Lactic Acid Does Not Directly Activate Hypothalamic-Pituitary Corticotroph Function (44351)

JOHN S. PETRIDES,*,† PATRICIA A. DEUSTER,* AND GREGORY P. MUELLER†,[1]

Departments of Military and Emergency Medicine and Physiology† Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814*

Abstract. **The role that metabolic products play in regulating the hypothalamic-pituitary-adrenal (HPA) axis during strenuous exercise is speculative. This investigation examined the extent to which lactic acid, a major metabolite of anaerobic exercise, directly affects hypothalamic-pituitary function. Specifically, β-endorphin secretion was measured from AtT-20 (D-16) mouse corticotroph tumor cells treated either acutely (15 min – 180 min) or chronically (1 day – 3 day) with physiologic levels of lactate (0.5×10^{-3} *M* to 5×10^{-2} *M*) or lactate in combination with the corticotroph releasing factors: corticotroph releasing hormone (CRH), arginine vasopressin (AVP), norepinephrine and/or epinephrine. Findings with AtT-20 cell cultures were shown to be representative of responses in primary cultures of rat anterior pituitary. Lactic acid did not alter the spontaneous release of β-endorphin by AtT-20 cells under either acute or chronic conditions. While CRH, norepinephrine, and epinephrine evoked significant increases in β-endorphin release, lactate, in combination with these secretagogues did not alter their effects. Similarly, lactic acid failed to alter basal or stimulated release of β-endorphin by primary cultures of rat anterior pituitary. The addition of lactate (3×10^{3} *M*) to rat hypothalamic explants did, however, produce a modest but significant reduction in spontaneous CRH release, suggesting that lactate may facilitate the return to basal secretion following exercise. The present findings show that physiologic concentrations of lactate have no effect, either alone or in combination with other pituitary secretagogues, on corticotroph secretion. Whereas a physiologic action for lactate within the hypothalamus is possible, the present findings indicate that lactate is an inhibitor of CRH release. Thus, lactate does not appear to play a direct role in the profound activation of the HPA axis that occurs in response to strenuous exercise.** [P.S.E.B.M. 1999, Vol 220]

It is well documented that physiologic stimuli including strenuous exercise, pain, and nonspecific stressors can profoundly activate the hypothalamic-pituitary-adrenal (HPA) axis in humans (1, 2). Although much is known about the neural and hormonal mechanisms that regulate the HPA axis during stress, roles played by metabolic factors in coordinating the function of the axis have not been clearly defined. This issue is especially relevant in light of the physiologic actions of glucocorticoids in mediating fuel mobilization and insulin resistance (3).

Several recent reports indicate that lactic acid, a principal metabolic product of anaerobic metabolism, may modulate the magnitude of HPA activation in response to exercise and other physiologic stimuli. For example, in humans, strong correlations exist between increases in circulating lactate and the degree of HPA activation due to high-intensity exercise and surgery (1, 4). In addition, plasma concentrations of growth hormone and prolactin have been shown to increase significantly in response to infusions of sodium lactate (5) as well as exercise (4). Therefore, it has been proposed that lactate may serve as a metabolic signal to coordinate neuroendocrine responses maintaining meta-

[1] To whom requests for reprints should be addressed at Department of Physiology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814–4799. E-mail: gmueller@usuhs.mil

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bolic homeostasis. Such a role could be dramatic during strenuous exercise when increases in metabolism and HPA function are profound.

The present investigation sought to determine the direct effects of lactic acid, alone and in combination with known corticotropin releasing factors, on the secretory responsiveness of pituitary corticotrophs. Cell culture experiments were first performed extensively with AtT-20 pituitary tumor cells, and findings were then confirmed in primary cultures of rat anterior pituitary lobe. In addition, hypothalamic explants were used to determine the direct effect of lactate on the release of CRH. Overall, the findings indicate that lactate does not have a direct role in mediating hypothalamic-pituitary corticotroph activation.

Materials and Methods

Animals. Adult male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 200–300 g were housed for at least 5 days prior to the onset of experiments (20°C; 12:12 hr light-dark cycle; food and water *ab libitum*).

