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NADH/NAD redox state of cytoplasmic glycolytic compartments in vascular smooth muscle

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Barron, John T., Liping Gu, and Joseph E. Parrillo. NADH/NAD redox state of cytoplasmic glycolytic compartments in vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 279: H2872–H2878, 2000.—The cytoplasmic NADH/NAD redox potential affects energy metabolism and contractile reactivity of vascular smooth muscle. NADH/NAD redox state in the cytosol is predominately determined by glycolysis, which in smooth muscle is separated into two functionally independent cytoplasmic compartments, one of which fuels the activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$. We examined the effect of varying the glycolytic compartments on cytosolic NADH/NAD redox state. Inhibition of $\text{Na}^+\text{-K}^+\text{-ATPase}$ by 10 μM ouabain resulted in decreased glycolysis and lactate production. Despite this, intracellular concentrations of the glycolytic metabolite redox couples of lactate/pyruvate and glycerol-3-phosphate/dihydroxyacetone phosphate (thus NADH/NAD) and the cytoplasmic redox state were unchanged. The constant concentration of the metabolite redox couples and redox potential was attributed to 1) decreased efflux of lactate and pyruvate due to decreased activity of monocarboxylate B-H^+ transporter secondary to decreased availability of H^+ for cotransport and 2) increased uptake of lactate (and perhaps pyruvate) from the extracellular space, probably mediated by the monocarboxylate- H^+ transporter, which was specifically linked to reduced activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$. We concluded that redox potentials of the two glycolytic compartments of the cytosol maintain equilibrium and that the cytoplasmic NADH/NAD redox potential remains constant in the steady state despite varying glycolytic flux in the cytosolic compartment for $\text{Na}^+\text{-K}^+\text{-ATPase}$.

$\text{Na}^+\text{-K}^+$ ATPase; lactic acid; metabolism; energetics; lactate transporter

THE CYTOPLASMIC NADH/NAD REDOX POTENTIAL has been shown to exert important effects on both energy metabolism and contractile reactivity of vascular smooth muscle (5, 6). Because NAD(H) serves as a cofactor for several enzymes, the NADH/NAD ratio in the cytosol governs the activity of several enzymatic reactions of intermediary metabolism. Furthermore, as an electrochemical potential, the cytoplasmic NADH/NAD redox potential affects the electrochemical gradient across the inner mitochondrial membrane because the mitochondrial transmembrane potential is essentially a

function of the difference of redox potentials between the intramitochondrial and cytoplasmic compartments (6, 28). As such, the cytoplasmic redox potential can exert important effects on mitochondrial oxidative metabolism (6). Therefore, it is of interest to examine the factors that determine the redox potential and state of NADH/NAD in the cytoplasm.

The NADH/NAD ratio and redox potential of the cytoplasm of vascular smooth muscle cells is predominately governed by the disposition of the cytosolic concentration ratios of the major end products of glycolysis, lactate and pyruvate, which are reductant and oxidant metabolites, respectively (5, 6, 19). The lactate/pyruvate redox couple is in equilibrium with the NADH/NAD redox couple as defined by the following relationship: $\text{lactate} + \text{NAD} \rightleftharpoons \text{pyruvate} + \text{NADH}$. These redox couples, in turn, are in equilibrium with other cytosolic metabolite redox couples, among which is the glycerol-3-phosphate/dihydroxyacetone phosphate couple. The interconversion of the respective reductant and oxidant species of this and other redox pairs is catalyzed by specific enzyme dehydrogenases and also requires NAD(H). Therefore, the concentration ratios of lactate/pyruvate and NADH/NAD, and thus the “poise” of the cytoplasmic redox potential, are a function of the relative rates of formation and removal of lactate and pyruvate from the cytoplasm and of the cytosolic pH. It has been shown under several conditions that alteration in glycolytic flux produces corresponding changes in the redox state of the cytoplasm (4–7). However, glycolytic flux in the cytoplasm of vascular smooth muscle cells is functionally compartmentalized (9, 11, 15, 20). That is, there are two glycolytic pathways with separate sets of glycolytic pathway enzymes (see Fig. 1). The glycolytic enzymes of one pathway have been shown to be associated with the plasma membrane, and the operation of this pathway provides the energy needed to drive membrane ion transport pumps ($\text{Na}^+\text{-K}^+\text{-ATPase}$) (9, 21, 22) (see Fig. 1). Activity of this pathway results in metabolism of glucose to lactic acid and is responsible for a substantial proportion of the total lactic acid production of the muscle. The glucose units traversing the other cyto-

