Role of Na\(^{+}\)-K\(^{+}\)-ATPase in insulin-induced lactate release by skeletal muscle

VALÉRIE NOVEL-CHATÉ, VALENTINE REY, RENÉ CHIOLÉRO, PHILIPPE SCHNEITER, XAVIER LEVERVE, ERIC JÉQUIER, and LUC TAPPY

Institute of Physiology, Medical School, University of Lausanne, 1005; Surgical Intensive Care Unit, University Hospital, 1011 Lausanne, Switzerland; and Laboratory of Fundamental and Applied Bioenergetics, University Joseph Fourier, 38041 Grenoble, France

Received 23 May 2000; accepted in final form 16 October 2000

Novel-Chaté, Valérie, Valentine Rey, René Chioléro, Philippe Schneiter, Xavier Leverve, Éric Jéquier, and Luc Tappy. Role of Na\(^{+}\)-K\(^{+}\)-ATPase in insulin-induced lactate release by skeletal muscle. Am J Physiol Endocrinol Metab 280: E296–E300, 2001.—Hyperinsulinemia increases lactate release by various organs and tissues. Whereas it has been shown that aerobic glycolysis is linked to Na\(^{+}\)-K\(^{+}\)-ATPase activity, we hypothesized that stimulation by insulin of skeletal muscle Na\(^{+}\)-K\(^{+}\)-ATPase is responsible for increased muscle lactate production. To test this hypothesis, we assessed muscle lactate release in healthy volunteers from the \([^{13}\text{C}]\)lactate concentration in the effluent dialysates of microdialysis probes inserted into the tibialis anterior muscles on both sides and infused with solutions containing 5 mmol/l [U-\(^{13}\text{C}\)]glucose. On one side, the microdialysis probe was intermittently infused with the same solution additioned with \(2.10^{-6}\) M ouabain. In the basal state, \([^{13}\text{C}]\)lactate concentration in the dialysate was not affected by ouabain. During a euglycemic-hyperinsulinemic clamp, \([^{13}\text{C}]\)lactate concentration increased by 135% in the dialysate without ouabain, and this stimulation was nearly entirely reversed by ouabain (56% inhibition compared with values in the dialysate collected from the contralateral probe). These data indicate that insulin stimulates muscle lactate release by activating Na\(^{+}\)-K\(^{+}\)-ATPase in healthy humans.

aerobic glycolysis; \([^{13}\text{C}]\)lactate; microdialysis; ouabain

LACTATE IS CONTINUOUSLY PRODUCED and utilized in virtually all organs and tissues in humans (2, 13). The lactate produced is derived mainly from glucose through glycolysis and reduction of pyruvate by the terminal glycolytic enzyme lactate dehydrogenase. In some tissues, such as red blood cells, the kidney medulla, or inflammatory tissues, lactate formation represents the major or sole pathway of glucose metabolism. In other tissues, such as skeletal muscle, adipose tissue, skin, or others, the pyruvate formed by glycolysis can either be released outside the cell as lactate or be further oxidized in the tricarboxylic cycle (13).Recent data suggest that glycolysis leads to formation of intracellular lactate, which can be transported into the mitochondria to undergo oxidation or be transported outside the cell (intracellular lactate shuttle concept) (3).

Hexokinase, the first enzyme involved in glycolytic degradation of glucose, is present in cells either within the cytosol or bound to proteins in the outer mitochondrial membrane (1, 8). Several observations indicate that the activity of the soluble hexokinase is, at least in part, linked to the activity of the plasma membrane ATP-dependent Na-K pumps. According to this scheme, the ATP produced from glycolysis is directly available to support the energy needs of the membrane Na-K pumps. A direct relationship between the rate of aerobic glycolysis and the activity of Na\(^{+}\)-K\(^{+}\)-ATPase is supported by the observation that inhibition of Na\(^{+}\)-K\(^{+}\)-ATPase by ouabain reduces lactate formation in various cell types in vitro (16, 19) whereas, on the other hand, stimulation of Na\(^{-}\)-K\(^{-}\)-ATPase by monensin increases lactate in skeletal muscle cells (11). It has also been shown that increased Na\(^{-}\)-K\(^{-}\)-ATPase activity was present in septic rats with hyperlactemia (11). In working skeletal muscle, lactate is produced not only from interstitial glucose but also in major part from glycogen degradation (17). Catecholamines increase lactate production in skeletal muscle, probably by stimulating glycogenolysis. A recent observation, however, indicates that stimulation of lactate production by epinephrine is secondary to Na\(^{-}\)-K\(^{-}\)-ATPase activation (12).

