Lactate clamp: a method to measure lactate utilization in vivo

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Gao, Jiaping, Mohammad A. Islam, Christine M. Brennan, Beth E. Dunning, and James E. Foley. Lactate clamp: a method to measure lactate utilization in vivo. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E729–E733, 1998.—A lactate clamp method has been developed to quantify the whole body lactate utilization in conscious, unstressed rats. Dichloroacetate (DCA), a known lactate utilization enhancer, was used to validate the method. Fasting lactate concentrations before the clamps were identical for DCA-treated (1 mmol/kg) and control groups (1.65 ± 0.37 vs. 1.65 ± 0.19 mM). The animals received a primed continuous lactate infusion for 90 min at variable rates to clamp the blood lactate concentration at 2 mM. The steady-state (60–90 min) lactate infusion rate, which represents the whole body lactate utilization in DCA-treated animals, was 144% higher than that in the control animals (13.2 ± 1.0 vs. 5.4 ± 1.1 mg·kg⁻¹·min⁻¹; P < 0.001). The markedly increased lactate infusion rate indicates an enhanced lactate flux by DCA. To determine whether the increased lactate infusion by DCA reflected reduced endogenous lactate production, lactate production was measured. The results indicate that endogenous lactate production was not affected by DCA. In conclusion, the lactate clamp provides a sensitive and reliable method to assess lactate utilization in vivo, a dynamic measurement that may not be clearly demonstrated by blood lactate concentrations per se.

Lactate infusion; lactate production; dichloroacetate

LACTATE IS BOTH an end product and a precursor of glucose metabolism. Lactate formed from glycolysis must be converted back to pyruvate before it can be metabolized. In a lactate-generative tissue such as skeletal muscle, lactate is either oxidized after being converted into pyruvate or released from the cells into the blood stream to be taken by other tissues, such as the liver. In the liver, lactate is oxidized to pyruvate, which is then converted into glucose via the gluconeogenic pathway. This lactate-associated gluconeogenic process, known as the Cori cycle, plays an important role in glucose homeostasis under postabsorptive conditions in healthy individuals but also contributes to hyperglycemia in diabetes mellitus (4, 7). Thus a knowledge of lactate utilization is important for a better understanding of carbohydrate metabolism. Although lactate utilization is somewhat reflected by the blood lactate concentration, the latter is simply the balance between the lactate production and utilization and may not adequately indicate the flux of lactate in either direction (3). Therefore, lactate utilization cannot be determined adequately from blood lactate concentrations per se. With the use of an isotope tracer technique, lactate turnover rate has been assessed in many studies (1, 10, 18). Whether the tracer technique truly measures lactate turnover rate remains controversial (12, 13). Furthermore, the method provides little information about the capacity to utilize lactate. To measure such ability directly, a lactate clamp method was developed in the present study to determine the whole body lactate utilization during a steady state of lactate infusion. Dichloroacetate (DCA) is a known enhancer of lactate utilization. DCA activates pyruvate dehydrogenase (PDH) by inhibiting PDH kinase, which deactivates PDH via phosphorylation (15). The activation of PDH accelerates the decarboxylation of pyruvate to form acetyl-CoA and thus facilitates the conversion of lactate into pyruvate in the tissues and, therefore, increases lactate utilization (17). In the present study, DCA was used to demonstrate the enhancement of lactate utilization and thereby validate the method. The results from this study illustrate that the lactate clamp is a sensitive and reliable method to assess lactate utilization in vivo.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Hilltop, Scottsdale, PA) at the age of 4 wk were housed in hanging wire-bottom cages and given access to water and food (diet containing 59% saturated fat, 20% carbohydrate, and 21% protein in calories) ad libitum. All procedures described below were approved by the Novartis Animal Care and Use Committee.

Cannulation procedure. At the age of 10 wk, the animals were vascularity cannulated for the study. The animals were anesthetized intraperitoneally with ketamine (67 mg/kg; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (6.7 mg/kg; Miles, Shawnee Miss, KS). Cannulas made from Micro-Renathane implantation tubing (0.033 in. OD × 0.014 in. ID; Braintree Scientific, Braintree, MA) were implanted into the jugular vein and left carotid artery and filled with saline containing 35% polyvinylpyrrolidone and 1,000 U/ml heparin. The cannulas were exteriorized at the nape of the neck and anchored to the skin.