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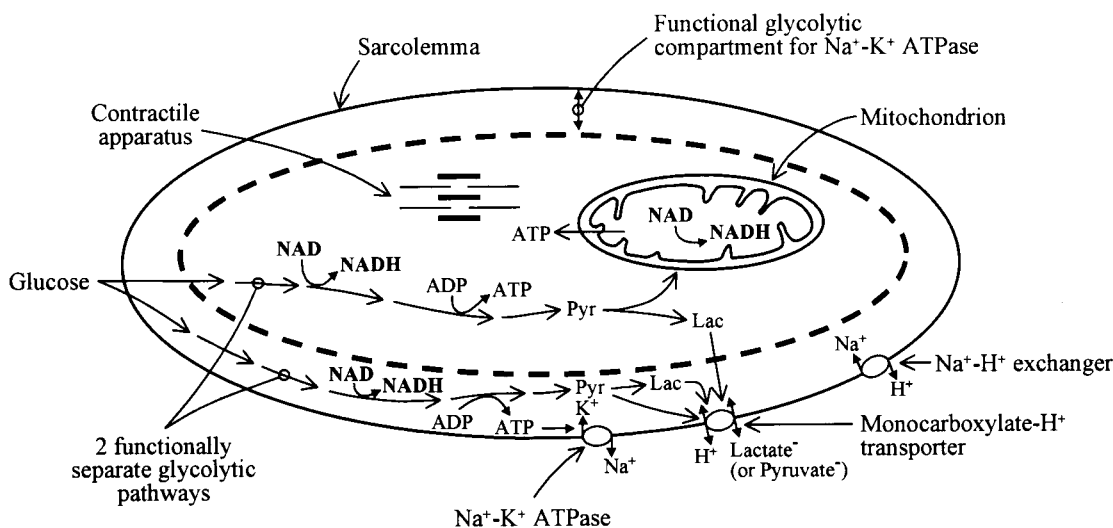


Fig. 1. Vascular smooth muscle cell glycolytic metabolism. Schematic diagram of vascular smooth muscle cell depicts functionally compartmented glycolytic pathways in the cytoplasm and certain ion transport systems in the sarcolemma. The diagram also depicts the mitochondrial pool of NAD(H) and two possible functionally separate pools of NAD(H) and metabolite redox pairs derived from glycolysis of the respective cytoplasmic compartments.

lic glycolytic pathway are partially channeled to oxidative metabolism (11, 20). The intermediary metabolites of the two glycolytic pathways do not mix (11, 16). This being the case, some question arises as to whether the NADH/NAD redox couples from the respective pathways are in equilibrium with one another. This is an important question because the two cytoplasmic glycolytic pathways can function and be varied independently of one another (15). Separate pools of metabolite redox couples that may also be functionally partitioned (Fig. 1) could conceivably constitute an electrochemical gradient between the two cytosolic compartments. The purpose of this investigation was to examine the influence of alteration of glycolytic flux in the cytosolic compartment serving the $\text{Na}^+\text{-K}^+\text{-ATPase}$ on the NADH/NAD redox state of the cytoplasm.

METHODS

Procurement and preparation of porcine carotid strips for study in organ baths were as described (2, 8). The strips were stretched to simulate 100 mmHg of mean arterial pressure. The incubation medium consisted of (in mM) 118 NaCl, 20 NaHCO_3 , 4.7 KCl, 1.2 KH_2PO_4 , 1.2 MgSO_4 , 1.6 CaCl_2 , and 5.6 glucose at 37°C. It was aerated with a gas mixture of 95% O_2 -5% CO_2 . The arteries were equilibrated for 1 h in this medium, after which time the passive tension was readjusted. The incubation continued for an additional 90 min, after which time the incubation medium was changed to contain radiolabeled isotopes of glucose for measurement of metabolic rates by sampling aliquots of incubation medium at various times for 90 additional minutes (total incubation time was 180 min). In some experiments, either glucose was deleted from the incubation medium or 0.1 mM sodium lactate was included, or both. In other experiments, 10 μM ouabain and other agents were added at 90 min. At the end of the incubation period, the arteries were removed, blotted, weighed, and frozen in liquid nitrogen for subsequent preparation of tissue extracts.

