Catecholamine response is attenuated during moderate-intensity exercise in response to the “lactate clamp”

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Fattor, Jill A., Benjamin F. Miller, Kevin A. Jacobs, and George A. Brooks. Catecholamine response is attenuated during moderate-intensity exercise in response to the “lactate clamp.” Am J Physiol Endocrinol Metab 288: E143–E147, 2005. First published August 24, 2004; doi:10.1152/ajpendo.00117.2004.—Catecholamine release is known to be regulated by feedback mechanisms. Norepinephrine (NE) and epinephrine (Epi) concentrations rise in response to stresses, such as exercise, that challenge blood glucose homeostasis. The purpose of this study was to assess the hypothesis that the lactate anion is involved in feedback control of catecholamine concentration. Six healthy active men (26 ± 2 yr, 82 ± 2 kg, 50.7 ± 1.4 kg·m⁻²·h⁻¹) were studied on five occasions after an overnight fast. Plasma concentrations of NE and Epi were determined during 90 min of rest and 90 min of exercise at 55% of peak V O₂ consumption (VO₂peak) two times with exogenous lactate infusion (lactate clamp, LC) and two times without LC (CON). The blood lactate profile (~4 mM) of a preliminary trial at 65% VO₂peak (65%) was matched during the subsequent LC trials. In resting men, plasma NE concentration was not different between trials, but during exercise all conditions were different with 65% > CON > LC (65%: 2,115 ± 166 pg/ml, CON: 1,573 ± 153 pg/ml, LC: 930 ± 174 pg/ml, P < 0.05). Plasma Epi concentrations at rest were different between conditions, with LC less than 65% and CON (65%: 68 ± 9 pg/ml, CON: 59 ± 7 pg/ml, LC: 38 ± 10 pg/ml, P < 0.05). During exercise, Epi concentration showed the same trend (65%: 262 ± 37 pg/ml, CON: 190 ± 34 pg/ml, LC: 113.2 ± 23 pg/ml, P < 0.05). In conclusion, lactate attenuates the catecholamine response during moderate-intensity exercise, likely by feedback inhibition.

norepinephrine; epinephrine; exogenous lactate infusion; sympathetic response; fuel sensing

LACTATE’S IMPORTANCE as an energy source and gluconeogenic precursor is made apparent by its proposed roles in the lactate shuttle (5, 6, 24, 29). Additionally, the role of lactate as a mediator of substrate partitioning has been recognized. Exogenous lactate infusion has been found to reduce both glucose uptake and utilization in rats in vivo (16) and glucose oxidation in isolated resting rat soleus muscle (23). As well, we recently reported that exogenous lactate infusion decreases glucose oxidation in humans during moderate-intensity exercise (21). Initially, we concluded that the site of action was largely peripheral because lactate decreased glucose disposal and oxidation through competitive inhibition and because skeletal muscle is the major site of glucose and lactate disposal. However, these peripheral interactions do not exclude the possibility of an additional mechanism of the lactate action, for example, that lactate attenuates the catecholamine response to physical activity.

A recent report by Borg et al. (4) provided initial support for a central role of lactate in indirectly mediating glucose rate of appearance (R a) by demonstrating that perfusion of the ventromedial hypothalamus (VMH) of rats with lactate suppressed hypoglycemic counterregulation, as assessed by catecholamine release. Presumably, the VMH sensor interpreted sufficiency of fuel supply by the presence of lactate. Several groups have used exogenous lactate infusions to determine the effect of lactate on cognitive dysfunction in hypoglycemia. Maran et al. (17, 18) and Veneman et al. (30) demonstrated that brain function is maintained during hypoglycemia with exogenous lactate infusion. However, whether catecholamine concentration would be lower in euglycemic exercising humans with exogenous lactate infusion is unknown.

Although plasma catecholamine concentration has been shown to correlate positively with blood lactate concentration (8), it is assumed that catecholamines, specifically epinephrine (Epi), are the independent variables and that blood lactate concentration is dependent on adrenergic stimulation of muscle glycogenolysis. Seldom is the converse considered, that is, that blood lactate concentration provides a feedback signal in the regulation of catecholamine release.

