Effect of lactic acid on plasma free fatty acids in pancreatectomized dogs

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MILLER, H. I., B. ISSEKUTZ, JR., P. PAUL, AND K. RODAHL. Effect of lactic acid on plasma free fatty acids in pancreatectomized dogs. Am. J. Physiol. 207(6): 1226-1230. 1964—Sodium lactate infusions into unanesthetized pancreatectomized dogs, with indwelling arterial and venous catheters, markedly decreased the plasma free fatty acid (FFA) level. Infusions of palmitate-1-Cl4 at constant rates showed that the rate of release of FFA was considerably reduced by the lactate. There was an inverse correlation between the logarithm of the plasma FFA concentrations and the logarithm of the blood lactate levels. Glucose infusion alone had no significant effect on the plasma FFA of the pancreatectomized dog. When both lactate and glucose were infused into the pancreatectomized dog, the plasma FFA was inversely correlated with the blood lactate level but not with the blood sugar. When the plasma FFA was elevated in normal dogs by norepinephrine infusion, the FFA-lowering effect of sodium lactate was not prevented. Neither acetylcholine nor nitroglycerine infusions had any marked effect on the plasma FFA. It is concluded that lactic acid has a direct effect on the release of FFA which does not require the presence of insulin and is independent of the blood glucose concentration.

plasma fatty acid turnover lactate, glucose, and FFA in diabetic dogs FFA release and lactacidemia pyruvate and FFA turnover

Our previous studies in unanesthetized dogs have shown that an inverse correlation exists between the logarithm of the lactate level of the blood and the logarithm of the free fatty acid (FFA) concentration of the plasma (6). Intravenous infusions of lactic acid or sodium lactate decreased the plasma FFA level, and in experiments carried out with palmitate-1-Cl4 lactate infusion, caused an increase in the specific activity of plasma FFA, thus indicating that the lactate infusion decreased the rate of release of FFA presumably from the adipose tissue (9). From these experiments it could not be determined, however, whether this was a direct effect of lactic acid or secondary to increased insulin production. Lactate infusions occasionally may induce a slow rise of the blood glucose concentration up to 90-115 mg/100 ml, which in turn could cause release of insulin (4) and hence concomitant decrease of the FFA turnover (1). Colwell and Lein (2) observed a negative correlation between the logarithm of the blood glucose level and the logarithm of the plasma FFA, and came to the conclusion "that in the normal dog, plasma fatty acid level is under some control by plasma glucose, particularly in the normal operating range of glucose concentration." However, under physiological circumstances, the blood lactate level shows considerably greater variations than the blood sugar; even in the resting dog a 100% fluctuation (between 7 and 14 mg/100 ml) is a frequent finding, and during exercise it may rise, unlike the blood sugar, eight- to tenfold within a few minutes. Therefore it seemed desirable to obtain further information about the effects of lactic acid on plasma FFA in the absence of insulin or in the presence of excess norepinephrine. It was of interest also to carry out comparative experiments with glucose and pyruvate infusions. Furthermore, since all these metabolites (glucose, lactate, pyruvate) have some vasodilating action which might cause a circulatory shunting of the adipose tissue, the effect of other vasodilating agents, such as acetylcholine and nitroglycerine, was also studied in a few experiments.

METHODS

Mongrel dogs weighing 10-15 kg were pancreatectomized in morphine-pentothal anesthesia. The animals were given 10-20 units of insulin daily, according to their needs to control glycosuria. They were kept on normal Purina dog chow fortified with meat, pancreatic tablets, and methionine. Three to four weeks after the operation, the dogs were lightly anesthetized with morphine-pentothal, and indwelling polyethylene catheters were placed into the carotid artery and into the jugular vein; no anticoagulant was used. After 3-4 days of recovery, the animals were trained to lie quietly on a table for several hours. Insulin was withheld 48 hr before the experiment. All experiments were carried out in a
postabsorptive state (18–20 hr fasting). After a standardization period of about 90 min, sodium-L (+) lactate (pH 7.4) was infused intravenously at a rate of 7–15 mg/kg min for 35–40 min, followed by a recovery period of 70–80 min. In other experiments the lactate was infused at a low rate of 3–4 mg/kg min for 190–200 min.