Glucose oxidation and glycolysis determinations were based on production of $^3\text{H}_2\text{O}$ from metabolism of $[6\text{-}^3\text{H}]\text{glu}$

coarse and $[5\text{-}^3\text{H}]\text{glucose}$, respectively (3). The $^3\text{H}_2\text{O}$ present in aliquots of incubation medium was separated from the remaining labeled substrate by using anion exchange column chromatography as previously described (2, 3). Lactic acid in aliquots of incubation medium was measured spectrophotometrically. O_2 consumption was measured by using a polarographic electrode embedded in a sealed organ bath (8). Perchloric acid extracts of the frozen carotid arteries were prepared as previously described (14). The following extracted tissue metabolites were assayed with the use of NAD-linked enzymatic fluorometric or spectrophotometric assays: lactate, pyruvate, glycerol-3-phosphate (G3P), and dihydroxyacetone phosphate (DHAP). Glycogen in whole tissue homogenates was measured (14).

All chemicals and enzymes were purchased from Sigma. $[5\text{-}^3\text{H}]\text{glucose}$ was purchased from Amersham, and $[6\text{-}^3\text{H}]\text{glucose}$ was purchased from NEN.

Statistics. When the means of two groups were compared, a Student's *t*-test was used. One-way ANOVA followed by the Bonferroni procedure was used to compare means of three or more groups. Repeated-measures ANOVA was used to assess the statistical significance of differences in metabolic rates and between different experimental conditions.

RESULTS

Cardiotonic glycosides have been shown to inhibit the production of lactic acid in vascular segments. This phenomenon has been attributed to inhibition of the membrane-associated $\text{Na}^+\text{-K}^+\text{-ATPase}$, which is fueled by glycolytic flux through glycolytic enzymes associated with the sarcolemmas of vascular smooth muscle cells (15, 20, 21). Figure 2 shows that treatment of carotid strips with 10 μM ouabain results in contraction of the muscles (due to membrane depolarization) and the expected diminution of both glycolysis and lactate production. In control arteries, glycolysis was $0.12 \pm 0.01 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n = 8$) and lactate production was $0.19 \pm 0.01 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n = 8$), whereas in the presence of ouabain, glycolysis was $0.07 \pm 0.01 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n = 10$, $P < 0.01$) and

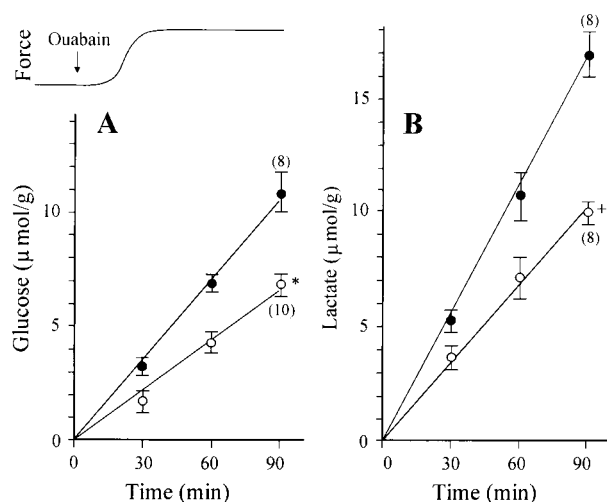


Fig. 2. Ouabain-sensitive and -insensitive glycolytic flux (A) and lactate production (B). *Inset*: schematic diagram of representative force recording upon treatment with ouabain. Values represent means \pm SE. Numbers in parentheses represent the number of experiments, each with 2 different arteries from different animals. ●, Control; ○, 10 μ M ouabain. * $P < 0.01$; † $P < 0.001$.

lactate production was $0.12 \pm 0.01 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n = 8$, $P < 0.001$). Although ouabain inhibited glycolysis, the rate of oxidation of extracellular glucose was not affected by ouabain treatment [$0.009 \pm 0.001 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n = 4$) vs. $0.011 \pm 0.001 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n = 7$); $P = \text{not significant}$]. Nevertheless, ouabain stimulated O_2 consumption. Basal O_2 consumption was $0.34 \pm 0.02 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, which subsequently increased to $0.40 \pm 0.02 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n = 6$, $P < 0.04$) with ouabain treatment. These results are consistent with previous results indicating that glucose metabolism in vascular smooth muscle is compartmentalized and that the end product of glycolysis in the compartment serving $\text{Na}^+ - \text{K}^+$ pump operation is lactate. That is, glucose units metabolized by this glycolytic compartment do not enter the oxidative pathway and are instead eliminated from the muscle predominantly in the form of lactate (15, 20) (see Fig. 1).

