

Two-dimensional Proton Echo-Planar Spectroscopic Imaging of Brain Metabolic Changes During Lactate-Induced Panic

Stephen R. Dager, MD; Seth D. Friedman, PhD; Aaron Heide, MD; Matthew E. Layton, MD, PhD; Todd Richards, PhD; Alan Artru, MD; Wayne Strauss, PhD; Cecil Hayes, PhD; Stefan Posse, PhD

Background: A fast, proton echo-planar spectroscopic imaging (PEPSI) technique, capable of simultaneously measuring metabolites from multiple brain regions, was used to investigate the anatomical distribution and magnitude of brain lactate responses to intravenous lactate infusion among subjects with panic disorder and control subjects.

Methods: Fifteen subjects with panic disorder and 10 control subjects were studied. All subjects were medication free and met *DSM-IV* criteria for panic disorder, or, for controls, no Axis I psychiatric disorder. Two-dimensional axial metabolite images having 1-cm³ spatial resolution were acquired at 6¹/₂-minute intervals during 3 conditions: a 20-minute baseline, 20-minute 0.5-mol/L sodium lactate infusion, and 15-minute postinfusion period.

Results: Intravenous lactate infusion increased brain lactate levels throughout the axial brain section studied in all subjects. Panic-disordered subjects had significantly

greater global brain lactate increases in response to lactate infusion. Lateralization of brain lactate response did not occur, nor were discrete regional loci of elevated lactate observed. Cerebrospinal fluid lactate changes corresponded to lactate changes in brain tissue. Severity of symptoms provoked by lactate infusion did not directly correlate with brain lactate response.

Conclusions: Greater overall rises in brain lactate among subjects with panic disorder compared with controls occurred in response to lactate infusion. We were unable to detect a distinct regional pattern for magnitude differences in brain lactate rise by which to identify a specific neuroanatomical substrate underlying a lactate-induced panic response. The wide anatomical distribution of these brain lactate increases suggest metabolic and/or neurovascular mechanisms for the abnormal rise in subjects with panic disorder.

Arch Gen Psychiatry. 1999;56:70-77

INDIVIDUALS with panic attacks have a specific vulnerability to precipitation of acute anxiety and/or physiological arousal by intravenous sodium lactate infusion.¹ Despite extensive investigation, mechanisms underlying this lactate sensitivity remain undetermined. One theory, that patients with panic disorder could be reacting to the effects of elevated brain lactate as a possible mechanism for lactate-induced panic, was postulated on the basis of study results in monkeys that directly measured lactate rises in cisternal fluid during intravenous lactate infusion.² To address this proposed relationship between lactate-induced panic and elevated brain lactate, a newly emerging technology, magnetic resonance spectroscopy (MRS),³ has been used to noninvasively measure brain lactate changes during lactate infusion. Magnetic resonance spectroscopy studies of rats⁴ and healthy human controls⁵ demonstrate progressive brain lactate rises in response to lactate infusion. These increases in brain lactate levels during lactate infusion occur in

subjects with panic disorder and are of greater magnitude and longer duration than in control subjects.^{6,7}

To date, most in vivo human MRS studies have used single-voxel MRS techniques that provide good time resolution but allow only a single predetermined region of interest (ROI) in the brain to be studied at one time.³ In previous single-voxel MRS studies,^{5,6,8} a large anatomical area (27 cm³), encompassing the insular cortex and adjacent regions, was chosen to be sampled on the basis of positron emission tomography studies indicating distinct blood flow abnormalities in that region among lactate-sensitive subjects with panic disorder.^{9,10} However, misinterpretation of extracranial signal artifact subsequently led to the retraction of those positron emission tomographic findings in panic disorder.¹¹ Thus, it remains uncertain whether lactate abnormalities detected among patients with panic disorder are specific to the insular cortex region^{6,7} or are representative of diffuse metabolic abnormalities. To address this

From the Departments of Psychiatry (Drs Dager, Friedman, and Layton), Bioengineering (Drs Dager, Richards, and Strauss), Radiology (Drs Richards and Hayes), and Anesthesiology (Dr Artru), and Diagnostic Imaging Sciences Center (Drs Dager, Heide, Richards, and Hayes), University of Washington School of Medicine, Seattle; and Center for MR Research, Institut für Medizin, Jülich, Germany (Dr Posse).