Pituitary Cell Cultures. Monolayers of AtT-20 (strain D16) cells were grown to 80% confluence by plating ≈ 450 000 cells in 35-mm wells 4–6 days before treatment (6). Prior to an experiment, cells were washed and allowed to equilibrate for 90 min in Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO/BRL, Gaithersburg, MD) containing 0.1% bovine serum albumin (BSA; fraction V, Sigma, St. Louis, MO) (DMEM/BSA). The medium was then replaced with DMEM/BSA containing experimental treatments. Following 0.25–72 hr incubation, media samples were transferred into 1.5-ml eppendorf tubes and centrifuged. Supernatants were collected and stored in polypropylene tubes at −20°C until assayed. Cells were harvested by scraping and sonicating in 2 N acetic acid (5 ml) and stored at −20°C until assayed for protein (Bradford) and β-endorphin.

Primary cell cultures were established with anterior pituitaries collected from normal male rats. Tissues were finely minced and enzymatically dissociated for 5 min at 37°C by delicately mixing in a solution of DMEM containing 10 mg/ml BSA, 4 mg/ml collagenase, 1 mg/ml hyaluronidase (Type IV-S), 0.01 mg/ml DNAase (Type I) (all enzymes from Sigma). Tissue fragments were collected by centrifugation, resuspended in DMEM containing 3 mg/ml trypsin(300,000 USP U/g; Sigma) and cells dispersed by titration through a polished pipet for 5 min at 37°C. Cells were harvested by centrifugation, rinsed once with DMEM containing 1 mg/ml lima bean trypsin inhibitor (Sigma), and washed twice with DMEM before being plated. Approximately 70,000 cells in 2 ml DMEM containing 10% horse serum and 2.5% fetal bovine serum were plated in 35-mm wells and cultured until ≈ 80% confluent (5–8 days).

Hypothalami Incubations. Incubations with explanted rat hypothalami were carried out as described by Calogero *et al.* (7). Hypothalami were carefully dissected and suspended on moveable nylon mesh platforms (nylon mesh monofilament 44 μ, Small Parts, Miami, FL) in wells (48 multiwell plates, Costar, Cambridge, MA) containing control media. Each plate was preincubated for 90 min at 37°C with 5% CO_2. The hypothalami were transferred in succession through three wells containing control media and then to experimental treatment media in the next two wells. Finally, the hypothalami underwent a provocative stimulus by potassium depolarization(60 m*M* KCl) to assess tissue responsiveness. Hypothalami were incubated for 20 min per well, an optimal incubation time established by Calogero *et al.* (7). Media samples were assayed directly for CRH.

Incubation Medium and Experimental Treatments. Pituitary cells were cultured in DMEM (GIBCO/BRL) containing 10% fetal bovine serum, 2% L-glutamine (200 m*M*), and 2% penicillin (5000 units/ml) – streptomycin(5000 μg/ml, GIBCO/BRL) (DMEM Complete) (6). Pituitary hormone release studies were carried out with DMEM/BSA. Experimental treatments were dissolved in DMEM/BSA to achieve final concentrations as follows: lactate (0.5×10^{-3}–5×10^{-2} *M*), CRH (10^{-11}–10^{-6}), AVP(10^{-8}–10^{-4} *M*), norepinephrine (10^{-8}–10^{-5} *M*) or epinephrine (10^{-8}–10^{-5} *M*). Data presented are representative of replicate experiments (3,4,5) which together encompassed the dose ranges above. Hypothalamic explants were incubated in medium 199 with modified Earl's salt (GIBCO/BRL), 0.1% BSA and 20 μ*M* bacitracin (Aldrich, Milwaukee, WI) containing either 0 *M*, 3×10^{-3} *M*, or 2×10^{-2} *M* lactate. All media were adjusted to pH 7.4 and fully gassed prior to addition to the cells.

Radioimmunoassays. Corticotroph responses were assessed by measuring β-endorphin, which is co-released with ACTH. Immunoreactive β-endorphin was measured by RIA as previously described (8). The sensitivity of the assay is ≈ 2.7 femtomoles. The C-55 antiserum used recognizes free and acetylated forms of β-endorphin$_{1-31}$ and β-endorphin$_{1-27}$ equally well but does not detect β-endorphin$_{1-16}$ (α-endorphin), β-endorphin$_{1-17}$ (γ-endorphin), methionine enkephalin, leucine enkephalin, α-melanocyte stimulating hormone, ACTH, CRH, or other nonopiate hypothalamic or pituitary hormones.