Under most conditions, the magnitude of lactic acid released into the extracellular bathing medium reflects the corresponding intracellular concentration of lactate. It also reflects the cytoplasmic NADH/NAD redox

state, i.e., increased lactic acid production ordinarily corresponds to an increase in the cytoplasmic NADH/NAD ratio (4, 6, 26, 29). Accordingly, the cytoplasmic NADH/NAD redox state was examined in the presence and absence of ouabain. The purpose of these series of experiments was to determine whether a reduction in the NADH/NAD redox potential in the ouabain-sensitive glycolytic compartment in the cytosol is reflected in the cytosolic glycolytic compartment not involving the operation of the $\text{Na}^+ - \text{K}^+$ -ATPase. Table 1 gives the concentrations, concentration ratios, and redox potentials of important metabolite redox couples, lactate/pyruvate, and G3P/DHAP. These metabolite redox pairs have been shown to be in equilibrium with one another and with the NADH/NAD redox couple (5, 19). Therefore, a change in these ratios reflects a change in the cytosolic redox potential and NADH/NAD. As shown in Table 1, ouabain treatment did not alter the levels of lactate, pyruvate, G3P, DHAP, and redox potential despite its effect to inhibit glycolysis and the production of lactic acid. The results are in contrast to the results of other studies that used K^+ depolarization as the contractile stimulus, which indicated that contraction of the arterial strips was associated with increased lactate production, an increased lactate content of the muscles (5, 7, 11, 15, 20), and an increase in the cytoplasmic NADH/NAD redox potential (5, 7). Furthermore, contraction by K^+ depolarization is associated with increased oxidation of glucose (3), whereas with depolarization-induced contraction by ouabain, glucose oxidation was unchanged (see above). Thus the metabolic changes observed with ouabain treatment are quite different from those due to contraction, and, therefore, they cannot be solely attributed to the energy metabolism of contractile activation of the muscle.

The converse experiments were also performed, in which the effect of ouabain on glycolysis and aerobic lactate production and on the concentration of metabolite redox couples was assessed in arteries in which the cytoplasmic NADH/NAD redox potential was already increased before the addition of ouabain. The purpose of these experiments was to determine whether an increase in NADH/NAD redox potential in the cytosolic compartment not involving operation of $\text{Na}^+ - \text{K}^+$ -ATPase would alter the NADH redox poten-

Table 1. Effect of ouabain on cytoplasmic metabolite redox couples and redox potential

Condition	Lactate Production, $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	[Lactate], $\mu\text{mol/g}$	[Pyruvate], $\mu\text{mol/g}$	[Lactate]/[Pyruvate]	[G3P], $\mu\text{mol/g}$	[DHAP], $\mu\text{mol/g}$	[G3P]/[DHAP]	E_h , mV
Control	0.19 ± 0.01	0.77 ± 0.06	0.15 ± 0.01	5.5 ± 0.4	0.21 ± 0.04	0.026 ± 0.003	8.0 ± 1.1	-220
Ouabain	$0.07 \pm 0.01^*$	0.74 ± 0.05	0.16 ± 0.01	4.7 ± 0.2	0.19 ± 0.03	0.026 ± 0.003	7.2 ± 0.6	-219
AOAA	$0.28 \pm 0.01^*$	$1.89 \pm 0.13^*$	$0.06 \pm 0.00^*$	$31.3 \pm 2.1^*$	$0.34 \pm 0.03^*$	$0.028 \pm 0.002^*$	$12.6 \pm 1.2^*$	-226
AOAA + ouabain	$0.20 \pm 0.01^\dagger$	$2.10 \pm 0.04^*$	$0.07 \pm 0.00^*$	$33.4 \pm 2.4^*$	$0.36 \pm 0.02^*$	$0.029 \pm 0.003^*$	$12.9 \pm 1.5^*$	-226

Values for lactate production rates are means \pm SE; $n = 8$ –10 experiments, each with 2 different carotid arteries from different animals. Values for intracellular metabolite concentrations are means \pm SE; $n = 8$ different arteries from different animals. AOAA, aminooxyacetic acid; G3P, glycerol-3-phosphate; DHAP, dihydroxyacetone phosphate; E_h , redox potential for [G3P]/[DHAP], using -192 mV as midpoint redox potential for the G3P dehydrogenase redox system (18, 28). * $P < 0.05$ compared with control; † $P < 0.05$ compared with AOAA.