Lactate clamp procedure. Five days after the surgery, the animals were randomly assigned to control or DCA treatment groups (n = 4/group). The animals were fasted at 10:30 AM on the day before the lactate clamp study. At 7:45 AM on the day
of the study, the animals were given dosing vehicle (0.5% carboxymethylcellulose with 0.2% Tween 80; Sigma, St. Louis, MO) or DCA (1 mmol/kg; Aldrich, Milwaukie, WI) in vehicle by oral gavage (10 ml/kg). At 9:30 AM, the animals were placed in plastic metabolic cages after their cannulas were connected to blood sampling tubing or lactate infusion syringes mounted on infusion pumps (Harvard Apparatus Syringe Infusion Pump 22, Harvard Apparatus, South Natick, MA). The lactate infusate contained 20% sodium lactate in saline, and the pH was adjusted to 7.0 with 20% lactic acid in saline (Sigma, St. Louis, MO). At 10:20 AM, a blood sample (20 µl) was taken via the arterial cannula for the measurement of fasting blood lactate and glucose concentrations before the clamp (0 h). After the 0-h blood sample was taken, 0.2 ml of heparinized saline (100 U/ml) was injected via sampling line to prevent blood clotting in the line during the clamp. At 10:30 AM, a primed lactate infusion at 56 mg·kg⁻¹·min⁻¹ was given for 2 min via the venous cannula to quickly raise blood lactate levels. After the primed infusion, a constant lactate infusion at 2 mg/min was given for 3 min before an adjustment of infusion rate was made based on blood lactate levels. Blood samples were taken via the arterial cannula at 5-min intervals for lactate measurement and at 15-min intervals for glucose measurement during the 90-min clamp. Blood lactate levels were maintained at ~2 mM via the adjustment of lactate infusion rates. Blood lactate concentrations were determined immediately after sample collection using an Analox GM7 Micro-Stat multi-assay analyzer (Analox Instruments, London, UK), and blood glucose concentrations were determined using an Analox GM9 glucose analyzer. The blood sample amounts are required for the lactate and glucose assays are 7 and 10 µl, respectively, and the total blood loss due to sampling during the clamp was <0.3 ml.

Measurement of lactate production. In separate experiments, the effect of DCA treatment on endogenous lactate production was determined in the animals that received identical diet, cannulation surgery, fasting, and dosing treatment to those in lactate clamp experiments. The animals (n = 5 for control group, n = 6 for DCA group) were infused with L-[¹⁴C(U)]lactate (Du Pont, Wilmington, DE). A bolus infusion of 5 µCi of [¹⁴C]lactate was administered within 2 min, followed by a constant infusion at 0.1 µCi/min for 43 min. Blood samples (150 µl each) were collected at 30, 40, and 45 min for determination of plasma lactate concentrations and ¹⁴C counts. The blood samples were immediately centrifuged in a refrigerated centrifuge at 1,000 g for 10 min to separate the plasma. Fifty microliters of plasma were mixed with 100 µl of 10% TCA and then centrifuged to obtain the supernatant. Fifty microliters of supernatant were aliquoted into a scintillation vial, in duplicate, to which 5 ml of scintillation fluid were added and then counted for 10 min in a Beckman scintillation counter (model LS 3801; Beckman Instruments, Irvine, CA).

Data analysis. Data are reported as means ± SE. The average lactate infusion rate (mg·kg⁻¹·min⁻¹) at the steady state (60–90 min) of the clamp was calculated and used as an index for the whole body lactate utilization. The average specific activity of plasma [¹⁴C]lactate (dpm/mg) during the last 15 min of [¹⁴C]lactate infusion experiments was calculated and used to determine the rate of lactate production (mg·kg⁻¹·min⁻¹), which was calculated as the rate of tracer lactate infusion (dpm/min) divided by the plasma specific activity of lactate (1).

Statistical analysis was performed to compare the control and DCA-treated groups with the use of a two-way ANOVA with repeated measures for blood lactate and glucose levels and lactate infusion rates during the clamps, and an unpaired t-test was performed for fasting blood lactate and glucose levels before the clamps, the average lactate infusion rates at the steady state, and the lactate production rates. Statistical significance was accepted at P < 0.05.

RESULTS

Body weights and fasting blood lactate and glucose concentrations before the clamps are shown in Table 1. Fasting blood lactate and glucose levels measured before the clamps were almost identical for both groups. Blood lactate concentrations for the control and DCA-treated groups during the clamps are shown in Fig. 1. Although the blood lactate concentrations before the clamps were not different between the two groups, the same amount of lactate infusion for the first 5 min of the clamp caused a more rapid increase in blood lactate level in the control group compared with the DCA-treated group. Blood lactate levels during the clamps for both groups were maintained around 2 mM, indicating that the target lactate level of the clamp was achieved.

Lactate infusion rate during the clamps was markedly higher in DCA-treated animals compared with the control rats (P < 0.01) (Fig. 2). The significant difference in lactate infusion rates between the two groups was maintained throughout the clamps. The average lactate infusion rate from 60 to 90 min is shown in Fig. 3. The average lactate infusion rate during that period of the clamp reflects the whole body lactate utilization rate at a steady state. In DCA-treated rats, the average lactate infusion rate was 144% higher than that in the control rats (P < 0.001).