Hyperinsulinemia stimulates lactate release by various tissues and organs (10) and increases plasma lactate concentration. Insulin also activates Na\(^{+}\)-K\(^{-}\)-ATPase through dephosphorylation of its \(\alpha\)-subunit in vitro (7, 20–22). It is therefore possible that the activity of Na\(^{-}\)-K\(^{-}\)-ATPase plays a major role in the regulation of lactate production in vivo. In this study, we assessed whether inhibition of Na\(^{-}\)-K\(^{-}\)-ATPase by ouabain inhibits muscle lactate production in humans in the basal state and during hyperinsulinemia. For this purpose, we monitored the effect of ouabain on the production of \([^{13}\text{C}]\)lactate released from \([^{13}\text{C}]\)glucose locally administered through microdialysis probes.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MUSCLE Na-K-ATPase AND LACTATE PRODUCTION

METHODS

Subjects. Six healthy human volunteers were recruited to take part in this study. They had a mean age of 26.8 ± 0.7 yr, height of 173.2 ± 4.5 cm, weight of 71.6 ± 6.7 kg, and body mass index of 23.6 ± 1.5. They were in good health, had no family history of diabetes or metabolic disorders, and were not currently taking any medication. The experimental protocol was approved by the ethical committee of Lausanne University Medical School, and every participant provided informed, written consent.

Experiments. All experiments began in the morning after an overnight fast. At their arrival in the metabolic investigation laboratory, subjects were weighed, measured, and placed in beds in a recumbent position. One indwelling venous cannula was inserted into a wrist vein on the right side. The right hand was then placed in a thermostabilized box heated to 50°C to achieve partial arterialization of venous blood. Blood samples were subsequently collected at various time intervals from this cannula. A second cannula was inserted into an antecubital vein of the left arm and was used for infusion of insulin, 20% dextrose, and potassium chloride. Microdialysis probes (CMA, Carnegie Medicine, Stockholm, Sweden) were inserted under local anesthesia (0.1 ml xylocaine 1%) in the tibialis muscle on each side. Probes were infused continuously with a lactate-free Ringer solution containing 5 mM [U-13C]glucose (Cambridge Isotope Laboratories, Cambridge, MA) and 1 mM [3,3,3-2H3]lactate (Cambridge Isotope Laboratories) at a rate of 2 μl/min by means of a CMA 100 microinfusion pump (Carnegie Medicine). Dialysate was collected at 30-min intervals.

After 30 min of stabilization, measurements were performed during two periods of 120 min. The first period (0–120 min) corresponded to basal conditions. During the second period (120–240 min), a hyperinsulinemic (1 mU kg−1·min−1)-euglycemic clamp was performed (6). Four millimoles per hour potassium chloride were infused during this period to prevent a decrease in plasma potassium concentration.

Through one microdialysis probe (ouabain), a lactate-free Ringer solution, containing 5 mM 98% enriched [U-13C]glucose, 1 mM 98% enriched [3,3,3-2H3]lactate, and 2.10−5 M ouabain, was infused from t = 60 to t = 120 min and from t = 180 to t = 240 min. The same solution but devoid of ouabain (ouabain−) was infused during the remaining time through this probe and during the total experiment through the contralateral probe.

Plasma glucose concentration was measured with a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). Plasma lactate concentration was measured enzymatically and plasma insulin concentration by radioimmunoassay, by use of a kit from Biodata (Biodata, Guidonia Montecelio, Italy).

Dialysate lactate concentration was measured in a CMA 600 Microdialysis analyzer (Carnegie Medicine).