SUBJECTS AND METHODS

SUBJECTS

Fifteen subjects with panic disorder (7 men and 8 women) aged 33.1 ± 11.7 years (all values are mean \pm SD), and 10 healthy control subjects (4 men and 6 women) aged 32.7 ± 7.4 years were studied. All subjects completed the study with the exception of 1 subject with panic disorder who stopped the study 10 minutes into the lactate infusion due to urinary urgency in the midst of a panic response (data from this subject are included through the first lactate infusion time point). In addition, incomplete MRS data sets were obtained for 3 subjects with panic disorder (2 with panic response to lactate infusion [panic responder] and 1 without panic response to lactate infusion [panic nonresponder]) and 1 control subject due to scanner malfunction.

All subjects were medication free for at least 1 month and were fasting at the time of MRS evaluation. Subjects with panic disorder had a diagnosis of panic disorder by structured psychiatric interview consistent with *DSM-IV* criteria,¹⁴ were free of other Axis I psychiatric diagnoses, and were actively experiencing panic attacks. Control subjects had no history of an Axis I psychiatric disorder. All subjects gave written informed consent for participation in the study, which was approved by the University of Washington Human Subjects Review Committee.

INFUSION PROTOCOL

Following insertion of an intravenous catheter by an experienced anesthesiologist, subjects were moved to the Diagnostic Imaging Sciences Center and placed in the supine position into the scanner for the lactate infusion protocol. After optimization of imaging parameters (see below), a 20-minute baseline period was followed by intravenous infusion of 0.5-mol/L sodium lactate over 20 minutes (10 mL/kg total),¹⁵⁻¹⁷ followed by a 15-minute recovery period. At baseline and immediately following study completion, the Acute Panic Inventory (API),¹⁸ a panic severity rating scale (0-10; 0 = no panic, 10 = extreme panic) and anxiety severity rating scale (0-4; 0 = no anxiety, 4 = extreme anxiety) were administered to subjects.

A carbon dioxide monitor (Puritan Bennett, Los Angeles, Calif) was used to continuously monitor end-tidal carbon dioxide ($P_{ET}CO_2$) at baseline, during, and after lactate infusion.

We have demonstrated that the $P_{ET}CO_2$ - $PaCO_2$ gradient within subjects is consistent under normal and hypocapnic conditions.⁸ In unpublished work (S.R.D. and A.A., 1994), sodium lactate infusion was found not to affect the $P_{ET}CO_2$ - $PaCO_2$ gradient.

IMAGE ACQUISITIONS

PEPSI studies were performed by using a clinical 1.5-T Signa whole-body scanner equipped with Genesis version 5.4 operating system (General Electric Medical Systems, Milwaukee, Wis). An experimental receive-only bird cage coil designed and built at the University of Washington, Seattle, was used, which provided approximately $\sqrt{2}$ enhanced signal-to-noise ratio over conventional quadrature head coils (C.H., unpublished data, 1996). The coil incorporated a built-in head holder to immobilize the head during lactate infusion.

High-resolution sagittal and axial T_1 -weighted magnetic resonance images were used for anatomical localization (repetition time, 600 milliseconds; echo time, 20 milliseconds; field of view, 25 cm; matrix, 256×128). An axial section at the level of the lateral ventricles was selected as the axial plane to be examined by PEPSI (**Figure 1**). This slice location was chosen to be consistent with prior single-voxel MRS studies of lactate infusion⁵⁻⁷ and hyperventilation.⁸

The PEPSI pulse sequence has been described in detail.¹³ Parameters used in data acquisition included echo time of 272 milliseconds, both to minimize lipid signal contribution and obtain an in-phase doublet of lactate⁵⁻⁸; repetition time of 2 seconds to acquire partially relaxed spectra during signal acquisition; 32×32 spatial matrix; 22-cm field of view; 20-mm slice thickness; 32-kHz spectral width; 16 384 complex data points for frame size (32 spatial points convoluted with 512 spectral points with nominal voxel size of 1 cm^3 , and 1.9-Hz/point spectral resolution); and 6 averages with acquisition of partial echoes to permit magnitude reconstruction. Following prescription and shimming, metabolic images were obtained during baseline (3 scans), lactate infusion (3 scans), and after infusion (2 scans).