Figure 4 shows blood glucose concentrations during the clamps. Although blood glucose concentrations be-

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Blood Lactate, mM</th>
<th>Blood Glucose, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>354 ± 14</td>
<td>1.65 ± 0.19</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>DCA treated</td>
<td>329 ± 16</td>
<td>1.65 ± 0.37</td>
<td>82 ± 11</td>
</tr>
</tbody>
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Values represent means ± SE for 4 animals/group. DCA, dichloroacetate.

Fig. 1. Blood lactate levels before and during lactate clamps in control and dichloroacetate (DCA)-treated rats. Values are means ± SE for 4 animals/group.
fore the clamps were not different between the control and DCA-treated groups, 15 min after the onset of the clamp blood glucose level in the control animals was elevated and maintained at a higher level during the rest of the clamps. In contrast, blood glucose level in DCA-treated animals was maintained at the basal level until the end of the clamps. There was a significant difference between the blood glucose levels of the control and DCA-treated groups during the clamps (P < 0.05). The ANOVA, however, showed that the drift of the glucose levels during the clamps was not statistically significant.

In the study for the measurement of lactate production, blood lactate levels at 0 and 45 min were, respectively, 1.46 ± 0.04 and 1.44 ± 0.09 mM for the control group and 1.35 ± 0.07 and 1.38 ± 0.07 mM for the DCA-treated group. The difference between the two groups in blood lactate levels at both time points was not statistically significant. Lactate production rates determined during the last 15 min of [14C]lactate infusion experiments are shown in Fig. 5. There was no significant difference in lactate production between the DCA-treated and the control groups.

**DISCUSSION**

Blood lactate concentration measurement has been frequently used as an indicator of lactate metabolism in vivo. Although the measurement is simple and provides useful information, it does not adequately reflect either lactate production or utilization, because blood lactate level is only a set point at which lactate production and utilization reach a balance. The present study has established the lactate clamp method to measure the whole body lactate utilization. The results from this study show that lactate utilization can be measured adequately by the clamp method, as demonstrated by a significant enhancement of lactate infusion rate in animals treated with DCA compared with the animals in the control group. This significantly higher lactate infusion rate was maintained throughout the clamp, providing a clear indication of enhanced lactate utilization.

To clamp the blood lactate at a desired level, it is necessary to rapidly raise and then maintain the blood lactate at a level that is higher than the basal lactate concentration. A very mild hyperlactatemia was achieved in the present study, which is within the physiological range of blood lactate. The 21% increase of blood lactate level from 1.65 mM at basal to 2.0 mM during the clamp is much smaller than the lactate elevations seen in studies using constant lactate infusion methods, in which lactate concentrations were raised to at least two- to fourfold of the basal concentration (8, 19). The minimal hyperlactatemia applied in...
the lactate clamp method provides a significant advantage over the more severe hyperlactatemia shown in the traditional constant lactate infusion methods. First, a severe hyperlactatemia could exert a profound effect on carbohydrate metabolism and electrolytic/acid-base balance, and, therefore, may affect the hormonal responses to the changes in metabolism. Second, without a stable blood lactate level being achieved, a constant lactate infusion may result in different levels of hyperlactatemia among individuals, which in turn may induce different levels of lactate removal and inhibition of endogenous lactate production and thus make the comparisons among the individuals less meaningful. To choose an optimal blood lactate level for the clamp, lactate clamp at 3 mM of blood lactate was also evaluated in our pilot studies. The results showed that the clamp at 2 mM was more sensitive to the DCA treatment than the clamp at 3 mM (unpublished data). It is important to maintain the experimental condition as close to the physiological condition as possible. However, to measure lactate utilization under various conditions, such as diabetes for example, it may be necessary to clamp blood lactate at levels other than 2 mM.

Isotope tracer technique has been applied to the measurement of lactate turnover rate (1, 10, 18). Lactate turnover rate is calculated by dividing the lactate tracer infusion rate by the lactate specific radioactivity in the blood. When a steady state is achieved during tracer infusion, rate of lactate turnover equals rate of lactate production and rate of lactate utilization (2). Although the lactate tracer dilution technique has been used extensively to assess lactate metabolism, the measurement of lactate turnover rate might be complicated because that lactate tracer is reversibly converted into pyruvate before being irreversibly removed from the system (13). The total loss of labeled lactate to pyruvate in blood amounts to ~10% of lactate disappearance at rest (12). Also, radioactivity of plasma $^{14}$CO$_2$ derived from lactate oxidation, although in a negligible amount, may need to be taken into consideration when the lactate turnover rate is calculated. Various compartmental models have been proposed to modify the calculation for the lactate turnover rates (12, 16). Although rapid isotopic exchange between lactate and pyruvate is taken into account in these models, the interpretation of lactate kinetic data obtained from the use of lactate tracer should be made with caution. Unlike the lactate tracer technique, the lactate clamp is a simple method to measure lactate utilization during a very mild hyperlactatemia. Aside from the simplicity of the measurement, the lactate clamp also provides the assessment for the capacity of lactate utilization, which cannot be determined by the tracer technique.