CRH concentrations were determined by RIA using anti-rat CRH antiserum TS-3 as previously described (7). The assay is sensitive to ≈ 5 picomoles. The antibody does not cross-react with thyrotropin-releasing hormone, somatostatin, substance P, vasoactive intestinal peptide (VIP), neuropeptide Y, ACTH, β-endorphin, vasopressin, oxytocin, luteinizing hormone, growth hormone, or many other unrelated peptides and proteins.

Statistical Analyses. Results were analyzed by one-way (dose response studies) and two-way (multiple treatment studies) ANOVA followed by Duncan's new multiple-range test for comparison of group means. Data are presented as mean ± standard error with significance set at $P \leq 0.05$.

Results

Dose Response and Time Course Effects of CRH and Lactate. Several preliminary experiments (n = 6) were conducted to assess the direct effects of lactate on corticotroph release *in vitro*. These showed that physiologic concentrations of lactate(1×10^3 M to 3×10^2 M) failed to alter the spontaneous release (15–240 min) or cellular content of β-endorphin in either AtT-20 cell or primary AL cultures. The positive control, CRH, markedly stimulated β-endorphin release in a dose-dependent manner. Representative findings are presented in Table I and extended by the results of the following studies.

Effects of Lactate in Combination with Lactate and CRH. The possibility that lactate may synergize with CRH to enhance stimulated secretion by AtT-20 cells and primary AL cell cultures was extensively investigated. Four separate experiments were performed using varying concentrations of lactate (1×10^{-3} M–5×10^{-3} M) both alone and in combination with CRH. The inactive isomer, D-lactate, was used as a control for the treatments. Figure 1 represents the overall findings of these experiments; additional findings are presented in Table I. Incubation for 3 hr with D- or L-lactate (3×10^{-3} M) alone, or in combination with increasing doses of CRH (10^{-11}, 10^{-10}, and 10^{-9} M), had no effect on β-endorphin release singly and failed to alter the dose–response effects of CRH on AtT-20 corticotroph secretion. The highest dose of CRH tested alone (10^{-9} M) significantly increased β-endorphin release by more than 5-fold ($P < 0.05$). Similar results were obtained with primary pituitary cultures. For example, in one experiment where CRH (10,8) increased β-END release from 4.7 ± 0.5 pmol/plate to 10.2 ± 0.8 pmol/plate over 4 hr, cells incubated in the presence of CRH with either 10^{-3} M or 3×10^{-3} M lactate released 12.6 ± 0.7 and 8.4 ± 0.4 pmol/plate (n = 4), respectively.

Effects of Lactate in Combination with Norepinephrine, Epinephrine, or AVP. Experiments were also conducted to determine the effects of L-lactate (0.5×10^{-3} M–5×10^{-3} M) in combination with the catecholamines, norepinephrine, and epinephrine (10^{-8}–10^{-4} M) on the spontaneous release of β-endorphin by AtT-20 cells and primary rat AL cell cultures. Data presented in Figure 2 represent the overall findings of these experiments. In this experiment, primary cultures of AL cells were treated with either L-lactate (5×10^{-3} M) alone or in combination with increasing doses of norepinephrine for 3 hr. L-lactate did

Table I. Effects of Lactate on CRH-Induced β-Endorphin Release from AtT-20 Cells

Treatment condition	β-Endorphin release (pmol/plate; n = 4) Incubation time	
	90 min	240 min
Control	2.6 ± 0.3	12.7 ± 1.0
Lactate (3×10^{-3} M)	2.2 ± 0.3	10.3 ± 2.0
CRH (10^{-8} M)	13.0 ± 1.7*	37.0 ± 3.2*
Lactate (3×10^{-3} M) + CRH (10^{-8} M)	11.8 ± 2.0*	35.8 ± 1.8*

Note. Data represent means ± SE.
* Values significantly different from their corresponding control value: $P \geq 0.05$.

