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The Interaction* between K Depletion and CO_2 Inhalation on Intracellular Bicarbonate Content (33028)

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In recent work from our laboratory (2) the plasma pCO_2 of control and K-deficient rats was altered between 20 and 90 mm Hg with a respirator and the intracellular pH (pH_i) of skeletal muscle measured by 5,5-dimethyl-2,-4-oxazolidinedione (DMO) distribution. Intracellular bicarbonate content ($[HCO_3^-]_i$ at the various pressures of CO_2 was calculated by substitution of pH_i values into the Henderson–Hasselbalch equation. The $[HCO_3^-]_i$ of low-K muscle (together with pH_i) was found generally to be lower than that of normal muscle.

An alternative method of obtaining $[HCO_3^-]_i$ is by derivation from the total CO_2 content of muscle. The CO_2-derived $[HCO_3^-]_i$ values for potassium deficient muscle have been reported from several laboratories. The data of one report (3) suggest that low-K $[HCO_3^-]_i$ is reduced to less than half the $[HCO_3^-]_i$ of normal muscle. This marked reduction is at variance with the slight reduction reported from another laboratory (4). More recently, Hudson and Relman (5), using a modified CO_2 method, found no significant change in the $[HCO_3^-]_i$ of muscle from potassium depleted rats. In view of these differences, it was decided to determine the CO_2-derived $[HCO_3^-]_i$ of our low-K rat muscle, the object being to see if the $[HCO_3^-]_i$ values so obtained would agree with, or at least parallel, values calculated from DMO determined pH_i.

Our recent work (2) showed also that the reduction in low-K $[HCO_3^-]_i$ (calculated from DMO derived pH_i) was not constant at all levels of pCO_2. As pCO_2 was increased, less HCO_3^- was generated in low-K than in normal muscle. The different rate of bicarbonate increase caused the slope representing low-K muscle in a pH–bicarbonate diagram [see Fig. 2 in Ref. (2)] to be significantly less than the slope representing control muscle. It was concluded from this slope difference that the *in vivo* buffer capacity of low-K muscle is less than that of normal muscle. The present experiments were designed to determine whether a similar, unequal intracellular bicarbonate increase would occur when deriving $[HCO_3^-]_i$ from tissue CO_2 content.

Methods. The present investigation consisted of four series of experiments with control or low-K male rats (Wistar strain) weighing 280–300 gm. The control and low-K regimens previously described (6) were

* The term interaction is used here in its statistical meaning. That is, to indicate the additivity (or lack of it) of the effect of two stresses, K depletion and CO_2 inhalation, on extracellular and intracellular bicarbonate content. See Snedecor (1) for discussion of the factorial experimental design.

used to prepare rats for experimentation. In the first series, control rats were exsanguinated immediately after being placed under sodium pentobarbital (50 mg/kg of body wt.) anesthesia. In the second series, control rats were placed in a chamber and exposed to a gas mixture containing 10% CO_2 and 90% air for 2 hours prior to exsanguination. The gas mixture was metered so that the atmosphere in the chamber would change every 8 min. A specially constructed head mask made it possible to continue the administration of the CO_2–air mixture after a rat was removed from the chamber for exsanguination. In the third and fourth series of experiments, the above protocol was repeated using rats that had been on the low-K regimen for 35 days. The procedures for exsanguination by direct cardiac puncture and the handling of freshly drawn blood have been described previously (2,6). In all four series, each experiment was performed singly and replicated five times.

Exsanguination required approximately 60 sec. Immediately afterwards, a sample of gastrocnemius muscle from each leg was transferred to a tared digestion tube containing ferric fluoride solution. With practice it was possible to excise and transfer a sample (0.8–1.2 gm) in 40 sec. These dulpicate muscle samples were analyzed without delay for their CO_2 contents using a manometric method (7). The values obtained agreed closely with those reported by Thompson and Brown (8) who killed rats by decapitation. Other gastrocnemius muscle samples were trimmed of fat and connective tissue in a humidor. The dry, fat-free solids remaining after determination of muscle water and lipid content were finely ground. The Cl content of weighed samples of ground, dried muscle was determined as described by Eichelberger and Bibler (9). Blood pH and plasma CO_2, Cl, and H_2O contents were determined as in a previous study (6).

