THE EFFECTS OF HYPOXIA AND REOXYGENATION ON FORCE DEVELOPMENT IN MYOCARDIA OF CARP AND RAINBOW TROUT: PROTECTIVE EFFECTS OF CO₂/HCO₃⁻.

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SUMMARY

Isometrically mounted and electrically paced myocardial ventricular strips of carp have a much higher capacity to develop force during severe hypoxia and to redevelop force after it than those of rainbow trout. When the concentrations of CO₂ and HCO₃⁻ in the solutions surrounding the strips were increased together, such that pH remained constant, the force developed during hypoxia increased. The concentration of CO₂ was raised from 0.4%; that of HCO₃⁻ from 0.25 mM. The effect was much more pronounced in the carp strips than in the trout strips.

With the carp strips, the force recovery upon reoxygenation was unaffected by the variations in CO₂ and HCO₃⁻. The trout strips, however, recovered better when CO₂ and HCO₃⁻ had been raised during either hypoxia or reoxygenation.

INTRODUCTION

The concentrations of H⁺, CO₂ and HCO₃⁻ are interrelated variables in the cellular environment. Of these concentrations only that of H⁺ proved to be of functional importance in having a negative inotropic effect in a study of an isolated heart preparation supplied with oxygen (Vaughan Williams & Whyte, 1967). During hypoxia the concentrations of CO₂ and HCO₃⁻ may be important in determining myocardial contractility. In this situation the cellular production of lactic acid and thus of H⁺ ions increases, and recently, CO₂ and HCO₃⁻ have been suggested to be constituents of mechanisms which increase the outflow of H⁺ ions from the cell (Boron & de Weer, 1976; Mainwood & Worsley-Brown, 1975; Strome, Clancy & Gonzalez, 1976). An intracellular acidosis is associated with a depressed anaerobic energy liberation (Ui, 1966) and probably also with a decreased functional capacity of the contractile elements themselves (Katz & Hecht, 1969; Williamson et al. 1976).

The present study concerns the influence of extracellular CO₂ and HCO₃⁻ on the contractility of isolated heart strips during severe hypoxia. The concentrations of CO₂ and HCO₃⁻ were increased together, such that pH remained constant. Myocardia of carp and rainbow trout were selected for two reasons. Firstly, the myo-
cardium of carp, in contrast to that of trout, was found in preliminary experiments have a high capacity to develop force during hypoxia. This capacity is correlated with the capacity for anaerobic energy liberation as shown in a study of hearts from marine fishes (Gesser & Poupa, 1974) and from turtles (Reeves, 1963). Secondly, the carp, a fish lives in ponds and swamps, probably experiences hypoxia together with an increase in the environmental total concentrations of CO$_2$ (Carter, 1934), and so may be expected to show metabolic adaptations to these conditions.

**MATERIAL AND METHODS**

Adult carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) of both sexes were used. They were kept in aquaria with filtered recirculating water at 15–20 °C. The Ringer solution used for the heart muscle preparations consisted of: 152 mM-Na$^+$, 2.93 mM-Ca$^{2+}$, 5.14 mM-K$^+$, 0.94 mM-Mg$^{2+}$, 2.00 mM-PO$_4^{3-}$, 0.94 mM-SO$_4^{2-}$, 5 mM Tris-HCl, 5 mM glucose, and Cl$^-$ and HCO$_3^-$ in varying concentrations. The sum of the Cl$^-$ and HCO$_3^-$ concentrations was always 161 mM, and the maximum HCO$_3^-$ concentration was 25 mM. The Ringer solution was equilibrated with different gas mixtures delivered from a gas mixing pump (Wösthoff type 1 M 301/a-F). When CO$_2$ and HCO$_3^-$ were used, the ratio of their concentrations was kept constant at 1% CO$_2$:6-25 mM-HCO$_3^-$ or at 4% CO$_2$:25 mM-HCO$_3^-$.

The Tris-HCl buffer stabilized the pH between 7.48-7.52 at 20 °C. The pH was continuously measured in the muscle bath with a glass electrode connected to a pH meter (Radiometer type PHM 71) allowing readings of 1/100 of a pH unit.

Each experimental fish was decapitated. The heart was rapidly excised and placed in Ringer solution containing 25 mM-HCO$_3^-$ equilibrated with 96% O$_2$ and 4% CO$_2$. A myocardial strip preparation, taken from a similar site on each ventricle, was mounted for isometric force recording in a bath containing 100 ml Ringer solution and equilibrated with oxygen and CO$_2$. The temperature of the bath was thermostatically controlled (Lauda type K2) at 20 °C. One end of the strip was firmly fixed with a plastic clip. The other end was tied with surgical silk to a force displacement transducer (Grass FT 03), connected to a recorder (Brush Mark 220). The strip was electrically paced at 12 beats/min with square pulses of 5 ms duration by a stimulator (Grass SD9) through two parallel platinum electrodes, one on each side of the strip. The voltage was about 20% above the minimum necessary to give a maximal mechanical response. Spontaneously beating strips were discarded.

