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RELATION BETWEEN NON-BICARBONATE BUFFER VALUE AND TOLERANCE TO CELLULAR ACIDOSIS: A COMPARATIVE STUDY OF MYOCARDIAL TISSUE

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SUMMARY

There is a large variation in the tolerance of myocardial tissue to cellular acidosis. Assuming the cytoplasmic acid-base status to be mainly a result of intracellular processes, this variation could be produced by variations in the tissue non-bicarbonate buffer value. In the myocardial tissue from nine vertebrate species, the non-bicarbonate buffer value did not correlate either with ability to develop tension under hypercapnic acidosis or with the indirectly estimated capacity for anaerobic glycolysis. Therefore, differences in myocardial tolerance to acidosis must be explained either by an active pH regulation or by other compensatory mechanisms.

INTRODUCTION

Intracellular acidosis decreases the ability of the myocardial cell to develop force (Williamson *et al.* 1975). An intracellular acidosis may occur due to an increase in p_{CO_2} or in the production of non-volatile acids, such as lactic acid, or both.

Under uniform extracellular conditions isolated myocardial strips from different vertebrate species show large differences in ability to work under hypercapnic acidosis (Poupa & Johansen, 1975; Gesser & Poupa, 1978). There are many possible causes for this variation. One may be a difference in the ability to remove an intracellular acid load. This could occur by an active extrusion of H^+ ions across the cell membrane or by a mechanism with equivalent effect, such as an uptake of bicarbonate. Such possibilities are strongly supported by recent evidence, although their functional importance is not clear (Strome, Clancy & Gonzales, 1976; Ellis & Thomas, 1976). The possibility still remains that the cytoplasmic acid-base status is mainly determined by mechanisms other than exchange processes across the cell membrane. Thus the cellular non-bicarbonate buffer value (β_a) could be of crucial importance in decreasing the effect of an acid load on the intracellular pH (Burton, 1978).

To get an idea of its functional importance, this parameter was compared with tolerance to hypercapnic acidosis and with both anaerobic and aerobic capacity in the cardiac ventricular tissue of nine species of vertebrates.

MATERIALS AND METHODS

The following species were studied: flounder (*Pleuronectes flesus* L.), cod (*Gadus morhua* L.), rainbow trout (*Salmo gairdneri* Richardson), carp (*Cyprinus carpio* L.), mud eel (*Synbranchus marmoratus* Bloch), clawed frog (*Xenopus laevis* Daudin), diving turtle (*Pseudemys scripta* L.), caiman (*Caiman crocodilus* Daudin) and albino rat (*Rattus norvegicus* Berkenh.).

Each animal was killed by a blow on the head. Its cardiac ventricle was rapidly isolated and subjected to different procedures.

The tissue non-bicarbonate buffer value (β_a) was determined essentially according to Heisler & Piiper (1971). The ventricle was rinsed, blotted dry, weighed and put in a solution containing 140 mM-KCl, 1·8 mM-$MgSO_4$, 1·0 mM-NaH_2PO_4, 4·0 mM-NaCl, 30 mM-$KHCO_3$ and 5·0 mM-Na succinate using 4 ml solution/g tissue. This solution was composed with the purpose to make it similar to the cytoplasmic fluid. Succinate was included as a mitochondrial substrate. The tissue was homogenized in a Potter Elvehjem glass Teflon homogenizer with 6 strokes of 15 s duration interspersed with 10 s periods. The temperature during these preparative steps was 0–4 °C. Two ml of the homogenate were transferred to shaking glass tonometers (Eschweiler, Kiel) incubated in a waterbath at 15 °C, unless otherwise stipulated. The homogenate was equilibrated with gases delivered by a gas mixing pump (Wösthoff, type 100). The mixtures were successively equilibrated to 1, 12 and 22% CO_2 in O_2 mixtures that were water-saturated. The partial pressure of CO_2 (p_{CO_2}) was obtained by multiplying respective percentage with the pressure of dry air, i.e. the pressure of air minus the partial pressure of water at saturation. The exposure to each p_{CO_2} lasted 35 min and the pH in the homogenate was measured after 30 and 35 min. The homogenates were considered to be equilibrated when the two pH values did not differ by more than 0·005 units. The pH electrode (Radiometer G K 2321 C) was connected to a pH meter (PHM-72) and pH was recorded on a servograph (Radiometer (REC 51) with a pH meter interface unit (REA-100)). Each pH measurement lasted 3 min during which time the tonometer was switched off. The concentrations of HCO_3^- ($c_{HCO_3^-}$) at different p_{CO_2}'s were calculated by the Henderson–Hasselbach equation. In the experimental pH range 7·8–6·8 the concentrations of free hydrogen ions and carbonate are negligibly small so that the non-bicarbonate buffer value of the homogenate is given by $\Delta c HCO_3^-/\Delta pH$. The constants used in the calculations of $c_{HCO_3^-}$ were $pK_a = 6{\cdot}222$, $\alpha_{CO_2} = 0{\cdot}0538$ mM/torr at 15 °C; $pK_a = 6{\cdot}173$, $\alpha_{CO_2} = 0{\cdot}0404$ mM/torr at 25 °C; and $pK_a = 6{\cdot}124$, $\alpha_{CO_2} = 0{\cdot}0319$ mM/torr at 35 °C. These values are given by Severinghaus (1965) for human plasma.