The current study was undertaken to evaluate the hypothesis that lactate is involved in feedback control of catecholamine concentration. Therefore, we determined catecholamine concentration during the lactate clamp (LC) procedure in resting and exercising men. If true, the hypothesis predicts that, with exogenous lactate infusion, the catecholamine concentration would be lower during an exercise bout sufficient to elevate metabolic rate but minimally affect blood lactate concentration. In addition, because catecholamines affect glucose R a and disappearance (R d) directly (i.e., by substituting other fuels for glucose in working muscle) and indirectly (i.e., by suppressing insulin action), we predicted that the catecholamine response would correlate with glucose R a and R d.

MATERIALS AND METHODS

Methodology regarding subject selection, study design, data analysis, and interpretation has been previously published (20). In the current report, we focus on the sympathetic response to the LC, with pertinent information from the previous report included for the convenience of readers.

Subjects. Six healthy men aged 18–32 yr were recruited from the Berkeley campus through posted notices and E-mail. Subjects were either competitive collegiate cyclists (n = 3) or moderately active (physically active 6 or more h/wk, n = 3). Subjects were included if they were nonsmokers, not taking medications affecting metabolism, free of injury and illness determined by a physical examination, had

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<25% body fat, and had normal lung function (at least 75% of forced vital capacity expired within 1 s). This study adhered to the principles of the Declaration of Helsinki, subjects gave their written and oral informed consent, and the Committee for the Protection of Human Subjects at the University of California, Berkeley, approved the studies (CPHS 2003–8–25).

**Initial screening.** Two graded-exercise tests on an electrically braked cycle ergometer (Monark 539E; Monark Exercise Laboratories) were used to determine peak O2 consumption (V\textsubscript{O2 peak}) and lactate threshold. Subjects warmed up and then started the test at 100 watts and increased workload 25–50 watts every 3 min until volitional exhaustion.Expired gases were analyzed using an indirect open-circuit system (CD3A for CO\textsubscript{2} and S3A1 for O\textsubscript{2}; Ametek, Paoli, PA) with inspiratory ventilation determined with a Fleisch pneumotachometer (Lausanne, Switzerland). Analog signals from the three sensors were digitized, and ventilatory parameters were calculated in real time (20, 21). The two exercise tests were performed at least 1 wk apart. The second test included inserting an indwelling catheter to draw arm-vein blood for the determination of blood lactate concentration and lactate threshold. Body fat was tested using the seven-site skinfold method (13). A Collins spirometer was used for pulmonary function testing (WE Collins, Braintree, MA).

**Study design.** At least 1 wk after initial screening, subjects were scheduled to complete five trials. The trials consisted of 90 min of rest followed by 90 min of exercise. The exercise intensity for the first trial was 65% of V\textsubscript{O2 peak} (65%). The subsequent trials were performed at 55% of V\textsubscript{O2 peak} with or without sodium-lactate infusion at a rate that maintained resting levels of blood lactate at 4 mM and exercising blood lactate levels matching the 65% trial; we have termed this associated measurement and lactate threshold. Body fat was maintained resting levels of blood lactate at 4 mM and exercising blood lactate levels during exercise matched to the individual’s 65% trial levels. The infusion rates during the second of the two 55% LC trials were matched to the first (20).

**Data collection and analysis.** Electrical activity of the heart was monitored using a 12-lead electrocardiogram (model Q750; Quinton, Bothell, WA), and heart rate (HR) was recorded every 5–15 min. Blood pressure was by auscultation every 15 min, and mean arterial pressure (MAP) was calculated as one-third the pulse pressure plus diastolic. Blood metabolites and hormones were analyzed from blood samples taken at 0, 60, 75, and 90 min of rest and 30, 45, 60, 75, and 90 min of exercise. Specifics on metabolites, insulin, and glucagon determination have been reported previously (20). Blood samples for catecholamine determinations were collected in chilled tubes containing glutathione and EGTA to prevent oxidation. Blood was immediately centrifuged at 3,000 g. Plasma was transferred to a storage tube and was frozen at −80°C until analysis. Catecholamines were extracted from the plasma using methods adapted from Anton and Sayre (1) using acid-washed WA-4 Alumina (Sigma) and 1.5 M Tris buffer containing 2% EGTA at a pH of 8.6. Perchloric acid (0.1 M) was used to elute the catecholamines. Finally, 100 μl of this eluent were injected in the HPLC system (Electrochemistry Separations Analysis, ESA, model LC/EC, 5200A; Coulochem, Clemsford, MA). The mobile phase was Cataphase 2 (ESA, Cambridge, MA), and the electrodes were set at +350, +50, and −350 mV. Standard catecholamine solutions were purchased from ESA. Chromatographs were analyzed using ESA’s 501 Data Chromatography System.