tial of the ouabain-sensitive compartment. To accomplish this, the arteries were treated with 0.4 mM aminoxyacetate (AOAA). This compound effectively inhibits the malate-aspartate shuttle, which is a cellular metabolic pathway that functions to remove NADH-associated reducing equivalents from the cytoplasm by promoting their transport into the mitochondria. Inhibition of the shuttle by AOAA results in an elevated cytosolic NADH/NAD ratio and redox potential (6, 24), as indicated by the increased concentration ratios of lactate/pyruvate and G3P/DHAP redox couples (Table 1). Using calculations based on the G3P dehydrogenase system and the respective equilibrium constant ($K_{G3PDH} = 1.3 \times 10^{-4}$; Refs. 26 and 29), the estimated free NADH/NAD in the cytoplasm was in the range of 1.0×10^{-3} in control arteries and 1.64×10^{-3} in arteries treated with AOAA. Arteries incubated with AOAA also demonstrate increased glycolysis and aerobic lactic acid production (Table 1). Subsequent addition of ouabain to AOAA-treated muscles resulted in the reduction of both glycolysis and aerobic lactate production. Despite this, there was no difference in the intracellular concentrations of lactate and pyruvate or in the concentration ratios of either lactate/pyruvate or G3P/DHAP in these arterial strips (Table 1). These results indicate that the redox systems of the two glycolytic compartments maintain equilibrium despite variation in glycolytic fluxes in the two compartments.

The constant intracellular lactate and pyruvate concentrations despite variation in total lactic acid production raised the possibility that the efflux of lactate from the tissue is carrier mediated and not simply reflective of passive diffusion of the accumulated lactate. (If the efflux of lactate were dependent simply on passive diffusion, then the intracellular concentration of lactate should have been proportional to the rate of lactate production.) In both cardiac and skeletal muscle, most of the lactate efflux is attributed to cotransport with H^+ by the monocarboxylate B- H^+ transporter (13). Whether this transporter is present in vascular smooth muscle was verified by experiments in which the arteries were treated with phloretin, an inhibitor of the transporter (12, 13). Figure 3 shows the effect of 0.6 mM phloretin on lactate elimination in porcine carotid strips. The concentration of 0.6 mM phloretin was used because it is the concentration that was previously shown to produce maximal inhibition of monocarboxylic acid transport in intact skeletal muscle preparations (12). As shown in Fig. 3, the rate of lactate elimination was significantly reduced in the presence of phloretin ($0.03 \pm 0.02 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, $n = 6$) compared with that in its absence ($0.19 \pm 0.01 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, $n = 8$; $P < 0.0001$). These results verify that the transporter is also present in the sarcolemmas of vascular smooth muscle cells.

Previous studies with membrane preparations from skeletal muscle indicated that efflux of lactate or pyruvate from intact skeletal muscle and skeletal muscle sarcolemmal preparations is sensitive to pH, consistent with the requirement of protons for cotransportation with the monocarboxylate species lactate or pyru-

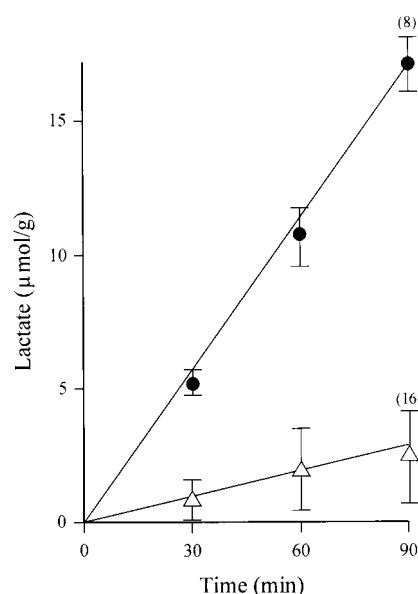


Fig. 3. Lactate efflux and inhibition of monocarboxylate B- H^+ transporter. Values represent means \pm SE. Numbers in parentheses represent the number of experiments, each with 2 different arteries from different animals. ●, Control; Δ , 0.6 mM phloretin. * $P < 0.0001$.

vate (12, 13, 23) (see Fig. 1). Intracellular pH, in turn, is importantly regulated by the Na^+/H^+ exchanger (1, 27). Additionally, cotransport of lactate and H^+ has been linked to the activity of the Na^+/H^+ exchanger (12, 23). Accordingly, the effect of amiloride, a specific inhibitor of the Na^+/H^+ exchanger, on lactate elimination was examined. For these studies 0.1 mM amiloride was used (23). In the presence of amiloride, the intracellular concentration of lactate was not different from that in the absence of amiloride (Table 2). However, with subsequent treatment with ouabain, the intracellular concentration of lactate was decreased compared with that under similar incubation conditions but in the absence of amiloride. The decreased concentration of lactate in amiloride-treated arteries was not attributed to an effect on glycolysis or total lactate production because amiloride did not affect these variables (data not shown). Thus amiloride, by inhibiting the Na^+/H^+ exchanger and causing acidification of the cytosol, apparently promoted the efflux of lactate when the Na^+ pump was inhibited. This hypothesis was tested by repeating the above experiments with amiloride and ouabain but with the addition of 0.6 mM phloretin, a specific inhibitor of the lactate B- H^+ cotransporter. When the cotransporter was inhibited by phloretin, the intracellular concentration of lactate returned to the level in the absence of amiloride, supporting the hypothesis that acidification of the cytosol promoted lactate efflux. (Note that the concentrations of pyruvate, G3P, and DHAP could not be determined in the above experiments because of interference of amiloride with the fluorometric assays due to a high background fluorescence.)