Palmitate-1-C14 bound to human albumin (specific activity about 10 &muEq) was infused intravenously throughout the entire experimental period (190–200 min) at a constant rate of 10–20 &muEq/kg min. In experiments on normal dogs, noradrenaline was infused intravenously at a constant rate of 0.4–0.5 &muEq/kg min. A double-lumen catheter was used for the lactate infusion in both types of experiments. Both acetycholine (0.3–0.6 &muEq/kg min) and nitroglycerine (6–8 &muEq/kg min) were infused intra-arterially to avoid a direct cardiac effect. At time intervals shown on Figs. 1, 2, and 4, arterial blood samples were taken for measurements of plasma FFA concentration (3) and radioactivity (g), blood lactate (12), plasma protein (8), and blood glucose (Glucostat method; Glucostat reagent, Worthington Biochemical Corp., Freehold, N. J.). In experiments with infusion of radiopalmitate, the radioactivity of the infusate (m&muEq/ml) was also measured (9). A Tri-Carb liquid-scintillation counter with an efficiency of about 65% was used for these measurements. The rate of release and the rate of uptake of FFA (m&muEq/kg min) were calculated according to the equations suggested by Steele (11) as in our previous studies (7).

RESULTS

Figure 1 shows the typical effect of sodium lactate infusion on the FFA turnover in a pancreatectomized dog. As the blood lactate level rose, the plasma FFA decreased and the specific activity of FFA increased. This is essentially the same response observed in normal dogs under similar experimental conditions (9). Calculation of the turnover rates revealed that lactate infusion decreased the rate of release from 32 &muEq/kg min to 20.5 &muEq/kg min. The rate of uptake (shaded area) followed the rate of release but with some delay. This delay caused the decrease of the plasma FFA concentration. A pronounced effect could be seen in the first 10 min of infusion when the blood lactate increased from 7 mg/100 ml to 23 mg/100 ml. The blood sugar level showed but little change; it rose from 300 to 470 mg/100 ml by a glucose infusion. This manipulation failed to change the course of plasma FFA. Since the large amount of glucose infused caused a considerable (about 10%) dilution of the plasma protein.
the plasma FFA level was expressed as microequivalents per gram protein. The logarithms of these values were plotted against the logarithm of the blood lactate (Fig. 2B) and the logarithm of blood sugar concentrations (Fig. 2C), respectively. A significant negative correlation was found only with the lactate concentration \( r = -0.98, P < .001 \) but not with that of glucose \( r = -0.40, P > .1 \).

Statistical evaluation of the 17 experiments carried out on normal dogs (140 samples) and of 9 experiments conducted on pancreatectomized animals (92 samples) showed that in both groups the same type of negative correlation existed between the logarithm of the plasma FFA level and the logarithm of the concentration of blood lactate (Fig. 3).

The regression equations, which describe this negative correlation for the range of 4–120 mg/100 ml lactate, are given in their exponential forms in the legend of Fig. 3. Both regression coefficients \( 0.59 \pm 0.05 \) for normal dogs and \( 0.41 \pm 0.07 \) for pancreatectomized dogs) are significant at the \( P < .001 \) level, but they do not differ significantly from one another. The difference between the two constants \( 1.74 \) and \( 2.88 \) are, however, significant at the \( P < .001 \) level; in other words, at the same lactate level the pancreatectomized animals had a higher FFA concentration than the normal dogs.

During exercise, when the blood lactate level rises, the blood norepinephrine concentration also is elevated (13). Therefore, it was necessary to investigate the effect of lactate on the norepinephrine-induced elevated FFA level of the normal dog. Figure 4 shows three experiments in which the plasma FFA was maintained at a moderately elevated level by infusion of norepinephrine. In two of these experiments sodium lactate, and in a third experiment acetylcholine, was infused. An increase of the blood lactate level from 7 to 35 mg/100 ml caused a 50–60% drop of the plasma FFA even in the presence of excess norepinephrine, whereas intra-arterial infusion of acetylcholine under similar conditions had a negligible effect (5% decrease).

Table 1 summarizes the results of the various experiments. This table shows the algebraic means of the values obtained before and at the end of the 35- to 40-min test infusions. Lactate infusions lowered the plasma FFA in both normal and pancreatectomized dogs and caused a 51–64% decrease also in dogs infused with norepinephrine. Acetylcholine (four experiments) and nitroglycerine (two experiments) did not alter the blood lactate concentrations and had a negligible effect on the plasma FFA. Sodium pyruvate was infused into three normal dogs and one pancreatectomized animal, the infusion rate ranging from 7.5 to 33.2 mg/kg min. Pyruvate proved to be very effective in all cases but it should be pointed out that pyruvate rapidly increased the blood lactate level, and therefore the FFA-lowering effect of the pyruvate could not be separated from that of lactate.

