pH$_i$, CONTRACTILITY AND Ca-BALANCE UNDER HYPERCAPNIC ACIDOSIS IN THE MYOCARDIUM OF DIFFERENT VERTEBRATE SPECIES

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SUMMARY

The influence of hypercapnic acidosis upon the heart was examined in four vertebrate species. The CO$_2$ in the tissue bath was increased from 2-7 to 15% at 12 °C for flounder (Platichthys flesus) and cod (Gadus morhua) and from 3 to 13% at 22 °C for turtle (Pseudemys scripta) and rainbow trout (Salmo gairdneri).

During hypercapnia, as previously described, there was a decline and recovery of contractility in heart strips of flounder and turtle, and a sustained decrease in cod and rainbow trout.

At high CO$_2$ the increase in contractile force following increases in the extracellular Ca-concentration were smaller for the cod myocardium than for the other myocardia.

The intracellular pH (pH$_i$), measured with the DMO method, in heart strips of turtle and trout was significantly lower at high than at low CO$_2$. This acidifying effect expressed as the increase in the intracellular concentration of hydrogen ions was larger in the turtle than in the trout myocardium.

Intracellular Ca-activity, measured by efflux of $^{45}$Ca from preloaded heart strips, was unaffected by high CO$_2$ in trout, but was raised in the other three species.

Thus the ability to counteract the negative inotropic effect of hypercapnia is apparently not due to cellular buffering or extrusion of hydrogen ions. More probably it involves (a) a release of intracellular Ca; (b) a positive inotropic effect of an increase in intracellular Ca-activity.

INTRODUCTION

In general, the contractility of the vertebrate heart is depressed by hypercapnic acidosis. This appears to be due mainly to an antagonism between hydrogen ions and the inotropic effect of Ca$^{2+}$ (Fabiato & Fabiato, 1978; Williamson et al. 1976). However, the myocardium of some vertebrates recovers contractility when the hypercapnia has lasted for a few minutes (Foëx & Fordham, 1972; Gesser & Poupa, 1978, 1979; Poupa, Gesser & Johansen, 1977). Evidence was obtained that this recovery involves a release of intracellular Ca-stores making more Ca available to the contractile system (Gesser & Poupa, 1978). In mammalian cardiac cells a large
transient decrease in pH followed by a partial recovery has been observed during hypercapnia (Ellis & Thomas, 1976). Thus, alternative explanations based on a removal of hydrogen ions from the cytoplasm cannot be excluded.

In this paper, these tentative explanations are further examined in heart tissue of flounder, turtle, cod and rainbow trout by measuring the influence of hypercapnia on the pH on the intracellular Ca-activity and on the relationship between the extracellular Ca\(^{2+}\) concentration and the contractility. The myocardia of flounder and turtle, in contrast to the other two, efficiently recover contractility during hypercapnia (Poupa et al. 1977; Gesser & Poupa, 1979).

**MATERIALS AND METHODS**

Heart strips were isolated from flounder (*Pleuronectes flesus* L.), cod (*Gadus morhua* L.), rainbow trout (*Salmo gairdneri* Richardson), and diving turtle (*Pseudemys scripta* L.). Each animal was killed by decapitation. Strips with a diameter less than 1 mm were rapidly prepared from the cardiac ventricle and used for recording of contractile force, pH or \(^{45}\)Ca-efflux. For the cardiac muscle of flounder and cod the physiological solution had a temperature of 12 °C and was composed of 150 mM NaCl, 5:25 mM KCl, 1:80 mM MgSO\(_4\). It was bubbled with a mixture of 85 % O\(_2\) and either 2:7 % CO\(_2\) and 12:3 % N\(_2\) or 15 % CO\(_2\). For rainbow trout and turtle heart the experimental temperature was 22 °C and the solution was composed of 125 mM NaCl, 5:14 mM KCl, 0:94 mM MgSO\(_4\) and bubbled with 87 % O\(_2\) and either 3 or 13 % CO\(_2\) (with 10 % N\(_2\) in the case of 3 % CO\(_2\)). Both solutions contained in addition 30 mM NaHCO\(_3\), 1 mM NaH\(_2\)PO\(_4\), 1 mM CaCl\(_2\) and 5 mM glucose. The gas mixture was delivered by two gas mixing pumps (Wösthoff 1 M-301/a-F) arranged in serial. The pH of both Ringers was 7:6 at low CO\(_2\) and 6:9 at high. The Ca\(^{2+}\) concentration of the bath was increased by adding different volumes of 1 M CaCl\(_2\). Radiochemicals were \(^{45}\)Ca (± 0:5 Ci/mmol) (Danish Atomic Energy Commission, Isotope Laboratory, Riso), \(^{14}\)C]DMO (5:5-dimethyloxazolidine-2:4-dione-2-\(^{14}\)C, 55 μCi/mmol) and \(^{3}\)H]inulin (1:21 Ci/mmol) (Radiochemical Centre Amersham, England).