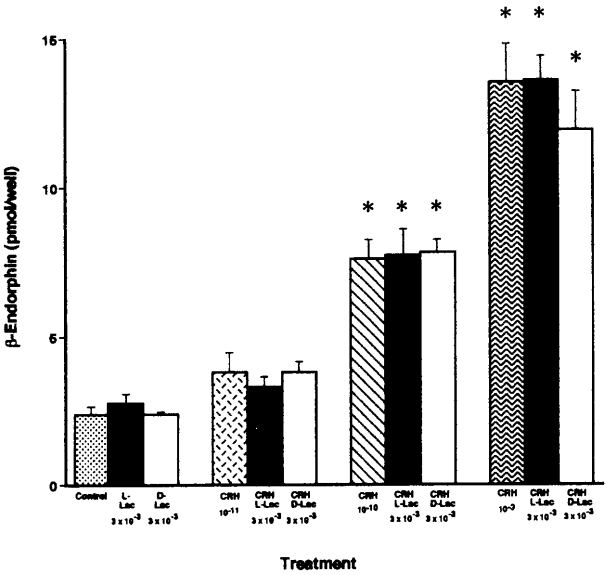

Figure 1. The effects of corticotropin-releasing hormone (CRH) and D- and L-lactate on the spontaneous release of β-endorphin from rat AtT-20 cells. Cultured cells were incubated for 3 hr with either CRH, lactate (D- and L-), or CRH and lactate combined at the indicated molar concentrations. Each group of three adjacent bars represents data from cells exposed to CRH at the concentration indicated under the first bar of the series. Basal release is represented by the control bar (n = 6 wells/treatment). * represents significant difference from control ($P < 0.05$).

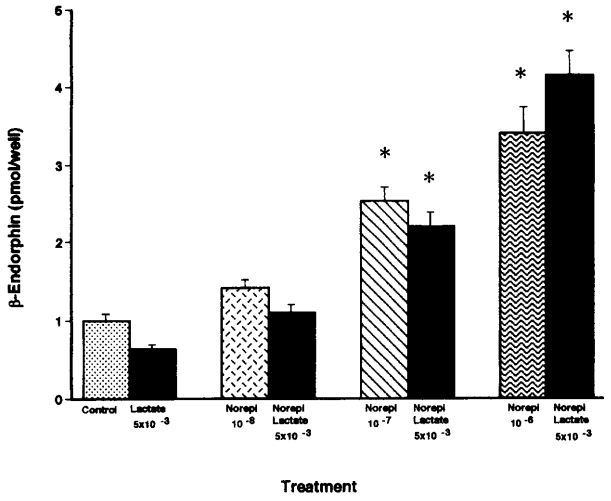

Figure 2. The effects of norepinephrine and L-lactate on the spontaneous release of β-endorphin by primary cultures of rat anterior pituitary cells. Cells were incubated for 3 hr with either norepinephrine (Norepi), lactate, or lactate and norepinephrine combined at the concentrations indicated. Each group of two adjacent bars represents data from cells exposed to norepinephrine at the concentration indicated under the first bar of the series. Basal release is represented by the control bar (n = 6 wells/treatment). * represents significant difference from control ($P < 0.05$).

not significantly alter basal release of β-endorphin from AL cells, whereas norepinephrine at concentrations of 10^{-7} *M* and 10^{-6} *M* significantly ($P < 0.05$) increased β-endorphin release two and three times over basal levels, respectively. As seen with CRH, L-lactate had no appreciable effect on corticotroph responses to norepinephrine. Similar experiments involving L-lactate (1×10^{-3} *M*–5×10^{-3} *M*) alone, or in combination with increasing doses of epinephrine (10^{-8}–10^{-4} *M*) or AVP (10^{-6}–10^{-4} *M*) yielded similar results. In all cases L-lactate failed to influence basal release or potentiate stimulated β-endorphin release from AtT-20 cells or primary cultures of AL cells. Additional representative findings are presented in Table II showing the failure of lactate to alter the dose response effects of epinephrine on β-endorphin release (3 h) from primary cultures of anterior pituitary cells.

Effects of Lactate in Combination with a "Cocktail" of CRFs. *In vivo* activation of pituitary corticotrophs is widely held to result from the simultaneous actions of several different corticotroph releasing factors. Because of this we also examined the effects of L-lactate in the presence of a "cocktail" of corticotroph activators consisting of CRH (10^{-10} *M*), norepinephrine (10^{-7} *M*), epinephrine (10^{-6} *M*), and AVP (10^{-10} *M*). The doses of secretogogues were selected to produce an intermediate corticotroph response upon which either enhancement or reduction could be observed. Representative results of experiments carried out with both AtT-20 and primary AL cultures are presented in Figure 3. Primary cultures of AL cells were treated with L-lactate (0.5×10^{-3} *M* and 5×10^{-3} *M*) alone and in combination with half-strength and full-strength "cocktail" for 3 hr. L-lactate, again had no direct effect on basal release of β-endorphin by AL cells, whereas the "cocktail" stimulated the release of β-endorphin in a dose-related manner. Lactate failed to potentiate the release of β-endorphin when combined with the "cocktail" and in fact, attenuated the stimulated release of β-endorphin produced by the high concentration of cocktail.