Table 1 also shows the result of glucose infusions in two diabetic dogs, the blood sugar rose from 250 to 280 mg/100 ml to the range of 700 mg/100 ml without causing any appreciable decrease of the plasma FFA. In general, whenever the blood lactate level was increased, the plasma FFA concentration declined regardless of whether this occurred at blood sugar levels of 70–90 mg/100 ml (normal dogs) or at 230–400 mg/100 ml (in diabetic animals).

**DISCUSSION**

These findings led to the conclusion that the effect of lactate on the FFA turnover was not mediated by insulin and was not due to an increase of the blood sugar level. Colwell and Lein (2), who infused glucose into four pancreatectomized dogs, found in two animals a significant negative correlation between the logarithm of plasma sugar and the logarithm of plasma FFA. In two of our experiments, glucose infusions into diabetic dogs failed to cause any appreciable decrease of plasma FFA (Table 1). In a third experiment, the FFA level was lowered by 36% (from 1.35 to 0.86 mEq/liter) but the blood lactate concentration rose in the course of the experiment from 12 to 37 mg/100 ml. If glucose infusions, elevating the blood sugar up to 700 mg/100 ml, failed to show any consistent effect on the plasma FFA of pancreatectomized dogs, then the effect of lactate infusions cannot be attributed to the slight increase (20–30 mg/100 ml) of blood sugar occasionally seen in these types of experiments.

Another possibility which deserves some consideration is that the circulatory effect of lactate infusions. Lactic acid is a well-known vasodilator which might increase the blood flow through the muscles with a compensatory vasoconstriction in the skin or in the splanchic areas including their adipose tissues. Such an effect could depress the rate of release of FFA from the adipose tissues. In this case, other more potent vasodilators, such as nitroglycerine or acetylcholine would be expected to cause a similar decrease of plasma FFA level. The circulatory effects of these two drugs infused intraarterially at a rate comparable to that applied in this study have been analyzed and published earlier (5).
FIG. 4. Three experiments on normal dogs. Effect of sodium lactate and acetylcholine, respectively, on the plasma FFA concentrations elevated by norepinephrine infusions.

Since neither of these vasodilators affected the plasma FFA level, it seems unlikely that the circulatory effect of the lactate infusion could be responsible for the decreased rate of release of FFA.

Although it may be premature to speculate about the mode of action of lactic acid, it should be pointed out that Peterson et al. (10) recently showed the existence of competition between pyruvate and dihydroxyacetone P for the DPNH₂ in ischemic muscle. This competition resulted in a striking rise both in lactate and in α-glycerophosphate concentrations. The oxidation of the excess lactate may give rise to excess DPNH₂, and whereas the resulting pyruvate could be fed into the Krebs cycle, the DPNH₂ would reduce the dihydroxyacetone-P to α-glycerophosphate which, in turn, can capture the FFA. One would expect that a similar mechanism in the adipose tissue would depress the rate of release of FFA, in a way resembling the effect of insulin but without requiring the presence of insulin. The observation that pyruvate has a similar effect does not rule out this possibility because pyruvate infusion sharply increases the blood lactate level.

### TABLE 1. Effects of infusions of lactate or pyruvate on plasma FFA in normal and in diabetic dogs

<table>
<thead>
<tr>
<th>Blood lactate, mg/100 ml</th>
<th>Blood glucose, mg/100 ml</th>
<th>Plasma FFA, mEq/L</th>
<th>Test Infusion†</th>
<th>Infusion Rates (Range), mg/kg min</th>
<th>At End of Test Infusion§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Na-lactate (17)</td>
<td>6.5-29.6 (29-124)</td>
<td>91 (70-115)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Na pyruvate (5)</td>
<td>7.49 33.2</td>
<td>48.8 (21-71)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Acetylcholine (4)</td>
<td>0.0003-0.0006</td>
<td>5.7 (5-6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nitroglycerine (2)</td>
<td>0.0006-0.0008</td>
<td>6.4 (6-7)</td>
</tr>
</tbody>
</table>

* Values are means ± se. † Figures in parentheses represent number of experiments. § Values are means, with ranges given in parentheses. $ The difference between the two means is significant (P < .001).
REFERENCES