To get the tissue non-bicarbonate buffer value the buffer value of the phosphates in the homogenization medium must be subtracted. The latter value was calculated using a pK_a of 6·68 for $H_2PO_4^-$. No correction for its temperature dependence was made. The influence of succinate ($pK_a = 5{\cdot}64$) was neglected as it maximally amounted to 5% of the non-bicarbonate buffer value of the homogenate. The water content of the hearts was determined by weighing tissue samples before and after drying at 110 °C for 24 h. The calculation of β_a in m-equiv/pH.kg wet weight was done according to Lykkeboe & Johansen (1975).

For *in vitro* determination of enzyme activities per gram tissue, a crude tissue

homogenate was made in 0·1 M sodium phosphate pH 7·4 using an Ultraturrax homogenizer. To estimate the anaerobic glycolytic capacity of the tissue the activity of pyruvate kinase (μmol/min) was measured according to Bücher & Pfleiderer (1955). In a study of different tissues from rabbit these two parameters were shown to be closely correlated (Simon & Robin, 1972). Furthermore a correlation was demonstrated in fish myocardial tissue between the pyruvate kinase activity and the ability to work under anaerobic conditions (Gesser & Poupa, 1974). The activity of cytochrome oxidase determined as the first order rate constant (min^{-1}) with respect to reduced cytochrome was determined according to Cooperstein & Lazarow (1951) in homogenates made 1% with desoxycholate. This activity was taken as an estimate of the tissue aerobic capacity since several studies (e.g. Simon & Robin, 1971) have shown these two parameters to be closely correlated.

To estimate the hypercapnic tolerance of the tissue, the development of contractile force was recorded under isometric conditions in isolated ventricular strips, or for rat papillary muscles, as described previously (Gesser & Poupa, 1978). The preparations were stimulated at a frequency of 12/min and stretched to produce maximal active force. A 'Ringer' for seawater vertebrates was used for flounder and cod (Gesser & Poupa, 1974). while one for freshwater living species was used for the other animals (Gesser & Poupa, 1978). The concentration of Ca^{2+} and HCO_3^- were 1 and 30 mM, respectively. The solutions were equilibrated with a gas containing 2·7% CO_2, 85% O_2 and 12·3% N_2. After stabilization of force development, CO_2 was increased to 15%, while N_2 was removed. The percentage of force left after 30 min at high CO_2 was taken as an estimate of the hypercapnic tolerance.

Each β_a value given represents one homogenate, which, however, often was obtained from several hearts to get a sufficient volume. This is in contrast to the other parameters, where each value represents one heart. Values are given as mean ± S.D. The degree of co-variation is estimated by the product moment correlation coefficient. The level of significance is set to 5%.