**Statistics.** For the purposes of this analysis, the two LC trials and the two control trials are combined and termed LC and CON, respectively. The resting data from the 65% trial were combined with the CON trial when there was no difference between them, and this is presented as CON. Data are presented as means ± SE. Significant differences were tested using repeated-measures ANOVA (Grad Pack 10.0; SPSS, Chicago, IL), and the α-level was set at 0.05. Significant results were further analyzed post hoc with the least significant difference test. Mean data are the last three time points of rest (60, 75, and 90 min) and the last three time points of exercise (60, 75, and 90 min). Pearson product-moment correlation coefficient was used to measure the relationship between variables.

**RESULTS**

**Subject characteristics.** Subject characteristics were previously reported (20) and are reproduced in Table 1 for convenience. Subjects’ fitness levels and diets did not change during the 5-wk testing period, and subjects remained weight stable throughout the testing period.

**LC.** During the resting phase, blood lactate was significantly elevated during the LC (2.80 ± 0.10 mM) compared with the combined resting phase of CON and 65% (0.82 ± 0.27 mM; Fig. 1). As well, the blood lactate concentration during the 55% LC trial (2.61 ± 0.11 mM) was not significantly different from that during the 65% exercise trial (2.45 ± 0.27 mM), and both were higher than CON (1.66 ± 0.09 mM). Over time, both LC and 65% blood lactate concentrations were higher than the CON at all exercising time points until 90 min, since the blood lactate concentration dropped over the exercise period after the initial increase at the start of exercise.

**Sympathetic response variables.** Resting HR during LC was significantly higher than CON (CON: 59 ± 3, LC: 67 ± 3 beats/min, P < 0.05; Table 2). There were no significant differences between the CON and LC for exercising HR (CON: 135 ± 12, LC: 140 ± 16 beats/min, P = 0.19; Table 2). As well, there were no significant differences in blood pressure between the two conditions during exercising time points (Table 2). As well, there were no significant differences in body temperature between the two conditions during exercising time points (Table 2). As well, there were no significant differences in blood pressure between the two conditions during exercising time points (Table 2). As well, there were no significant differences in body temperature between the two conditions during exercising time points (Table 2).
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Fig. 1. Mean blood lactate concentration ([lactate]) at rest and exercise with and without the lactate clamp (LC, + SE). Shown are data for controls (CON) and LC at 55% peak O$_2$ consumption (V˙O$_{2\text{peak}}$); 65% indicates 65% V˙O$_{2\text{peak}}$. *P < 0.05, significantly different from resting CON (+) and significantly different from CON (*).

Rest Exercise

[65%, CON, LC]

5.00 4.50 4.00 3.50 3.00 2.50 2.00 1.50 1.00 0.50 0.00

mM

Values are means ± SE; n = 6 subjects. CON, controls; LC, lactate clamp. CON and LC exercise at 55% V˙O$_{2\text{peak}}$. *P < 0.05, significantly different from resting CON (†) and significantly different from 65% (‡). All variables were significantly higher during exercise compared with rest (P < 0.05).

Table 2. Cardiovascular response during rest and exercise with and without exogenous lactate infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest</th>
<th>Exercise</th>
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<tr>
<td></td>
<td>65%</td>
<td>CON</td>
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<td>HR, beats/ min</td>
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<tr>
<td>MAP</td>
<td>59±2</td>
<td>59±6</td>
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<td>mmHg</td>
<td>90.2±1.7</td>
<td>89.1±1.8</td>
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Values are means ± SE; n = 6 subjects. CON, controls; LC, lactate clamp. CON and LC exercise at 55% V˙O$_{2\text{peak}}$. *P < 0.05, significantly different from resting CON (†) and significantly different from 65% (‡). All variables were significantly higher during exercise compared with rest (P < 0.05).