After 15 min at a low applied stretch the strip was stretched to the apex of the curve of contractile force to length. After an additional period of 15–30 min of stabilization, hypoxia was induced by replacing O$_2$ with N$_2$ in the gas mixture. During the stabilization period 4% and 25 mM, or 1% and 6-25 mM, of CO$_2$ and HCO$_3^-$, respectively, was present in the Ringer solution. In some of the experiments these concentrations were left unchanged. In others, about 30 s before onset of hypoxia CO$_2$ was removed from the gas mixture while simultaneously the Ringer solution was exchanged for one without HCO$_3^-$.

After 30 min of hypoxia the prehypoxic conditions of O$_2$, CO$_2$ and HCO$_3^-$ were restored. All solutions were pre-equilibrated with the appropriate gas mixtures.

The force developed during hypoxia and reoxygenation was calculated as
and myocardial hypoxia

Percentage of that developed after stabilization just before the onset of hypoxia. Some strips developed force irregularly with successive contractions during hypoxia. For these strips the force value given represents a mean of the value obtained upon four stimulations. Before concluding an experiment the length of the strip at the apex of the length–contractile force curve was measured. The strip was then blotted and weighed. The cross sectional area was calculated assuming cylindrical form and a specific gravity of 1.00. Absolute force values in mN/mm² could therefore be calculated. Data are presented as mean ± S.E. Differences were analyzed with Student’s t test. The level of significance was set at 5%.

RESULTS

After stabilization in well oxygenated conditions the forces developed by the strip preparations were 6.87 ± 0.78 mN/mm² (n = 21) for carp and 7.16 ± 0.69 mN/mm² (n = 25) for trout. The total concentration of CO₂ and HCO₃⁻ in the Ringer during the stabilization period had no effect on the force developed. The cross sectional area of the strips was 1.02 ± 0.43 mm² for carp and 1.10 ± 0.40 mm² for trout.

Fig. 1 shows that the force development of the myocardium of both carp and trout decreased during 30 min hypoxia. In the carp, this decrease was strongly affected by the muscle bath concentrations of CO₂ and HCO₃⁻. In the presence of 4% CO₂ and 25 mM-HCO₃⁻ the force levelled off at the end of the hypoxic period at about 80% of the pre-hypoxic value. When 1% CO₂ and 6.25 mM-HCO₃⁻ were used the force was still declining at the end of hypoxia, at which time it had reached a value of 34% of the original. In the absence of CO₂ and HCO₃⁻ the force decrease was even more accentuated, and the final value reached was 10% of the pre-hypoxic force. In the trout the decrease was less affected by CO₂ and HCO₃⁻. In their absence, or in the presence of 1% CO₂ and 6.25 mM-HCO₃⁻, there was a decrease to about 10% after 30 min hypoxia. After hypoxia in the presence of 4% CO₂ and 25 mM-HCO₃⁻, there was a decrease to about 30% of the prehypoxic value. For both species there was a tendency for the myocardium to develop force irregularity with successive contractions when CO₂ and HCO₃⁻ were absent.

Fig. 2 shows recovery of force development during re-oxygenation for 30 min in the presence of 1% CO₂ and 6.25 mM-HCO₃⁻; the 30 min of hypoxia having either been in the absence of CO₂ and HCO₃⁻ or in the presence of 1% CO₂ and 6.25 mM-HCO₃⁻. The trout myocardium recovered more rapidly and to a greater extent after CO₂ and HCO₃⁻ had been present in the bath than after the bath had been CO₂ and HCO₃⁻ free. The carp myocardium recovered without showing a dependence on the CO₂ and HCO₃⁻ concentration of the bath during hypoxia.