RESULTS

p_{CO_2} was equilibrated within 30 min in every measurement. β_a determined in the interval 1–12% CO_2 was not significantly different from that determined between 12 and 22% CO_2. The mean value of these two β_a values, the tissue water content, the activity of cytochrome oxidase and pyruvate kinase and the tolerance to hypercapnic acidosis for the nine vertebrate species are given in Table 1. β_a varies between 58 m-equiv/pH.kg for cod and 28 m-equiv/pH.kg for carp. Except for the water content a wide variation was also found for the other parameters. Cytochrome oxidase was 1445 ml/min.g for rat and 88 ml/min.g for mud eel. Pyruvate kinase was 121 μmol/min.g for carp and 57 μmol/min.g for mud eel, and the hypercapnic tolerance varied from 125% for flounder to 38% for carp.

To what extent does tolerance to acidosis, or to capacity for cellular lactic acid production, covary with β_a? A comparison of force development under hypercapnia with β_a did not reveal any significant correlation ($r = -0{\cdot}33$). Neither did the tissue capacity for anaerobic glycolysis, estimated by the correlation of the activity of pyruvate kinase with β_a ($r = -0{\cdot}11$). During the measurement of β_a, a positive

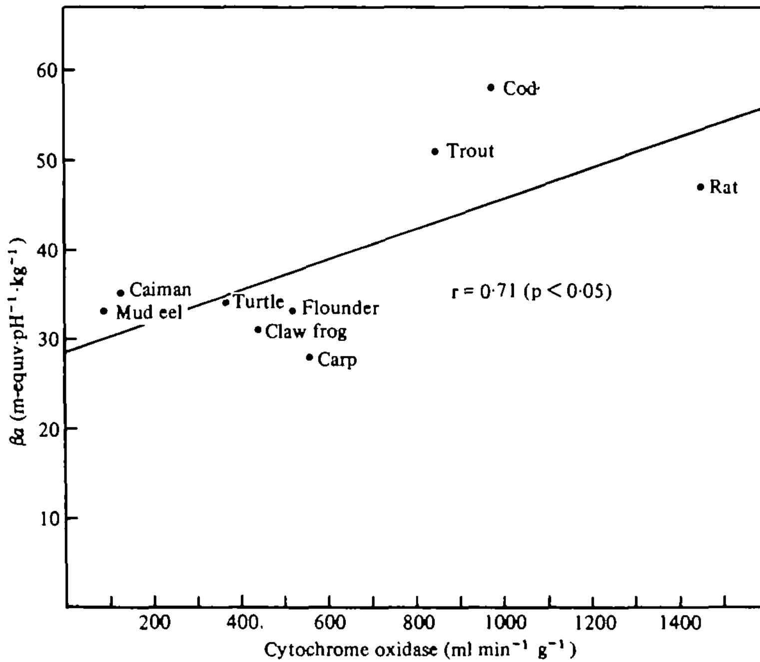

Fig. 1. Relation of β_a (non-bicarbonate buffer value) to activity of cytochrome oxidase for nine vertebrate species.

relationship between respiratory capacity and β_a was indicated. During the time when the pH electrode was inserted in the homogenate, a small drop in pH could be detected and the extent of this seemed to be larger the higher β_a. This drop in pH is probably caused by stoppage of the tonometer during the pH measurement resulting in an impaired gas exchange and an accumulation of metabolically formed CO_2 in the aqueous phase.

Therefore the aerobic capacity of the tissue as estimated by the activity of cytochrome oxidase was compared with β_a (Fig. 1). The correlation ($r = 0{\cdot}71$) between the two parameters is significant ($P < 0{\cdot}05$).

During the measurement of β_a, which lasts for at least 90 min, the homogenate is probably metabolically active. Such an activity may conceivably affect the β_a value. For instance mitochondria are able to accumulate Ca^{2+} ions in exchange for H^+ ions (Bartley & Amoore, 1958), and metabolic processes may result in a production or consumption of acid substances (Siesjø, 1973). To investigate the importance of such metabolic processes different interventions were made. β_a was determined for five flounder and five cod myocardial homogenates under anaerobic conditions obtained by exchanging O_2 for N_2 in the gas mixture. The values obtained were for flounder $28{\cdot}0 \pm 1{\cdot}8$ m-equiv/pH.kg and for cod $56{\cdot}7 \pm 2{\cdot}7$ m-equiv/pH.kg. These values do not differ significantly from those obtained under aerobic conditions (Table 1).

