts the modest bradycardia of hypoxia in guinea pigs into the extremely low heart rate that is commonly observed in muskrats. ATP-sensitive potassium channels may have an important role in muskrats as well. Blockade of the channels during hypoxia was not well tolerated by the muskrat heart; contracture ensued within 5 min of glibenclamide administration in two of five animals. Administration of ATP-sensitive potassium channel agonists to muskrat hearts during hypoxia also appears to damage the myocardium compared with vehicle administration (Fig. 7A). These results suggest that myocardial damage during hypoxia can be produced by either diminished or augmented ATP-sensitive potassium channel opening. Evidence from radiolabelled glibenclamine binding to cardiac membrane preparations indicates that muskrat hearts have significantly greater numbers of these channels than do guinea pig hearts (McKean *et al.* 1993*a*). The mechanism of isolated muskrat heart bradycardia during hypoxia has yet to be explained but may involve adenosine and ATP-sensitive potassium channels, both of which modulate extracellular potassium concentrations in the heart. Although adenosine and

ATP-sensitive potassium channels may play important roles, the mechanism of bradycardia in the muskrat could involve multiple factors and it cannot be explained entirely by either of these mechanisms.

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