Table II. Effects of Lactate on Epinephrine-Induced β-Endorphin Release from Anterior Pituitary Cells

Treatment condition	β-Endorphin release (pmol/plate; $n = 4$)
Control	0.32 ± 0.04
Lactate (5×10^{-3} *M*)	0.47 ± 0.11
Epinephrine (10^{-7} *M*)	0.51 ± 0.05
Epinephrine (10^{-7} *M*) + Lactate (5×10^{-3} *M*)	0.68 ± 0.09
Epinephrine (10^{-6} *M*)	1.96 ± 0.17*
Epinephrine (10^{-6} *M*) + Lactate (5×10^{-3} *M*)	2.36 ± 0.22*
Epinephrine (10^{-5} *M*)	2.30 ± 0.19*
Epinephrine (10^{-5} *M*) + Lactate (5×10^{-3} *M*)	2.74 ± 0.19*

Note. Data represent means ± SE.
* Values significantly different from control; $P \geq 0.05$.

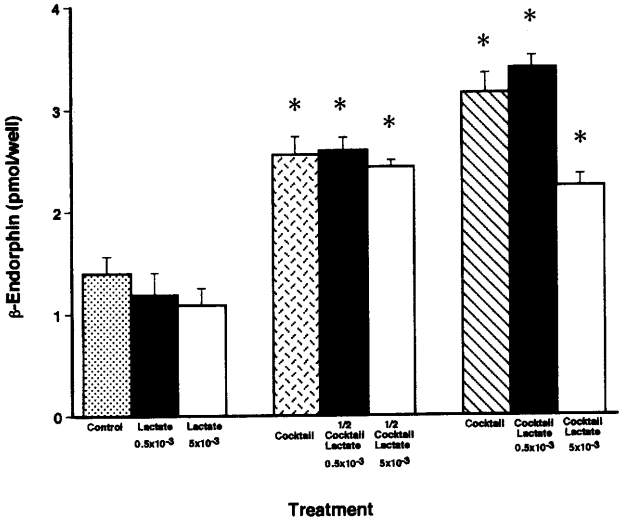

Figure 3. The effects of L-lactate together with a combined treatment of CRH, AVP, norepinephrine, and epinephrine on the release of β-endorphin from primary cultures of rat anterior pituitary cells. Full-strength "cocktail" contained CRH (10^{-10} *M*), norepinephrine (10^{-7} M), epinephrine (10^{-6} *M*), and AVP (10^{-10} *M*). Cells were incubated for 3 hr with either L-lactate, "cocktail", or a combination of the two at the indicated concentrations. Basal release is represented by the control bar ($n = 6$ wells/treatment). * represents significant difference from control ($P < 0.05$).

Long-Term Effects of Lactate on Corticotroph Release. Additional studies were conducted to examine the longer-term effects of lactate on both basal release and cellular content of β-endorphin in AtT-20 cells. Three separate experiments revealed that physiologic concentrations of lactate (1×10^{-3} *M*–5×10^{-2} *M*) had no effect on the release or cellular content of β-endorphin over periods of 1, 2, or 3 days. Table III show the results of an experiment conducted over 48 hr.

Effects of Lactate on CRH Release. An investigation was performed to determine the effects of L-lactate on the release of CRH by rat hypothalamic explants. As shown in Figure 4, hypothalamic explants exhibited a spontaneous release of basal CRH of 14.3 ± 0.5 pmol/100 μl over the 30 min incubation period. Incubation for 30 min with L-lactate at physiologic concentrations significantly decreased basal release of CRH by hypothalamic cells ($P < 0.05$). In contrast, cellular depolarization by KCl (60 m*M*) stimulated the release of CRH 2-fold over basal levels ($P < 0.05$).