One of two flounder cardiac homogenates was exposed to anaerobic and the other to aerobic conditions. Both were made 0·5 mM with respect to iodoacetate, a substance which in this concentration is known to inhibit glycolysis. The β_a values

Table 1

The non-bicarbonate buffer value (β_a), m-equiv/pH.kg wet weight, water content, % of tissue wet weight, cytochrome oxidase activity (CO), ml/min.g, pyruvate kinase activity (PK), μmol substrate/min.g, and hypercapnic tolerance, % force, left after 30 min at 15% CO_2, for cardiac tissue of nine vertebrate species. Values are mean ± S.D. For the β_a values *n* signifies number of homogenates. For the other parameters it signifies number of animals.

Species	β_a	Water content	CO	PK	Hypercapnic tolerance
Cod	57·9 ± 6·7 *n* = 14	82·3 ± 1·1 *n* = 4	974 ± 230 *n* = 3	77 ± 14 *n* = 3	43 ± 13 *n* = 3
Trout	50·9 ± 6·4 *n* = 16	80·2 ± 0·2 *n* = 3	846 ± 138 *n* = 3	94 ± 3 *n* = 3	71 ± 12 *n* = 5
Rat	47·1 ± 2·9 *n* = 16	78·8 ± 1·8 *n* = 3	1445 ± 223 *n* = 3	66 ± 9 *n* = 3	92 ± 6 *n* = 3
Caiman	34·5 ± 0·5 *n* = 2	83·4 *n* = 1	136 *n* = 1	67 *n* = 1	100 *n* = 1
Mud eel	33·3 ± 1·1 *n* = 2	79·8 *n* = 1	88 *n* = 1	57 *n* = 1	64 *n* = 1
Turtle	33·6 ± 4·0 *n* = 10	82·1 ± 1·7 *n* = 3	366 *n* = 1	63 *n* = 1	80 *n* = 1
Flounder	33·0 ± 6·1 *n* = 14	81·4 ± 1·2 *n* = 3	520 ± 32 *n* = 2	103 ± 29 *n* = 2	125 ± 12 *n* = 7
Clawed frog	31·0 ± 5·6 *n* = 6	81·9 ± 0·3 *n* = 2	440 ± 64 *n* = 2	61 ± 11 *n* = 2	124 *n* = 2
Carp	27·8 ± 18 *n* = 8	78·9 ± 1·6 *n* = 3	556 ± 41 *n* = 2	121 ± 13 *n* = 2	38 *n* = 2

obtained were 27·0 and 25·1 m-equiv/pH.kg, respectively. Thus the measured β_a do not seem to be affected by any production of lactic acid.

The influence of the mitochondrial Ca^{2+} metabolism was examined by taking two samples from each of three flounder myocardial homogenates. In each case one sample was equilibrated with 1% CO_2 and made 4 mM with $CaCl_2$ while the other served as control. The β_a values obtained were 30·9 ± 1·5 m-equiv/pH.kg with added Ca^{2+} and 32·7 ± 6·8 m-equiv/pH.kg without. In another approach, β_a was determined in two samples of each of two flounder cardiac homogenates containing 4 mM-$CaCl_2$. One of each sample pair was made 0·01 mM with Ruthenium red. This substance is an inhibitor of the mitochondrial Ca^{2+} uptake (Ash & Bygrave, 1977; Crompton, Capano & Carafoli, 1976). In the absence of Ruthenium red, the β_a values were 30·9 and 31·2 m-equiv/pH.kg; in its presence they were 32·3 and 32·6 m-equiv/pH.kg. Thus no effect of the mitochondrial Ca^{2+} metabolism could be demonstrated.

In the present study β_a was measured in an homogenate prepared in a solution high in K^+ and low in Na^+, thus simulating intracellular conditions. In earlier studies (Heisler & Piiper, 1971; Lykkeboe & Johansen, 1975) an 'extracellular' solution, i.e. one high in Na^+ and low in K^+, was used. This difference may be of importance, since the concentration of Na^+ may affect metabolism (e.g. Crompton *et al.* 1976). β_a was therefore measured in five myocardial homogenates of flounder prepared in an 'extracellular' solution, where KCl was decreased from 140 to 5 mM, while NaCl was increased from 4 to 138 mM and $KHCO_3$ was exchanged for $NaHCO_3$.