Because lactate elimination was depressed in arteries in which $Na^+-K^+-ATPase$ was inhibited, it seemed possible that inhibition of $Na^+-K^+-ATPase$ might also

Table 2. Effect of intracellular acidification on lactate content in carotid arteries

	Control	Ouabain	Amiloride	Ouabain + Amiloride	Ouabain + Amiloride + Phloretin
Lactate, $\mu\text{mol/g}$	0.77 ± 0.06	0.74 ± 0.05	0.85 ± 0.04	$0.45 \pm 0.03^*$	0.66 ± 0.04

Values are means \pm SE; $n = 7$ – 10 arteries from different animals. $*P < 0.05$ compared with control.

promote a reciprocal effect to stimulate the uptake of lactate from the extracellular space. This phenomenon, if operative, could contribute to the constant concentration of lactate in the cytoplasm despite the reduced glycolysis and aerobic lactate production. This hypothesis was tested by performing experiments in which carotid arteries were incubated with medium containing sodium lactate, and the effect of inhibition of the Na^+ - K^+ -ATPase on the uptake of lactate from the incubation medium was assessed. To simplify measurement of lactate uptake, we used glucose-free medium in these experiments so that the cellular production of lactic acid and the attendant release of lactate into the extracellular medium could be minimized. Any lactate produced would originate primarily from endogenous glycogen stores, which in substrate-depleted arteries would be minimal. Figure 4 shows the production of lactate by arteries incubated in glucose-free medium for 90 min and then contracted with ouabain for an additional 90 min, with or without 0.1 mM lactate included in the bathing medium. An increase in the lactate content of the incubation medium would indicate that lactic acid was produced from breakdown

of the residual glycogen stores, whereas a decrease in the concentration of lactate in the bathing medium originally containing 0.1 M lactate would indicate that net uptake of lactate had occurred. By the end of 90 min of treatment with ouabain in glucose-free medium, $1.94 \pm 0.19 \mu\text{mol/g}$ lactate was formed ($n = 4$), while during the same time $\sim 1.56 \mu\text{mol}$ glucosyl units/g glycogen was degraded; glycogen content was $1.90 \pm 0.24 \mu\text{mol}$ glucosyl units/g ($n = 7$) before challenge with ouabain, but it then decreased to $0.34 \pm 0.05 \mu\text{mol/g}$ glucosyl units ($n = 4$, $P < 0.001$) during the subsequent 90 min. In contrast, in experiments in which arteries were incubated in glucose-free medium containing 0.1 mM lactate, no net lactate production was observed upon challenge with ouabain, despite the fact that a comparable quantity of glycogen had been catabolized ($\sim 1.59 \mu\text{mol}$ glucosyl units/g) over the same period [glycogen content decreased from 1.90 ± 0.24 ($n = 7$) to $0.3 \pm 0.01 \mu\text{mol}$ glucosyl units/g ($n = 4$); $P < 0.001$]. In fact, there was a net decrease in the concentration of lactate in the incubation medium, indicating that net uptake of lactate had occurred. By the end of the 90-min treatment with ouabain, the lactate content of the incubation medium had decreased by $1.12 \pm 0.31 \mu\text{mol/g}$ ($n = 18$; $P < 0.002$). If it is assumed that the glycogen catabolism that occurred contributed to the lactate content of the incubation medium, then it is calculated that net lactate uptake was $\sim 3.1 \mu\text{mol/g}$ under these conditions. These results are contrasted with those of experiments in which arteries were similarly incubated in glucose-free medium containing lactate but that were contracted instead for 90 min by 100 μM norepinephrine (NE). Net lactate uptake could not be demonstrated upon challenge with NE. Instead, lactate in the bathing medium increased by $2.14 \pm 0.54 \mu\text{mol/g}$ ($n = 5$), which was associated with a $0.99 \mu\text{mol}$ glucosyl units/g breakdown of endogenous glycogen over the same 90-min period [glycogen content decreased from 1.90 ± 0.24 to $0.91 \pm 0.14 \mu\text{mol}$ glucosyl units/g ($n = 4$); $P < 0.02$]. Differences in the pattern of lactate uptake under the three experimental conditions were not attributed to differences in contractile force generated by the arterial strips because maximal isometric force was the same in all three conditions (see Fig. 4 legend). Net uptake of lactate was also not demonstrable with KCl-induced contractions under similar conditions (data not shown).