Table III. Long-Term Effects of Lactate on β-Endorphin Release from AtT-20 Cells

Treatment condition	β-Endorphin release (pmol/plate; $n = 5$)
Control	51 ± 2
Lactate (10^{-3} *M*)	49 ± 1
Lactate (3×10^{-3} *M*)	50 ± 2
Lactate (10^{-2} *M*)	52 ± 1
Lactate (3×10^{-2} *M*)	54 ± 1

Note. Data represent mean ± SE for β-Endorphin release over 48-hr culture.

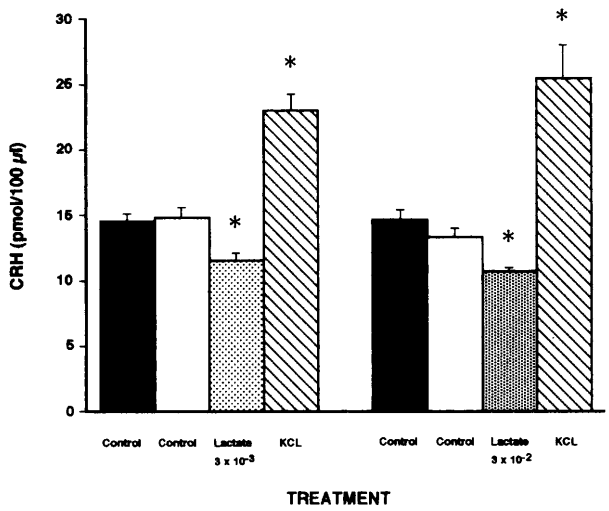

Figure 4. The effects of L-lactate and 60-m*M* KCl on the release of corticotropin-releasing hormone (CRH) from rat hypothalamic explants. Hypothalami were equilibrated in control medium and then exposed to L-lactate (3×10^{-3} *M* or 3×10^{-2} *M*) followed by 60 m*M* KCl. Basal release is represented by the control bar ($n = 6$ wells/treatment). * represents significant difference from control ($P < 0.05$).

Discussion

The role metabolic products play in regulating HPA function remains to be fully defined. Several lines of evidence indicate that lactic acid, a principal product of anaerobic metabolism, may contribute to HPA activation under conditions of strenuous exercise. During exercise in normal subjects, parallel increases in mean concentrations of circulating lactate, ACTH, and cortisol occur in an intensity-dependent manner (3, 4). Moreover, at the same intensity of exercise, differences among individual subjects clearly reveal a direct correlation between the magnitude of one's plasma lactate response and degree of HPA activation (9). Thus, the present investigation sought to determine if lactic acid plays a physiologic role in regulating the HPA axis. Overall, the findings show that physiologic concentrations of lactic acid do not stimulate HPA function by acting either directly on the pituitary corticotrophs or hypothalamic neurons secreting CRH.

An extensive series of *in vitro* experiments demonstrated that lactate has no direct effects that stimulate corticotroph function, acting either alone or in combination with other established releasing factors including CRH, norepinephrine, epinephrine, or AVP. In a consistent manner, lactic acid also failed to directly stimulate the release of CRH from rat hypothalamic explants. In fact, lactate tended to inhibit the spontaneous release of CRH by cultured explants. While it is possible that lactate may facilitate the return to basal HPA function following exercise, an extensive *in vivo* investigation is needed to establish this role. Overall, on the basis of the present findings, it is concluded that lactate does not play a major role in controlling basal or stimulated HPA function through direct actions at the level of pituitary or hypothalamus.

The present findings do not preclude a role for lactate in regulation of the HPA axis. Rather, they suggest that its actions may be mediated *via* extra-hypothalamic brain centers, which increase the drive for CRH, AVP, and/or adrenal catecholamine release. Actions for lactate in the central nervous system (CNS) have been postulated based upon the findings of studies on anxiety and panic (10). It is well recognized that infusion of lactate elicits panic attacks in individuals with panic disorders (11) and that humans most susceptible to panic attacks exhibit higher levels of serum lactate during exercise as compared to those who are less susceptible (12). Primates exhibiting elevations in blood lactate levels also demonstrate parallel increases in CNS lactate concentrations. These findings are consistent with the notion that lactate has a role in mediating CNS responses. Recent evidence in rats has further defined a potential mechanism involved by indicating the organum vasculosum of the lamina terminalis (OVLT) as the specific site where lactate may be sensed by the brain (13). The extent to which the OVLT or other circumventricular organs participate in lactate-mediated neuroendocrine responses to exercise and other stimuli remains to be determined (13, 14).