Dialysates [2H3]- and [13C]-lactate were measured by gas-chromatography-mass-spectrometry on a Hewlett Packard Instrument (GC 5890/MS 5971, Hewlett Packard, Palo Alto, CA) after derivatization to lactate-N-propylamide-heptafluorobutyrate. Samples were analyzed in electron impact mode with selective monitoring of mass-to-charge ratio (m/z) 241, 243, and 244 (10). The analyzed ion retains carbons 2 and 3 of the lactate molecule; hence, two heavy isotopes from [13C3]lactate (mass m + 2) and the three 2H of [2H3]lactate (mass m + 3). Standard curves with several dilutions of pure [2H3]lactate and [13C3]lactate with unlabeled lactate were used to determine isotopic enrichments in dialysate samples. In two subjects, [3,3,3-2H3]lactate was infused alone to evaluate whether loss of 2H from [2H]-lactate occurred to any significant extent. The relative abundance of lactate at m/z 243 remained unchanged after infusion of [3,3,3-2H3]lactate, indicating that loss of tracer from [2H]-lactate did not interfere with monitoring of [13C]-labeled lactate (data not shown).

Dialysate [13C]-lactate concentration was calculated by multiplying dialysate lactate concentration with dialysate [13C]-lactate enrichment.

Relative recovery of lactate was assessed with the internal reference method (15) by adding [2H3]lactate in the perfusate. This procedure assumes no significant reentry of labeled lactate in the dialysate. Interstitial lactate concentration was then calculated as (dialysate lactate concentration)/relative recovery.

Statistics. Data were analyzed using a two-way analysis of variance for repeated measurements and paired t-tests with Bonferroni’s correction to compare results obtained in probes infused with and without ouabain.

RESULTS

In the basal state, plasma glucose concentration was 5.2 ± 0.1 mM/l, and plasma insulin concentration was 52.5 ± 10.2 pmol/l. During insulin infusion, plasma insulin concentration was increased to 590.4 ± 52.2 pmol/l (P < 0.001), whereas plasma glucose remained clamped at 5.1 ± 0.1 mM/l. Plasma lactate concentration increased from 1.01 ± 0.16 mM/l in the basal state to 1.33 ± 0.09 mM/l during hyperinsulinemia (P < 0.05).

Lactate concentration and [13C]-lactate/[2H3]-lactate enrichment during the time course of the experiments are shown in Fig. 1. In the dialysate collected from the control probe (ouabain−), [13C]-lactate increased slightly from 60 to 120 min (P < 0.05) and then increased further during hyperinsulinemia at 180 and 240 min (P < 0.05). Similar trends were observed with interstitial lactate concentration, although the results were not significant.

In the dialysate collected from the probe infused intermittently with ouabain, ouabain had no effect on [13C]-lactate concentration or on interstitial lactate concentration in the basal state. Ouabain, however, reduced [13C]-lactate concentration by 56% (P < 0.05) and interstitial lactate concentration by 24% (not significant) compared with values obtained in the contralateral probe infused with ouabain-free Ringer buffer during hyperinsulinemia (Fig. 2).

DISCUSSION

The present data demonstrate for the first time in humans that stimulation of Na+–K+–ATPase by hyperinsulinemia is associated with an increased lactate production in skeletal muscle. This is consistent with several previous studies performed on isolated cells (16, 19) and in animals (11), showing that aerobic glycolysis is coupled with Na+–K+–ATPase activity and with reports showing that insulin activates Na+–K+–ATPase activity (20, 21). Ouabain was infused locally through microdialysis probes inserted into the tibialis anterior muscle. This local delivery of the drug allowed the use of a high concentration of ouabain without adverse systemic effects. Although we cannot assess
the actual concentration of ouabain in the interstitium with this procedure, the concentration of ouabain in the perfusion fluid was one order of magnitude above the concentration that produces nearly complete inhibition of Na\(^+\)-K\(^+\)-ATPase in vitro. It is, therefore, likely that efficient inhibition of Na\(^+\)-K\(^+\)-ATPase was obtained in the vicinity of the microdialysis probe.

Stimulation of lactate production in skeletal muscle cells is expected to increase the interstitial muscle lactate concentration. Several other factors, however, influence interstitial lactate concentration. Skeletal muscle blood flow is known to increase during hyperinsulinemia (14) and may therefore limit any increase in interstitial lactate concentration. It is also recognized that lactate is both produced and utilized by skeletal muscle fibers (5), and it is therefore possible that an increase in lactate production is masked by a simultaneous stimulation of lactate utilization. The procedure we used in this study largely avoids these shortcomings linked to monitoring of interstitial lactate concentration. By infusing \(^{13}\text{C}\)lactate through the probe, we locally labeled the interstitial glucose that represents the immediate precursor for lactate synthesis. This allows use of \(^{13}\text{C}\)lactate concentration in the effluent dialysate as a semiquantitative index of aerobic glycolysis. Using this approach, we previously showed that hyperinsulinemia and hyperglycemia stimulate lactate production in subcutaneous adipose tissue of human volunteers (10).