IMAGE PROCESSING

Raw data traces from individual excitations were averaged, and separate even and odd echo data were com-

Continued on next page

issue, recent advances in MRS imaging now allow the simultaneous spatial mapping of metabolites within multiple brain regions to investigate both global and regional-specific metabolic changes.^{12,13}

The purpose of the present study was 2-fold: (1) to test the hypothesis that global brain metabolic abnormalities occur in patients with panic disorder during and following lactate infusion, and (2) to ascertain whether regional-specific brain lactate elevations occur that provide a marker for specific neuroanatomical substrate underlying lactate-induced panic. To undertake this research, we used a recently developed 2-dimensional fast spectroscopic imaging technique, proton echo-planar spectroscopic imaging (PEPSI),¹³ configured to have a spatial resolution of 1 cm^3 and a $6\frac{1}{2}$ -minute time resolution, to serially measure brain lactate changes during lactate infusion.

RESULTS

Twelve of 15 subjects with panic disorder, but no control subjects, experienced a panic response to lactate infusion using criteria of sufficient API-rated symptoms to fulfill *DSM-IV* criteria for a panic attack and onset of moderate to severe feelings of panic that we have previously applied to studies of lactate infusion.^{1,6,7,17}

Compared with control subjects, subjects with panic disorders showed significantly elevated API scores, and panic severity and anxiety severity scores at baseline and in response to lactate infusion, as shown in **Table 1**.

RM-ANOVA of $P_{ET}CO_2$ levels found no significant difference in ventilatory response between diagnostic groups (group effect: $F_{1,19} = 2.02$, $P = .17$). $P_{ET}CO_2$ levels did decrease over time in both groups (scan effect: $F_{7,133} = 4.53$, $P < .001$; $\epsilon = 0.43$, GG- $P < .001$); however, no interaction was

bined for reconstruction into a 3-dimensional matrix (1 dimension for spectral encoding and 2 dimensions for spatial encoding), as previously described.¹³ The spectral dimension was filtered by applying a trapezoidal filter with a ramp width of 50 points on both sides of the 256 complex raw data vector. This filter was used as an almost complete echo was collected in the time domain. A sine-bell function spatial filter was applied in k-space to reduce residual lipid bleed in lactate metabolic images. For *N*-acetylaspartate (NAA) images, a gaussian filter was applied with full width as half-maximum of 11 points of 32 in the spatial domain. Magnitude spectra were computed and screened for validity using an automated computer program, or “mask,” to provide a consistent, conservative approach for defining usable spectra based on metabolite line width and resonant frequency. This approach removed voxels having poorly optimized magnetic field homogeneity or lipid suppression¹⁹ (as shown in **Figure 2**). Individual masks, created for each of the 8 PEPSI acquisitions, were combined to form a composite mask, incorporating only voxels that were valid across all scans, to ensure that identical voxel sets were analyzed. Metabolic maps were calculated from the spectral resonances of choline, creatine, NAA, and lactate. For this study, only lactate and NAA metabolic imaging data are reported. Magnetic field inhomogeneity shifts (B_0 -shifts) were corrected by setting the maximum point of the NAA peak to 2.0 ppm for each spectrum. Following baseline correction determined on a voxel-by-voxel basis using 32 points to the right of 0.0 ppm, metabolic maps were calculated from the spectral resonances of NAA and lactate by integrating spectral regions that extended 0.09 ppm to the left and to the right side of each resonance.¹⁹

The NAA spectral peak was used as an internal reference (Lac/NAA) to standardize measurements for differences in magnet shimming between studies and across the entire slice and at each time point within studies.²⁰⁻²² In previous work, NAA signal remained stable during lactate infusion and hyperventilation.⁵⁻⁸ Because partial volume effects in ventricular ROIs make NAA an unsuitable internal reference, absolute lactate signal intensity was used to assess cerebrospinal fluid (CSF) response. Although using lactate intensity without reference to an internal standard is not ideal,^{20,21} we wanted to investigate whether diagnostic groups exhibited a differential time course of lactate response within CSF.

Whole brain lactate levels, expressed as Lac/NAA ratios, were calculated automatically for combined voxels from

the mask. For regional analyses, ROIs were defined using guidelines of Damasio and Damasio²³: frontal lobe (left [L] and right [R]), anterior cingulate, anterior CSF (positioned at midline within the horns of the lateral ventricles), basal ganglia (L/R), thalamus (L/R), insula (L/R), parietal white matter (L/R), parieto-occipital tissue (L/R), posterior CSF, splenium, and central occipital lobe determined from the high-resolution image coregistered with metabolite images. Because voxels acquired in this study are relatively large (1 cm³ nominal voxel size) and square in configuration, the best approximation of the correct anatomical contours was used (see Figure 1 for representative ROI placement). An invalid spectrum falling within an anatomical region was excluded. Additionally, the number of sampled voxels for each region was the same across individuals to keep the signal-to-noise ratio similar.