The contractile force of cardiac strips developed upon electrical stimulation (12 pulses/min) was recorded under isometric conditions as described previously (Gesser & Poupa, 1978). The strips were stretched until no further increase in contractile force was observed, and they were allowed to stabilize for at least 15 min.

The pH\(_i\) of unstretched, resting cardiac strips of turtle and trout was determined from the distribution of DMO between the extracellular and intracellular water (Wadell & Butler, 1959). The strips were kept for 30–60 min at low CO\(_2\) in an unlabelled Ringer. They were then transferred to a Ringer (0:5 ml/strip) containing 0:2 μCi \(^{14}\)C]DMO and 1 μCi \(^{3}\)H]inulin together with 1 mM of the unlabelled forms of DMO and inulin. The Ringer was aerated with either high or low CO\(_2\). To allow the substances to attain steady-state distribution, incubation lasted 30–60 min for turtle and 60–90 min for trout. Thereafter the strips were washed for less than a second in an unlabelled Ringer, blotted with filter paper and cut into two pieces. After weighing, one piece was dried for about 24 h at 110 °C to obtain its total water content. The other was used for radioactivity determinations, after first boiling in 2 ml distilled water for about 45 min. After the addition of 200 μl 50 % trichloric acid (TCA) and centrifugation...
tion, 1 ml of the solution was transferred to a vial for scintillation counting and mixed with 10 ml of a solution obtained by mixing 0.53 l toluene, 0.33 l Triton-x-100, 0.13 l ethanol and 1.33 g Permablend III (Packard). 200 μl of the radioactive incubation solutions were added to 1800 μl water and prepared for radioactivity determinations as above with boiling etc. Radioactivity was measured by scintillation spectrophotometry (Beckman LS 250). The pH was obtained knowing the total tissue water and using inulin as an extracellular marker. The pK₁ of DMO was calculated using the formula given by Malan, Wilson & Reeves (1976).

Intracellular Ca-activity was measured by efflux of ⁴⁶Ca (Ashley, Caldwell & Lowe, 1972; Schatzmann, 1973) in resting, unstretched strips. Each strip was incubated for 2 h in 1 ml Ringer with 4 μCi ⁴⁶Ca/ml. After being washed three times for 20 min in about 15 ml of an unlabelled Ringer, it was transferred through a series of baths of 2 m Ringer (for a total of 10 min) at either high or low CO₂. Finally the strip was digested overnight in 30% H₂O₂ at 110°C. The residual was dissolved in 2 ml H₂O. To this solution and to each of the 2 ml portions of the incubation series 0.2 ml 50% TCA was added. One ml of each solution was transferred to a plastic vial containing 10 ml of the Triton-100/toluene scintillation solution. From measurements of the radioactivity the efflux rate was calculated as the fraction of the ⁴⁶Ca²⁺ contained in the tissue washed out per minute.

The results are presented as mean ± SE, and Student’s t-test was applied to evaluate differences. The level of significance was 0.05.
RESULTS

Contractile force under hypercapnic acidosis

The contractile force developed upon electrical stimulation of myocardial strips decreased rapidly, to around 70% in 5 min, after CO₂ was increased (Fig. 1). Myocardia of cod and trout then continued to lose force, while those from flounder and turtle recovered force. The flounder myocardium achieved a level well above the original.

Contractile force and the extracellular Ca²⁺

At both high and low CO₂, greater force was developed as Ca²⁺ concentrations were raised, especially in the range 1–3 mM, which is probably the physiological range (Prosser, 1973; Ruben & Bennett, 1981). In cod and trout high CO₂ depressed the inotropic effect of Ca²⁺. At 1 mM-Ca²⁺ in flounder and turtle high CO₂ enhanced the force. At higher Ca²⁺ concentrations, high CO₂ depressed the force for flounder but had no significant effect for turtle (Fig. 2).

pH₄

The pH₄ of myocardia from turtle and trout was lower at high than at low CO₂ (Table 1). The intracellular concentration of hydrogen ions rose by \(1.4 \times 10^{-8}\) M for turtle and \(6.4 \times 10^{-8}\) M for trout. Thus, the acidifying effect of the increase in CO₂ was larger for the turtle than for the trout myocardium.
Table 1. $pH_i$, total and extracellular water content given as % of tissue wet weight, and intracellular concentration of hydrogen ions ($[H^+]_{ic}$) calculated as antilog $-pH$, at low and high $CO_2$ for cardiac tissue of turtle and trout

The $pH_i$ of a tissue sample was calculated using the values of total and extracellular water from the same sample. Values are mean ± SE; $n$ = number of animals.