The $[HCO_3^-]_i$ of skeletal muscle was calculated by the method of Wallace and Hastings (10). With the data obtained it was possible also to estimate pH_i based on the cellular contents of HCO_3^- and carbonic acid (10). A recent paper by Butler *et al.*

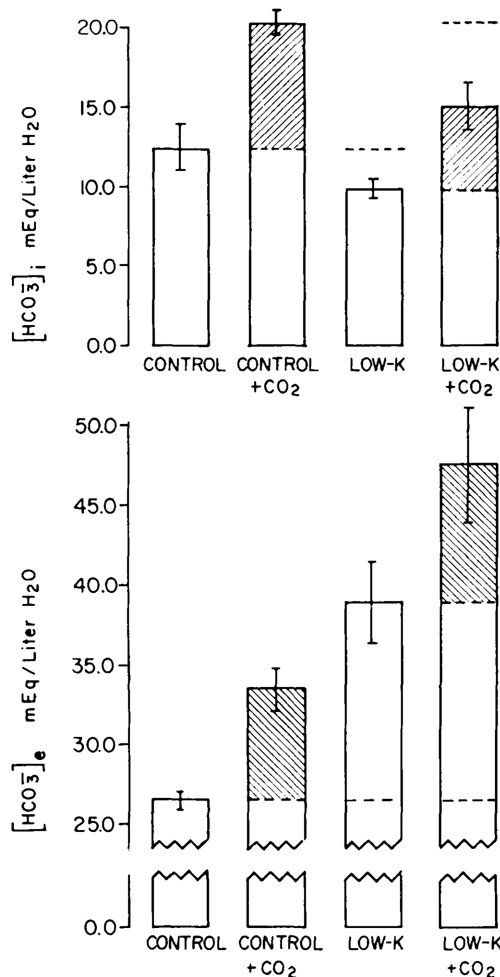

FIG. 1. The effect of CO_2 inhalation and K depletion on extracellular and intracellular bicarbonate content. (lower) The additivity of the effects of CO_2 inhalation and K depletion on *extracellular* bicarbonate content when these two stresses were applied together. (upper) The *lack* of additivity (the interaction) of the two stresses on *intracellular* bicarbonate content. See text for further discussion.

(11) provides new evidence that calculation of intracellular pH from acid-labile CO_2 appears valid.

Results. In this work two stresses, K deprivation and CO_2 inhalation, were applied singly and together. By this procedure it was possible to compare the HCO_3^- increment due to CO_2 inhalation in muscle and extracellular fluid of control and low-K rats. The shaded areas on the bars in Fig. 1 represent these HCO_3^- increments.

The lower portion of Fig. 1 shows the average extracellular HCO_3^- concentration ($[HCO_3^-]_e$) of control and stressed rats. The $[HCO_3^-]_e$ of control rats inhaling CO_2 increased by 7.1 meq/liter. This increase reflects the extracellular respiratory acidosis resulting from inhalation of CO_2. The $[HCO_3^-]_e$ increase of low-K rats reflected the extracellular alkalosis accompanying potassium deficiency. When K-deficient rats inhaled the CO_2–air mixture there was a further increase in $[HCO_3^-]_e$. This increase (8.7 meq/liter) was essentially the same as the HCO_3^- increment observed when control rats inhaled CO_2. Statistical analysis indicated there was no real difference in the effects of CO_2 inhalation on $[HCO_3^-]_e$ when (i) acting alone, and (ii) when superimposed on K deficiency; that is, the interaction between the two main effects (K deprivation and CO_2 inhalation) was not significant $F = 0.49$; significance at the 5% level of probability requires an F value > 4.75).

The upper portion of Fig. 1 shows the *intracellular* HCO_3^- changes associated with the applied stresses. The distance between the top of the "low-K" bar and the broken line above it represents the amount by which the $[HCO_3^-]_i$ of low-K rats (breathing room air) was lower (2.6 meq/liter) than the $[HCO_3^-]_i$ of control rats (also breathing room air). The mean $[HCO_3^-]_i$ values for the control and low-K groups were found to be significantly ($p < .01$) different. The distance between the top of the "low-K + CO_2" bar and the broken line above it represents the $[HCO_3^-]_i$ decrease in low-K rats inhaling CO_2 (5.6 meq/liter). The mean values for the control + CO_2 and the low-K + CO_2 groups were also found to be significantly ($p < .001$) different.

The $[HCO_3^-]_i$ of low-K animals inhaling CO_2 increased 5.2 meq/liter. This HCO_3^- increment was considerably less than the increment (8.1 meq/liter) resulting from Co_2 inhalation by control rats. Analysis of variance showed there was significant interaction ($F = 9.20$) of the two main effects on intracellular HCO_3^-. In other words, a real difference existed between the effects of CO_2 inhalation (i) when acting alone, and (ii) when in the presence of K-deficiency. The unequal shaded areas on the bars representing $[HCO_3^-]_i$ show this.