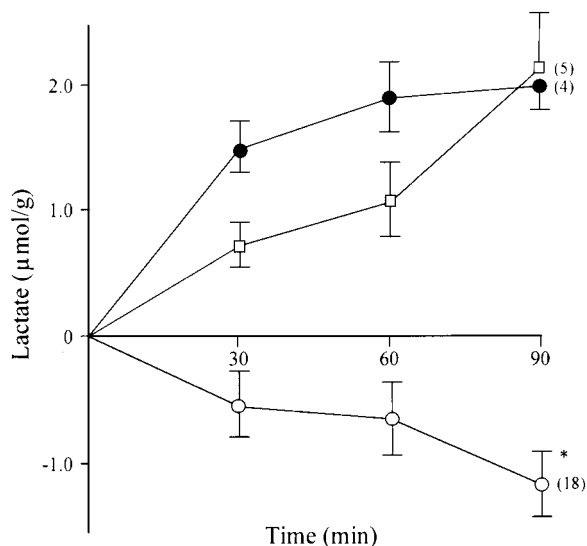


Fig. 4. Uptake of lactate associated with inhibition of Na^+ - K^+ -ATPase by ouabain. Carotid arteries were incubated in either glucose-free medium plus ouabain (\bullet) or glucose-free medium containing 0.1 mM sodium lactate plus ouabain (\circ). In glucose-free medium containing lactate, a net decrease in lactate content of the incubation medium was observed with ouabain. For comparison, carotid arteries were incubated in glucose-free medium and 0.1 mM lactate and then contracted with 100 μM norepinephrine (\square). There was no difference in the maximal isometric contractile force generated by the arterial strips under the 3 experimental conditions (36 ± 5 , 40 ± 3 , and 46 ± 5 g, respectively; $P = \text{not significant}$). Values represent means \pm SE. Numbers in parentheses represents the number of experiments, each with 2 different arteries from different animals. $*P < 0.002$.

DISCUSSION

The two glycolytic compartments in the cytosol of vascular smooth muscle cells are functionally compartmentalized and can operate and vary independently from one another (11, 15, 20). The central observation of the present investigation with respect to the cyto-

lic compartmentation of glycolytic pathways is that, even when the glycolytic flux and attendant lactate production is reduced in the compartment subserving the activity of the plasma-membrane associated $\text{Na}^+\text{-K}^+\text{-ATPase}$, the cytosolic NADH/NAD redox potential remained unchanged in the steady state. This implies that there is no associated compartmentation of NAD-associated-reducing equivalents and, as a consequence, no functional electrochemical gradient between the two compartments in the cytosol. It does not exclude the possibility that such an electrochemical gradient between the two glycolytic compartments would not initially develop under non-steady-state conditions, i.e., under conditions in which the metabolic fluxes of the two compartments are varied and before homeostatic mechanisms that would normalize the redox potential in the $\text{Na}^+\text{-K}^+\text{-ATPase}$ -associated compartment become operative, and thus dissipate any electrochemical gradient. Physiological or pharmacological modulation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity by hormones and/or vasoactive agonists (10, 17) may indeed initially produce a subcytosolic electrochemical redox gradient if the rate of alteration of the metabolic fluxes in the two compartments exceeds the rate in which the homeostatic mechanisms can normalize the subcytosolic redox potential.

We have identified two homeostatic mechanisms that acted to normalize the cytosolic NADH redox state upon decreased glycolytic flux caused by inhibition of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ by ouabain. The homeostatic mechanisms appear to be specifically linked to the activity of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ because it was previously demonstrated that a similar reduction of glycolytic flux by means not involving diminished $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity resulted in a change in the NADH redox potential of the cytoplasm (6, 8). The first mechanism is reduced extrusion of lactate (and presumably pyruvate) from the interior of the cell across the sarcolemma to the extracellular space. The transport of both lactate and pyruvate is mediated by the monocarboxylate transporter, which has a requirement for H^+ for its activity (13). Both H^+ and a monocarboxylic acid species, in this case either lactate or pyruvate, become bound to the transporter and are transported and released on the opposite site of the membrane. The monocarboxylate transporter mediates both extrusion and uptake of monocarboxylic acid species (12, 23) (see Fig. 1). With reduced intracellular production of lactate anions and protons upon inhibition of the Na^+ pump by ouabain, one would expect a relative increase in the pH in the subcytosolic compartment serving the $\text{Na}^+\text{-K}^+\text{-ATPase}$. Furthermore, reduced hydrolysis of ATP to $\text{ADP} + \text{H}^+$ resulting from reduced activity of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ would also decrease the availability of protons in this subcytosolic compartment, thereby contributing to reduced activity of the transporter. Therefore, even though the local lactate and H^+ concentrations would initially decrease upon inhibition of the $\text{Na}^+\text{-K}^+\text{-ATPase}$, with reduced activity of the monocarboxylate- H^+ transporter, the subcytosolic compartment concentrations of H^+ , lactate, and pyruvate would, in