In basal conditions, \(^{13}\text{C}\)lactate concentration in the dialysate increased between the 1st and 2nd hour of measurement. This may be explained by the time required to achieve the equilibrium locally after starting the infusion of \(^{13}\text{C}\)glucose. During the 2nd hour of basal measurements, ouabain was infused through one probe while infusion of ouabain-free solution was continued in the contralateral probe. Interstitial lactate and \(^{13}\text{C}\)lactate dialysate concentrations remained, however, identical in the dialysate collected from both probes, suggesting that activity of Na\(^+\)-K\(^+\)-ATPase was not a major determinant of lactate production in skeletal muscle under basal conditions.

During hyperinsulinemia, \(^{13}\text{C}\)lactate concentration increased significantly in the dialysate collected from the probe infused with ouabain-free solution, indicating that insulin stimulated glucose conversion into lactate in skeletal muscle. This is consistent with several reports that showed a stimulation of lactate production in skeletal muscle during hyperinsulinemia (5, 9, 10). In the other probe, infusion was changed to...
Muscle Na-K-ATPase and lactate production

E299

ouabain-free solution during the 1st hour of hyperinsulinemia. The insulin-induced increase in interstitial 
$[^{13}C]$lactate was comparable with that observed in the contralateral probe, consistent with a rapidly reversible inhibition of the Na-K activity by ouabain. In contrast, when infusion of ouabain was restored during the 2nd hour of hyperinsulinemia, we observed a significant decrease in $[^{13}C]$lactate concentration in the collected dialysate. This clearly indicates that local lactate release was sharply decreased when Na-K-ATPase was inhibited. The increase in $[^{13}C]$lactate concentration in the interstitium may be secondary to enhanced muscle glucose uptake and glycolysis or to a decreased pyruvate oxidation with subsequent conversion of pyruvate into lactate. Muscle glucose uptake or oxidation cannot be evaluated from our measurements. Because the increase in $[^{13}C]$lactate concentration was inhibited by ouabain, and because there is no evidence that ouabain inhibits glucose oxidation or pyruvate dehydrogenase activity, at least in cardiac muscle (4), we favor the former hypothesis. A decrease in the cytosolic concentration of ATP with a concomitant increase in ADP concentration in the immediate vicinity of activated Na-K-ATPase may be responsible for activation of the key glycolytic enzyme phosphofructokinase (18).

From these results, we can only speculate on the cell types in which Na-K-ATPase activity increased in response to hyperinsulinemia and on the possible functional consequences of this activation. It is possible that the Na-K-ATPase was activated in both skeletal muscle fibers and vascular smooth muscle cells, but because the former represents the bulk of the tissue, we assume that our results are representative of mainly the skeletal muscle. Our observation was done in the tibialis anterior, a muscle composed mainly of fast-twitch glycolytic fibers. It is, therefore, possible that activation of Na-K-ATPase-linked glycolysis occurs essentially in glycolytic muscles. Results obtained in rats, however, indicate that monensin, which activates Na-K-ATPase by increasing intracellular Na$^+$ concentration, also stimulates glycolysis in the soleus, a muscle with a large proportion of oxidative fibers (12). Finally, we cannot exclude the possibility that an activation occurred in the vascular smooth muscle cells as well. If that was the case, hyperpolarization caused by Na-K-ATPase activity may have reduced the excitability of these cells and may have participated in the insulin-induced vasodilation through this mechanism.

In conclusion, the present data indicate that 1) hyperinsulinemia increases lactate release by skeletal muscle fibers of healthy humans, and this effect is linked with insulin-induced activation of Na-K-ATPase; and 2) the relationship between Na-K-ATPase activity and local lactate release can be investigated in vivo with the use of microdialysis and tracer technology. It remains to be determined whether Na-K-ATPase activity plays a significant role in the increased lactate production observed in patients with type 2 diabetes or in other conditions associated with hyperlactatemia.

This work was supported by grants from the Swiss National Science Foundation (FNSR no. 3200–053737.98 to R. Chioleéro and no. 32–45387.95 to E. Jéquier).

REFERENCES