The data presented in the top part of Table I indicate that control rats breathing CO_2 (Series II) exhibited extracellular respiratory acidosis and that the low-K rats breathing room air (Series III) had a typical hypochloremic extracellular metabolic alkalosis. There was practically no difference in the extent to which plasma pH (pH_p) was elevated by K deficiency in rats breathing normally (Series I vs Series III) and in rats inhaling the CO_2–air mixture (Series II vs Series IV). Similarly, the reduction of pH_p due to CO_2 inhalation was essentially the same in control and low-K rats. Results similar to these were reported and discussed previously (2).

The muscle pH values listed in the lower part of Table I show that, intracellularly, there was a different pattern of response to CO_2 inhalation. Control muscle pH was reduced 0.13 by CO_2 inhalation; but the reduction was greater (0.17) when low-K rats inhaled the same CO_2–air mixture for the same period of time.

The values at the bottom of Table I show that both control and low-K muscle combined considerable CO_2 (Series II and IV); but the amount combined by low-K muscle was less ($p < .05$). This is not an unexpected result in view of the abundant evidence (6, 12,13) that low-K muscle is more acid than normal muscle.

Discussion. In the present work the CO_2-derived $[HCO_3^-]_i$ of low-K muscle was 81% of the value obtained for control muscle. The $[HCO_3^-]_i$ values obtained by calculation from the DMO pH_i data previously published (6) for our control and low-K muscle were 8.9 and 7.2 meq/kg of cell H_2O, respectively. Both these values are lower than the corresponding CO_2-derived values[1] for $[HCO_3^-]_i$ in Table I. However-

[1] Similarly, in a study (14) in which there was direct comparison in the same muscle preparation of intracellular pH values calculated from distribution

TABLE I. Average[a] Plasma and Muscle Acid–Base Data.

Series Treatment	I Control	II Control + CO_2	III Low-K	IV Low-K + CO_2
		Plasma		
pCO_2 (mm Hg)	39.2 ± 3.6	87.2 ± 4.8	42.9 ± 3.6	92.1 ± 4.7
CO_2 (m*M*)	25.4 ± 0.3	32.9 ± 1.1	36.3 ± 2.2	45.5 ± 3.1
Cl (meq/liter)	99.4 ± 2.9	99.8 ± 1.9	89.5 ± 1.9	83.7 ± 4.4
$[H^+]$ (10^{-5} meq[b])	3.88 ± 0.13	6.89 ± 0.11	2.90 ± 0.18	5.26 ± 0.12
pH[c]	7.41	7.16	7.54	7.28
		Muscle		
pH_i[c]	7.06	6.93	6.91	6.74
$[H^+]$ (10^{-5} meq[d])	8.8 ± 1.2	11.8 ± 0.7	12.2 ± 1.2	18.1 ± 2.6
$[HCO_3^-]_i$ (meq[e])	12.5 ± 1.4	20.6 ± 0.9	9.9 ± 0.6	15.0 ± 1.6
CO_2 (mmol[f])	12.2 ± 0.7	19.4 ± 0.7	12.8 ± 0.9	17.3 ± 1.6

[a] Average of 5 experiments ± SD.
[b] Per liter of plasma.
[c] Because pH is a logarithmic function, individual values were converted to hydrogen ion concentration. Mean values for hydrogen ion concentration were then reconverted to pH units.
[d] Per liter of intracellular fluid.
[e] Per kilogram of cell water.
[f] Per kilogram of fresh tissue.

er, on a percentage basis, the reduction in $[HCO_3^-]_i$ as determined by either method was almost identical.

We have attempted to reconcile the 20% reduction in $[HCO_3^-]_i$ observed in our K-deficient rats (depleted 35 days) with the much more marked reduction, 55%, reported by Gardner *et al.* (3) whose rats were depleted 40 days. Table II shows differences between some of their data and ours. These differences may appear to be subtle; but they do in fact account for the greater reduction in $[HCO_3^-]_i$ reported by them. This may be demonstrated by recalculating their data substituting our low-K plasma CO_2, plasma pH and muscle CO_2 values singly and then in all possible combinations. A relatively small reduction in the total CO_2 content of low-K muscle results in a comparatively large reduction in $[HCO_3^-]_i$ all other things being equal. In the present experiments and in the other work cited, except that of Gardner *et al.* (3), the differences between the muscle CO_2 contents of control and low-K rats were no greater than 0.6 mmol/kg of fresh tissue.