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<th>Tissue</th>
<th>Extracellular</th>
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<tr>
<td>pH$_i$</td>
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</tr>
<tr>
<td>3% CO$_2$</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>13% CO$_2$</td>
<td>13%</td>
<td>13%</td>
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<tr>
<td>Turtle</td>
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<td>6.74±0.06</td>
<td>7.7±0.6</td>
</tr>
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<td>$n = 8$</td>
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<tr>
<td>Trout</td>
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<td>6.99±0.09</td>
<td>4.8±0.4</td>
</tr>
<tr>
<td>$n = 14$</td>
<td>$n = 17$</td>
<td>$n = 14$</td>
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The total and the extracellular water were not affected by the difference in CO$_2$ except that the extracellular water of the trout myocardium was somewhat lower at high CO$_2$.

$^{45}$Ca-efflux

Increase in CO$_2$ resulted in an increased $^{45}$Ca-efflux rate in all myocardia except for that of trout (Fig. 3).

DISCUSSION

As observed earlier the myocardium generally loses force when made hypercapnic. In some myocardia, however, like those of flounder and turtle, contractile force is recovered in spite of a continuing acidosis (Foëx & Fordham, 1972; Gesser & Poupa, 1978, 1979; Poupa et al. 1977).

Mammal myocardial cells have been found to respond to hypercapnia by a sharp decrease in pH$_i$ followed after a few minutes by a partial recovery (Ellis & Thomas, 1976). Fry & Poole-Wilson (1981) used this observation in explaining their finding of a partial recovery of force in the hypercapnic myocardium of guinea pig and rabbit. More generally they suggested that the myocardial contractile force under respiratory or metabolic acidosis mainly varies in parallel with the pH$_i$. The results of the present study, however, suggest that the force recovery under hypercapnia is not solely due to a recovery of pH$_i$.

Hypercapnia induced a lasting acidification of the myocardium, which was more pronounced in the turtle than in the trout. This is compatible with the finding of a lower non-bicarbonate buffer value in the turtle than in the trout myocardium (Damm Hansen & Gesser, 1980). In spite of these differences the turtle myocardium, in contrast to that of trout, recovered force efficiently under hypercapnia.

It has earlier been hypothesized that hypercapnia causes a release of intracellular Ca-stores. This would result in an enlarged pool of Ca$^{2+}$ available for contraction, counteracting the negative inotropic effects of hydrogen ions (Gesser & Poupa, 1978). This hypothesis is supported by the estimated increase in intracellular Ca-activity in
myocardia of flounder and turtle, both of which showed efficient force recovery, and by the lack of change in the trout myocardium, which did not recover.

Observations for the cod heart, however, do not support the above hypothesis. During hypercapnia no recovery of force could be observed in spite of a sharp rise in the estimated concentration of intracellular Ca. Perhaps factors other than the cytoplasmic Ca-activity limit the contractile force developed by the hypercapnic cod myocardium. This is supported by the finding that the positive inotropic effect of an increased extracellular Ca-concentration was much less expressed in the cod myocardium than in the other myocardia under hypercapnia.
At concentrations of extracellular Ca\(^{2+}\) above 3 mM the force of the flounder myocardium attained a maximal value well below those recorded at low CO\(_2\). Thus, above a certain level of force development in this tissue, hypercapnia results in an inhibition of the contractility which cannot be removed by Ca\(^{2+}\). This is in accordance with the previous finding of a more expressed recovery at a low than at a high extracellular Ca-concentration (Gesser & Poupa, 1979). At variance with this situation the force of the turtle myocardium levelled off at similar values at low and high CO\(_2\). In the trout myocardium no levelling off of force could be observed within the Ca-range tested.

The hypothesis that the recovery of force under hypercapnia is due to an increased intracellular Ca-activity must, however, be viewed cautiously. Hess & Weingardt (1980) found a simultaneous decrease in both Ca-activity and pH\(_i\) in sheep Purkinje cells during 5 min of hypercapnia. It should, however, be recalled that the recovery of force can only be observed for hypercapnic periods lasting more than about 5 min. A release of let us say mitochondrial Ca (Gesser & Poupa, 1978) may be a relatively slow process.

To conclude, the variation in ability to maintain myocardial force under hypercapnic acidosis does not appear to be due to a varying ability to regulate intracellular pH. Rather it appears to depend on a varying release of intracellular Ca\(^{2+}\)-stores combined with a varying inotropic response to increases in the cytoplasmic Ca-activity.

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REFERENCES