It is also possible that the condition of strenuous exercise unmasks a mechanism through which lactate may alter HPA function. Such a mechanism might involve either central or peripheral sites of action since there is precedent for lactate to act in both manners to control respiration (15). Actions of lactate could involve paracrine or even intracine mechanisms. Physiologic changes in the pH of blood or cerebral spinal fluid might provide the basis for unmasking a latent mechanism by which lactate controls the functioning of the HPA axis. The present experiments were carried out under a constant condition of pH and would not therefore have revealed such a mechanism even if it normally occurred at the level of the pituitary or hypothalamus.

In summary, the present findings show that physiologic concentrations of lactate do not alter hypothalamic/pituitary corticotroph function through actions directly on the pituitary or hypothalamus. Thus a physiologic role for lactate in mediating HPA activation would have to occur through actions at extra-pituitary and hypothalamic sites or *via* a condition-dependent mechanism that is unmasked by strenuous exercise.

1. Calogero AE, Norton JA, Sheppard BC, Listwack SJ, Cromack DT, Wall R, Jenson RT, Chrousos GP. Pulsatile activation of the hypothalamic-pituitary-adrenal axis during major surgery. Metabolism **41:**839–845, 1992.
2. Farrell PA, Garthwaite TL, Gustafson AB. Plasma adrenocorticotropin and cortisol responses to submaximal and exhaustive exercise. J Appl Physiol **55:**1441–1444, 1983.
3. Galbo H. Hormonal and Metabolic Adaptations to Exercise. New York: George Thieme Verlag, 46–51, 1983.
4. Luger A, Deuster PA, Kyle SB, Gallucci WT, Montgomery LC, Gold PW, Loriaux DL, Chrousos GP. Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise: Physiologic adaptations to physical training. N Engl J Med **316:**1309–1315, 1987.

5. Luger A, Watschinger B, Deuster P, Svoboda T, Clodi M, Chrousos G. Plasma growth hormone and prolactin responses to graded levels of exercise and to a lactate infusion. Neuroendocrinology **56:**112–117, 1992.
6. Hook V, Heisler S, Sabol S, Axelrod J. Corticotropin releasing factor stimulates adrenocorticotropin and β-endorphin release from AtT-20 mouse pituitary tumor cells. Biochem Biophy Res Commun **106:**1364–1371, 1982.
7. Calogero AE, Bernardini R, Margioris AN, Bagdy G, Gallucci WT, Munson PJ, Tamarkin L, Tomai TP, Brady L, Gold PW, Chrousos GP. Effects of serotonergic agonists and antagonists on corticotropin-releasing hormone secretion by explanted rat hypothalami. Peptides **10:**189–200, 1989.
8. Mueller GP. Attenuated pituitary β-endorphin release in estrogen-treated rats. Proc Soc Exp Biol Med **165:**75–81, 1980.
9. Petrides JS, Mueller GP, Kalogeros KT, Chrousos GP, Gold PW, Deuster PA. Exercise-induced activation of the hypothalamic-pituitary-adrenal axis: Marked differences in the sensitivity to glucocorticoid suppression. J Clin Endocrinol Metab **79:**377–383, 1994.
10. Hollander D, Liebowitz MR, Gorman JM, Cohen B, Freyer A, Klein DF. Cortisol and sodium-lactate-induced panic. Arch Gen Psychiatry **46:**135–140, 1989.
11. Pitts FN, McClure JN. Lactate metabolism in anxiety neurosis. N Engl J Med **277:**1329–1336, 1967.
12. Jones M, Mellersh V. Comparison of exercise response in anxiety states and normal controls. Psychocom Med **8:**180–187, 1946.
13. Shekhar A, Keim SR. The circumventricular organs form a potential neural pathway for lactate sensitivity: Implication for panic disorder. J Neurosci **17:**9726–9735, 1997.
14. Dager SR, Rainey JM, Kenny MA, Artu AA, Metzger GD, Bowden DM. Central nervous system effects of lactate infusion in primates. Biol Psychiatry **27:**193–204, 1990.
15. Petrozzino JJ, Scardella AT, Santiago TV, Edelman NH. Dichloroacetate blocks endogenous opioid effects during inspiratory flow-resistive loading. J Appl Physiol **72:**590–596, 1992.