That Eckel *et al.* (4) found no significant $[HCO_3^-]_i$ reduction in rats depleted of K for 8 (Series I) or 17 (Series II) days is in accord with our recent study relating pH_i to chronicity of K depletion (6). After 14 days on a low-K regimen our rats exhibited no change in pH_i and $[HCO_3^-]_i$ despite a 1/3 reduction in muscle K content. However, rats on the low-K regimen for 35 days did exhibit significantly reduced pH_i and $[HCO_3^-]_i$. It appears likely that a significant reduction in $[HCO_3^-]_i$ would have been obtained by Eckel *et al.* had their Series I and II animals been depleted a longer period.

of CO_2 and that of DMO, the DMO pH_i values were lower.

TABLE II. A Comparison of Previously Published Acid–Base Data for Low-K Rats with Those Obtained in This Study.

	Gardner *et al.* (3)	Δ[a]
Plasma CO_2 (m*M*)	42.1	+5.8
Plasma pH	7.49	—0.05
Muscle CO_2 (mmol/kg[b])	10.8	—2.0

[a] This column shows the extent to which the data of Gardner *et al.* (3) are greater or less than the corresponding data listed in Table I. These differences, operating in concert, account for the much greater reduction in $[HCO_3^-]_i$ obtained in their work.
[b] Per kilogram of cell water.

The data of Hudson and Relman (5) showing a reduced pH_i during K-deficiency that was ***not*** accompanied by a significant $[HCO_3^-]_i$ reduction are at variance with this work. We can offer no explanation for the different results.

The parallel reduction in CO_2-derived $[HCO_3^-]_i$ and DMO pH_i-derived $[HCO_3^-]_i$ during K deficiency provides further evidence that elevation of pCO_2 in compensation for extracellular alkalosis does not play a significant role in reducing the pH_i of K-depleted muscle as has been suggested (15). If there were significant diffusion of CO_2 into the K-depleted cell, $[HCO_3^-]_i$ would be elevated rather than reduced. Proof that $[HCO_3^-]_i$ is reduced when intracellular acidosis accompanies K deficiency is provided not only by our parallel results from two independent methods, but also by recently published works (2,11) in which the pH_i of normal and low-K rats was determined at pressures of pCO_2 ranging up to 100 and 150 mm Hg. In both reports low-K pH_i was consistently lower than control pH_i at any given pCO_2 (within the range studied). This constitutes further evidence that the acidity of low-K cells must be primarily of metabolic (rather than of respiratory) origin.

The smaller rise in $[HCO_3^-]_i$ and the greater pH_i fall in low-K rats inhaling CO_2 bears out our earlier finding (2), based on an entirely different protocol, that the K-depleted cell is less able to buffer acidity produced by CO_2 inhalation. The reason(s) for the reduced buffer capacity of acidotic low-K muscle is not clear. However, the significantly reduced nonbicarbonate buffer capacity demonstrated previously (2), together with the intracellular HCO_3^- data reported here, strongly suggests that the acidotic low-K cell is defending against H^+ ions whose origin is metabolic. Recent work (6) showed that transfer of H^+ into the cell from the extracellular compartment is not the cause of the intracellular acidity associated with K depletion. The alternative explanations are that during K deficiency there may be diminished extrusion of H^+ ions (13), increased production of H^+ ions or a combination of both.

Summary. Intracellular bicarbonate content ($[HCO_3^-]_i$) of rat skeletal muscle was derived from CO_2 distribution in animals subjected to CO_2 inhalation and K deficiency applied singly and together. The objectives were to determine (i) whether or not $[HCO_3^-]_i$ would be reduced in acidotic, low-K muscle, and (ii) whether the $[HCO_3^-]_i$ increase due to CO_2 inhalation would be the same in normal and K-deficient animals. There was a 20% reduction in the CO_2-derived $[HCO_3^-]_i$ of low-K muscle. A 20% reduction was also obtained when deriving $[HCO_3^-]_i$ from DMO pH_i. The $[HCO_3^-]_i$ increment due to CO_2 inhalation was sufficiently less in low-K muscle to yield, upon analysis of variance, a statistical interaction between the two stresses applied. This interaction was indicative of less buffering by low-K muscle. The data strongly suggest that the acidotic, low-K cell is defending against H^+ whose origin is metabolic.

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