Fig. 3 illustrates the recovery of force after 30 min of hypoxia during which CO₂ and HCO₃⁻ had been absent from the Ringer solution. The trout myocardium recovered more force if the reoxygenation occurred in the presence of 4% CO₂ and 25 mM-HCO₃⁻ than in the presence of 1% CO₂ and 6.25 mM-HCO₃⁻. The force recovery of the carp heart was similar in the two conditions. It can be seen from Figs. 2 and 3 that the carp myocardium recovers force more rapidly and to a greater extent than the trout myocardium.
Fig. 1. Force development (mean ± S.E.) during hypoxia in different concentrations of carbon dioxide and bicarbonate: ○, 4% CO₂; ■, 3.1% CO₂ + 10 mm-HCO₃⁻; △, 3.1% CO₂ + 23 mm-HCO₃⁻. The fractions indicate the proportion of preparations in which force was developed irregularly with successive contractions.
Fig. 2. Force recovery (mean ± S.E.) in the presence of 1% CO₂ and 6.25 mm-HCO₃⁻, after 30 min of hypoxia in either 1% CO₂, 6.25 mm-HCO₃⁻ (●—●) or in absence of these substances (○—○). Fractions indicate as for Fig. 1.
Fig. 3. Force recovery (mean ± s.e.) in either 4% CO₂, 25 mm-HCO₃⁻ (●—●) or 1% CO₂, 6.25 mm-HCO₃⁻ (○—○) after 30 min of hypoxia in the absence of CO₁ and HCO₅⁻. Fractions indicate as for Fig. 1.
In some experiments the period of recovery was extended to 90 min. The trout heart, however, never reached the initial force level of 100%. It was probably irreversibly damaged during hypoxia.

DISCUSSION

Myocardial force development during hypoxia was kept at a much higher level in the carp than in the trout. There was also a more rapid and complete recovery in the carp. This presumably reflects the adaptation of the carp to waters in which oxygen availability is frequently very low, and the lack of such adaptation in the trout. The cause of the overshoot in contractile force seen initially during recovery for carp heart (Fig. 2) is not known, but is being studied in this laboratory.

During hypoxia the carp myocardium would presumably profit more than the trout myocardium from factors counteracting an intracellular acidosis, because a high hypoxic force development is thought to be associated with a high rate of formation of lactic acid and hence of hydrogen ions (Gesser & Poupa, 1974; Reeves, 1963). An excess of these ions inside the myocardial cell has been claimed to depress the glycolytic capacity (Ui, 1966), and the uptake of Ca$^{2+}$ by the cell (Williamson et al. 1976). Furthermore, an intracellular acidosis is alleged to inhibit in a competitive way the binding of Ca$^{2+}$ to troponin (Katz & Hecht, 1969).

Counteraction of intracellular acidosis may be expected to be produced by an increase in extracellular HCO$_3^-$ concentration. Such an increase is likely to raise the outflow of undissociated lactic acid from muscle cells (Mainwood & Worsley-Brown, 1975), and, furthermore, CO$_2$ and HCO$_3^-$ seem to take part in a mechanism equivalent in effect to an active transport of H$^+$ ions out from the cell (Boron & de Weer, 1976; Strome et al. 1976). It may be, therefore, that the effect of a raised CO$_2$ and HCO$_3^-$ concentration upon the carp myocardium during hypoxia, to reduce the depression of force development, may be explained by a counteraction of intracellular acidosis.

During the introductory stabilization period, CO$_2$ and HCO$_3^-$ levels in the bath were either 4% and 25 mM or 1% and 6-25 mM. This difference did not affect the force values obtained before the onset of hypoxia. Neither did it affect the force developed during hypoxia, when CO$_2$ and HCO$_3^-$ had been removed. Thus, the contractility during hypoxia seems to be a function of the absolute levels of CO$_2$ and HCO$_3^-$ and not of the extent of change in these parameters.

The cause of the greater effect of hypoxia upon the trout than the carp myocardium, and the means by which CO$_2$ and HCO$_3^-$ aid recovery in the trout is a subject inviting further studies.

In interpreting the results it must be remembered that a complete absence of CO$_2$ and HCO$_3^-$ in the extracellular fluid is alien to an in vivo situation. 1% CO$_2$ and 6-25 mM HCO$_3^-$ are within the range found in fish arterial blood while 4% and 25 mM align to values typical for terrestrial vertebrates (Howell, 1970). The values in the immediate vicinity of the fish myocardium are hard to estimate. The heart muscle cells in fish are to a great extent nourished by blood returning from central systemic veins, likely to be higher in CO$_2$, HCO$_3^-$ and H$^+$-ions than the arterial blood leaving the gills.
It is of interest that for the carp a decrease in ambient oxygen availability might be concomitant with a sharp increase in CO$_2$ and HCO$_3^-$ in the surrounding water (Carter, 1934). Such an increase will cause elevated concentrations of the same substances in the blood (Janssen & Randall, 1975) and hence may counteract the effects of hypoxia. Also, in habitual divers, oxygen lack is combined with a raise in CO$_2$ and HCO$_3^-$ in the blood due to the interrupted gas exchange with the environment (Scholander, 1940).

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REFERENCES