the steady state, all return to the levels measured before glycolytic flux was reduced by ouabain. Because the lactate/pyruvate redox couple is in equilibrium with the NADH/NAD couple, the cytosolic NADH/NAD redox potential would remain unchanged despite significant reduction in glycolytic flux and the attendant lactate and pyruvate formation. This hypothesis was supported by the results of experiments in which the cytoplasm of the cells was acidified with amiloride and then treated with ouabain. Under these conditions, lactate levels were reduced to levels commensurate with the reduction in the rate of glycolysis and lactate formation. Cotreatment with phloretin, an inhibitor of the monocarboxylate transporter, returned the intracellular lactate concentration to the level of that in the absence of amiloride.

A second homeostatic mechanism that may normalize the cytosolic NADH redox potential with inhibition of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ is uptake of lactate and pyruvate from the incubation medium into the cytosol. It was demonstrated that there was net uptake of lactate by the carotid arteries when they were incubated in glucose-free medium containing 0.1 mM lactate and then contracted by ouabain. Contraction induced by norepinephrine or KCl, which would be expected to be associated with increased activity of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ (9, 15, 17, 20, 21), did not result in demonstrable uptake of lactate from the incubation medium under similar experimental conditions. Uptake of lactate was probably mediated by the monocarboxylate B-H^+ transporter because it may transport lactate together with H^+ bidirectionally across the plasma membrane (Fig. 1). The direction and magnitude of lactate transport would depend on the corresponding lactate and proton concentration gradients (12, 23). As in the case with lactate efflux described above, inhibition of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ could result in relative proton deficiency in the subcytosolic compartment, producing a more inwardly directed H^+ gradient. If an inwardly directed lactate gradient existed, as would occur if the arterial muscles were incubated in glucose-free medium containing lactate or in regular medium in which higher concentrations of lactate were present, net uptake of lactate and H^+ would take place. Alternatively, simultaneous uptake and efflux of lactate has been reported in skeletal muscle (25), but the mechanism of this phenomenon is unclear, unless a functional compartmentation of lactate transport were operative analogous to the known functional compartmentation of glucose and lactate metabolism in smooth muscle.

The nature of the functional compartmentation of carbohydrate metabolism in the cytosol of vascular smooth muscle cells is not defined. On one hand, it is possible that the cytosolic intracellular membrane system constitutes a physical barrier that separates the metabolites and products of the two metabolic compartments. On the other hand, it has been proposed that, underlying the functional compartmentation of metabolic systems in the cytosol, there is a "reaction-diffusion" mechanism wherein the rates of reactions of the two glycolytic fluxes exceed the local diffusion rates

of the metabolites of the respective systems (11). In any case, it is unlikely that the compartmentation of metabolism in the cytosol is absolute because it has been demonstrated that the two pathways may be subject to feedback regulation of one another (8). It has also been reported that, even though the Na^+ pump is ordinarily fueled by the pathway for aerobic glycolysis and lactate production, energy support from oxidative metabolism is needed at high Na^+ pump rates (9). Therefore, the two compartments are not totally independent from one another, and the results of our studies indicate that at least the NADH/NAD redox potentials are equal in the steady state.

In summary, we determined in this investigation that, despite the functional compartmentation of cytoplasmic glycolytic fluxes produced by differential activity of the Na^+ - K^+ -ATPase, there was no difference in corresponding NADH/NAD redox potentials of the two compartments in the steady state. Homeostatic mechanisms involving the monocarboxylate transporter are engaged to normalize the concentrations of lactate and pyruvate, and thus the redox potential, of the cytosolic glycolytic compartment serving the Na^+ pump. Evidence presented indicates that the activity of the monocarboxylate transporter may be functionally linked either directly or indirectly to the activity of the Na^+ - K^+ -ATPase, possibly in concert with the Na^+ / H^+ exchanger.

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