143 ± 1, LC: 142 ± 1 beats/min, but 65% was higher than both (159 ± 4 beats/min, P < 0.05). MAP was not different at rest (CON: 89.1 ± 1.6, LC: 88.1 ± 1.7 mmHg) but was significantly lower during exercise with LC compared with CON (CON: 95.1 ± 1.6, LC: 90.1 ± 1.9 mmHg, P < 0.05).

As expected, the ANOVA revealed a significant main effect of exercise on both plasma NE and Epi concentrations, with CON higher than LC (65%: 68 ± 9 pg/ml, CON: 59 ± 7 pg/ml, LC: 38 ± 10 pg/ml, P < 0.05). During exercise, Epi concentrations showed the same trend as in resting subjects (65%: 262 ± 37 pg/ml, CON: 190 ± 34 pg/ml, LC: 113 ± 23 pg/ml, P < 0.05). There was also a condition-by-time effect, with LC significantly lower than CON at all exercising time points for NE and the last two time points for Epi (P < 0.05). During the last hour of exercise, NE concentration was significantly higher in 65% than both CON and LC, and Epi was higher in 65% than LC.

The sympathetic response variables NE, Epi, MAP, and HR were significantly correlated (r > 0.8, P < 0.05) with previously reported glucose flux rates from this study (20) when all conditions are considered. When isolating exercise conditions (rest data excluded), NE and Epi concentrations correlated moderately with glucose R$_d$ (NE: r = 0.5, Epi: r = 0.5, P < 0.05) and R$_g$ (NE: r = 0.6, Epi: r = 0.6, P < 0.05). The HR and MAP relationship with NE and Epi concentration was not significant with rest excluded.

DISCUSSION

We examined the effects of exogenously infused lactate on the catecholamine responses in men resting and engaged in an exercise bout that ordinarily has a minimal effect on blood lactate concentration. Our novel finding was that catecholamine concentrations were lower during exercise with the infusion of exogenous lactate despite euglycemia throughout the exercise bout. As well, MAP was correspondingly lower during the exogenous lactate infusion (clamp) procedure. This lends evidence to the feedback control of catecholamines by peripheral infusion of CHO fuel in the form of lactate. Feed-back control of catecholamines and lactate-catecholamine-glucose interactions will be given consideration.

Feedback control of catecholamines. Recent reports support the role of lactate as a potential mediator of catecholamine secretion. Specifically, studies on the VMH directly implicate lactate’s involvement in the counterregulatory process. Borg et al. used local perfusion of the rat VMH with lactate (4) or glucose (3) during a hypoglycemic clamp to demonstrate that sensors in the VMH respond to both fuels and as such attenuate the counterregulatory response. Studies by Yang et al. (34) support this finding in that the glucose-responsive neuron in the rat VMH was not only responsive to glucose, but also to lactate as well as mannose, galactose, glyceraldehyde, and glycerol, but not pyruvate. These data suggest that the glucose sensor in the VMH may be better termed “metabolic sensor” (15). The portal vein is also a potential site of metabolic sensing. Nijima (22) has demonstrated that several different metabolites infused in the portal vein can influence vagus nerve discharge rates. Donovan and colleagues (10, 12) also found that the Epi response was 50% lower when the portal vein of rats and dogs was perfused with glucose during peripheral hypoglycemia, demonstrating feedback control of the catecholamines. In addition to these animal models, Maran et al. (17, 18) and Veneman et al. (30) showed that peripheral venous infusion of lactate with systemic hypoglycemia in humans lowered catecholamine concentration after lactate infusion compared with hypoglycemia alone. Results of these studies are in agreement with those of the current investigation in that Epi and norepinephrine (NE) were lower with the lactate infusion.