β_a obtained was 27·6 ± 2·2 m-equiv/pH.kg and thus not significantly different from that obtained in an 'intracellular' solution (Table 1).

The temperature dependence of active as compared to passive processes are normally much higher. β_a of rat myocardium was determined at 15, 25 and 35 °C with three homogenates at each temperature. The values obtained were 45·3 ± 1·6, 48·5 ± 2·5 and 46·1 ± 3·8 m-equiv/pH.kg, respectively, revealing absence of significant temperature-dependence.

DISCUSSION

Various methods have been employed for determinations of buffer values in different animal tissues (review by Burton, 1978). Using CO_2 titration of homogenates Lai, Atterbery & Brown (1973) reported a value of β_a = 33 m-equiv/pH.kg in rat ventricular tissue, while Ellis & Thomas (1976) obtained a value of 76·6 m-equiv/pH.kg.l intracellular water in the same tissue, using an intracellular pH electrode in cells titrated with CO_2. This value, if recalculated according to Burton (1976), amounts to about 50 m-equiv/pH.kg. The value of 47 m-equiv/pH.kg found in the present study is within this range, but substantially higher than the value obtained by Lai *et al.* (1973).

The experiments with different interventions in metabolic processes indicate that estimates of β_a are not affected by these particular processes.

The lack of correlation between β_a and hypercapnic tolerance shows that a variation in this tolerance in hearts from different species must be explained by mechanisms other than a variation in the passive non-bicarbonate buffering value.

Lykkeboe & Johansen (1975) found a very high buffer value in the chicken breast muscle, 75 m-equiv/pH.kg, and they suggested that it might be related to an ability of this poorly perfused tissue to perform work depending on anaerobic metabolism. As many cellular processes are inhibited by acidosis, a cell with a high β_a would conceivably, during intermittent heavy exercise or oxygen lack, be able to tolerate a higher intracellular lactic acid concentration than a cell with a lower β_a. In the present study, however, the buffer value did not show any correlation with the pyruvate kinase activity. This activity has been stated to give a good estimate of the tissue capacity for anaerobic glycolysis (Simon & Robin, 1972). In addition the pyruvate kinase activity is correlated with the cardiac anaerobic work performance (Gesser & Poupa, 1974). These findings indicate that the non-bicarbonate buffer value is of no or very little importance for the capacity to maintain a high rate of anaerobic glycolysis.

The absence of a correlation between non-bicarbonate buffer value and either hypercapnic tolerance or capacity for anaerobic glycolysis, indicates that the myocardial cell does not act as a passive acid-base system. Various investigators have observed changes in pH of cardiac tissues, which they only could account for by assuming a transport of H^+ or HCO_3^- across the cardiac cell membrane (Lai *et al.* 1973; Ellis & Thomas, 1976; Strome *et al.* 1976). Differences in the ability for active regulation of cell pH may conceivably explain the species differences concerning (1) hypercapnic tolerance and (2) the ability to maintain high rates of acid production under hypoxia.

As to other possibilities, there is evidence (Gesser & Poupa, 1978) that Ca^{2+} is released from intracellular stores during hypercapnic acidosis. As H^+ is believed to compete with Ca^{2+} for sites on the contractile proteins (Williamson *et al.* 1975), such a release of Ca^{2+} would decrease the inhibitory effect of H^+ on contractility.

In the present study the observed relation between β_a and the activity of cytochrome oxidase suggests that the non-bicarbonate buffer value is associated with the aerobic capacity of the tissue. Inducing anoxia in the homogenate of both the 'low' aerobic flounder and the 'high' aerobic cod did not, however, influence β_a. This indicates that β_a is not directly related to respiratory activity. It may rather be that a high capacity for aerobic metabolism is positively associated with the concentration of some substances, which increases the non-bicarbonate buffer value. The present study could be extended to investigate whether the buffer substances are localized in the mitochondria, the sarcoplasmic reticulum or the cytosol.

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