During the clamp, the lactate appearing in the blood may originate from both endogenous and exogenous sources, i.e., the lactate produced from tissues and the lactate infused. Thus the increased lactate infusion rate during the clamps in DCA-treated animals may reflect reduced endogenous lactate production, i.e., the lactate utilization in animals treated with DCA might be overestimated by using lactate infusion rate as an indicator. To investigate this possibility, the effect of DCA treatment on endogenous lactate production was measured. The results for lactate production rate determined from the $[^{14}$C]lactate infusion experiments clearly show that the treatment with DCA did not alter lactate production rate in these animals. The lactate production rate during the clamps was not measured in the present study to keep the clamp method relatively simple. It has been reported that infusion of lactate at a rate of 140 mg kg$^{-1}$h$^{-1}$ completely inhibited endogenous lactate production in humans (14). The lactate infusion rates in the present study were much higher (averaged at 306 ± 60 and 738 ± 36 mg kg$^{-1}$h$^{-1}$ for the control and DCA-treated groups, respectively), and a complete inhibition of endogenous lactate production could be expected. Although it is difficult to compare studies using different species, in light of the small amount of lactate production under basal condition and the same blood lactate levels during the clamps in both groups in the present study, even if the lactate production was not completely shut down, it is very unlikely that the difference between the control and DCA-treated groups in lactate production rate, if any, would have much impact on lactate infusion rate during the clamps. Therefore, the lactate infusion rate during the clamps may closely represent the rate of the whole body lactate utilization. There is a controversy about the lactate turnover measurement using arterial (venous-arterial procedure) or venous (arterial-venous procedure) lactate specific activity (1, 10). We did not compare the two procedures in the present study. However, the purpose of the lactate turnover measurement was to address whether there was a difference between the control and DCA-treated groups. The outcome may not be affected by using either procedure, although values obtained from the two procedures may be different.

Although DCA is known to increase lactate utilization by its inhibition of PDH kinase, as demonstrated by increased PDH activity in various tissues (17), the effect of DCA on blood lactate concentration in animals was not always apparent. We have routinely observed that the decrease in blood lactate concentration in DCA-treated (1 mmol/kg, maximally effective dose) rats is no more than 30%, and the effect of DCA usually peaks between 2 and 4 h after the treatment (unpublished data). In the present study, the blood lactate concentration before the clamp was not even affected by the DCA treatment. Thus measuring blood lactate concentration per se is not a reliable way to assess lactate utilization. Despite the lack of effect on blood lactate concentration before the clamps, the DCA treatment markedly increased lactate infusion rate by 2.4-fold during the clamps. The significant and consistent stimulation of lactate removal during the clamps after the DCA treatment suggests that lactate flux is greatly enhanced by the treatment. It also suggests that lactate infusion rate during the clamp is a sensitive and reliable measurement for assessing lactate utilization in vivo.
In addition to the change in lactate infusion rate, blood glucose concentration in DCA-treated animals during the clamps was significantly lower than that in the control animals, despite the similar blood glucose levels for the two groups before the clamps. It has been hypothesized that lactate formed in one tissue could distribute through interstitium and circulation to other tissues to be oxidized or used for other metabolic processes, such as gluconeogenesis, a lactate shuttle theory supported by several lines of evidence (3). A lower blood glucose level and a greater lactate infusion rate during the clamps in the DCA-treated group suggest that the excess lactate was oxidized rather than being used as a precursor for gluconeogenesis in the liver. It has been reported that DCA effectively inhibits gluconeogenesis from lactate in isolated hepatocytes by limiting substrate availability as well as the direct inhibition from oxalate, a metabolite of DCA (6).

Although hepatic glucose production during the lactate clamp was not measured, our recent study measuring hepatic glucose production after an oral lactate administration showed that DCA treatment prevented a significant elevation of hyperlactatemia-stimulated hepatic glucose production in rats (9). Thus, when combined with the measurement of hepatic glucose production during the clamps, the lactate clamp method may provide a useful assessment for the status of the Cori cycle, especially during starvation, or with certain metabolic disorders, such as diabetes mellitus (4, 7).

In conclusion, the lactate clamp provides a sensitive and reliable method to assess in vivo lactate utilization, enzyme deficiency, or other dysfunctions (11). The pharmacologic actions of dichloroacetate and 2-chloropropionate. Arch. Biochem. Biophys. 198: 145–152, 1979.