Although blood glucose concentration was normal and constant during exercise (65%: 4.5 ± 0.1, CON: 4.6 ± 0.1, LC: 4.3 ± 0.1 mM), there was over a twofold increase in glucose R$_d$ in the transition from rest to exercise. In the current study, peripheral lactate infusion resulted in attenuation of the sympathetic response to exercise where both NE and Epi were 41% lower during exercise with LC compared with CON. With LC, we delivered an average of 2.98 ± 0.13 mg·kg$^{-1}$·min$^{-1}$ (averaged 1.02 ± 0.04 kcal/min) of lactate during the resting phase and 2.76 ± 0.22 mg·kg$^{-1}$·min$^{-1}$ (averaged 0.93 ± 0.08 kcal/min) during the exercise phase, which was sufficient to provide a feedback signal to attenuate the sympathetic response during exercise after a 12-h fast.
Lactate-catecholamine-glucose interactions. The priority of glucose homeostasis is demonstrated by the complexity of mechanisms working in concert to maintain precise control of blood glucose concentration. Both central and peripheral mechanisms control the supply and demand of glucose and alternative substrates, thus supporting glucose homeostasis. Central mechanisms include sympathetic activation and subsequent catecholamine release, which elicit increased gluconeogenesis (32), hepatic (33) and skeletal muscle (31) glycogenolysis, and lipolysis in adipose tissue. These responses affect glucose $R_g$ and $R_d$ directly as well as indirectly by suppressing insulin action (7). Consequently, it is not surprising that we (7) and others (26) have shown significant correlations between glucose $R_g$ and circulating NE in exercising men. In the current study, glucose concentrations were unchanged during exercise both with and without the LC. However, as previously reported (20), both glucose $R_g$ and $R_d$ were lower with the LC. Indeed, parameters of glucose kinetics and catecholamine were correlated. Although a correlation cannot denote a causative relationship, these results can be interpreted to suggest that catecholamines participate in a mediating role in glucose kinetics. Besides Epi and NE, exogenous lactate infusion had no effect on other measured glucoregulatory hormones (20).

Results with exogenous lactate infusion in exercising men are informative, since LC lowers both catecholamines and glucose flux.

An interesting result was that Epi was lower at rest with the LC, indicating that lactate may also influence the adrenal gland as well. Paradoxically, $V_{O_2}$ and HR were higher at rest with LC. We suspect that the higher metabolic rate is the result of increased CHO storage and the suspected thermic effect of exogenous lactate described by Ferrannini et al. (11), and that the parasympathetic nervous system dominates at rest; thus the lower Epi was of no consequence to HR regulation. It is also possible that the influence at rest may have affected Epi during exercise.

Alternative mechanisms. The literature offers little support for the possibility that catecholamines were lower during LC because of changes to catecholamine $R_d$. Variables such as age (19) and hypoxia (14) have been associated with changes in catecholamine kinetics. However, understanding of the regulation of clearance is incomplete. Given the lack of data concerning the regulation of catecholamine clearance during exercise and the supporting data of lactate anion influencing the glucose-responsive neurons, for the present, we are left with the tentative conclusion that the lactate anion was sensed either by the VMH, the portal vein, or the adrenal gland directly or elsewhere, signaling abundant fuel supply.

It should be noted that there were fluid balance shifts with the sodium-lactate infusion. However, when NE and Epi concentrations were corrected for plasma volume changes, the catecholamine relationship between the LC and CON trials persisted. However, given that Roy et al. (27) showed that NE concentration is higher during exercise with prior dehydration compared with no dehydration, the fluid balance shifts were of concern and may have affected our results via influence on plasma volume.

Summary and conclusion. Lactate is an important metabolic intermediate that represents a significant fuel source and gluconeogenic precursor. As well, it appears that blood lactate level can signal metabolic and endocrine responses. Specifically, exogenous lactate infusion was effective in attenuating sympathetic responses to exercise. Decreased sympathetic drive was evidenced by the lower catecholamine concentrations and MAP during exercise with the LC. Presumably, this catecholamine attenuation was one mechanism responsible for the changes in glucose metabolism seen during the LC compared with the CON condition. As well, it is clear that catecholamine-glucose-lactate interactions display components of both feedback and feedforward regulation. A dose-response investigation that varies both lactate load and exercise intensity would elucidate the relative contribution of fuel availability to the regulation of the sympathetic drive.

ACKNOWLEDGMENTS

We thank our subjects for time and dedication to this project.

Fig. 2. Plasma norepinephrine (A) and epinephrine (B) concentrations across time. Shown are data for CON and LC at 55% $V_{O_2\text{peak}}$; 65% indicates 65% $V_{O_2\text{peak}}$. Insets are mean rest and exercise for each condition. $P < 0.05$, significantly different from CON (*) and significantly different from 65% (#).

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