STATISTICAL ANALYSES

SPSS software (version 6.1.1; SPSS Inc, Chicago, Ill) was used for statistical analyses. Clinical rating scales were compared between diagnostic groups by independent *t* tests. Differences in $P_{ET}CO_2$ were compared by repeated-measures analysis of variance (RM-ANOVA). Additionally, correlational analyses between $P_{ET}CO_2$ and brain lactate levels were performed for heuristic purposes. RM-ANOVA also was used to compare group differences in global brain Lac/NAA across the 8 PEPSI acquisitions. For RM-ANOVAs reaching significance, independent *t* tests using pooled variances were applied to compare signal Lac/NAA between diagnostic groups at each time point. Paired *t* tests were used to assess whether control and subjects with panic disorder showed a similar rate of lactate response during and after infusion. RM-ANOVA was used to compare regional Lac/NAA and lactate signal amplitude changes. One-way ANOVAs were used for post hoc comparisons. As previous work found significant brain lactate elevations among subjects with panic disorder, which forms the basis for this work, 1-tailed *P* values were used as a statistical criterion in analyses of whole brain lactate. For regional analyses, 2-tailed *P* values were used, as no a priori hypotheses were posited. Both unadjusted *P* values and Geisser-Greenhouse (GG)²⁴—corrected *P* values are reported for all RM-ANOVAs, although homogeneity of variance was satisfied in the majority of analyses. For post hoc analyses, both uncorrected and Bonferroni²⁵—corrected *P* values are reported.

present (group \times scan: $F_{7,133} = 0.17$, $P = .99$). To investigate the concordance between brain lactate levels and $P_{ET}CO_2$ levels at each time point by group, correlational analyses were performed, which demonstrated an inverse relationship for both groups during the first scan after infusion (control group: $r = -0.83$, $P = .02$; panic group: $r = -0.63$, $P = .04$) but not at other time points; this significance was lost with Bonferroni correction.

Sequential lactate and NAA images acquired from a subject with panic disorder who reacted to lactate are shown in Figure 2 and **Figure 3**. Representative spectra at baseline and at completion of infusion are shown in **Figure 4**. Data on NAA demonstrated no significant group effects ($F_{1,18} = 1.04$, $P = .32$), scan effects ($F_{7,126} = 0.73$, $P = .65$), or group \times scan effects ($F_{7,126} = 1.66$, $P = .12$).

Significant differences in brain Lac/NAA were observed between subjects with panic disorder and con-

rol subjects: group ($F_{1,18} = 5.77$, $P = .01$), across scans ($F_{7,126} = 12.09$, $P < .001$; $\epsilon = 0.24$, GG- $P < .001$), and group \times scan ($F_{7,126} = 2.70$, $P = .006$; GG- $P = .05$). Because lactate sensitivity may demarcate a specific subgroup of subjects with panic disorder who have metabolic abnormalities,¹⁷ and consistent with our analytic approach in prior MRS studies,^{6,7} we analyzed brain Lac/NAA changes among groups differentiated by lactate response.¹ Comparison of subjects with panic disorder who panicked in response to lactate infusion (panic responders) with controls revealed significant differences: group ($F_{1,16} = 8.12$, $P = .006$), across scans ($F_{7,112} = 12.44$, $P < .001$; $\epsilon = 0.23$, GG- $P < .001$), group \times scan ($F_{7,112} = 3.32$, $P = .002$; GG- $P = .03$), as shown in **Figure 5**. No significant differences were observed between panic nonresponders and controls (group: $F_{1,9} = 0.02$, $P = .88$; across scans: $F_{7,63} = 5.92$, $P = .001$; group \times scan: $F_{7,63} = 0.28$, $P = .96$),

or panic nonresponders and panic responders (group: $F_{1,9} = 1.85, P = .21$; scan: $F_{7,63} = 3.38, P = .004$; group \times scan: $F_{7,63} = 0.57, P = .78$). Because only panic responders were significantly different from controls, and in keeping with our past approach toward differentiating panic-disordered subjects by lactate response, panic nonresponders were excluded in subsequent analyses.

Independent t tests revealed elevated brain Lac/NAA among panic responders, compared with controls, at the first baseline, during lactate infusion, and after infusion as shown in **Table 2**. When the Bonferroni correction for multiple tests was applied, group differences remained statistically significant at scans 5 and 6 during lactate infusion and after infusion at scan 8, with other time points indicating trend differences. To investigate whether controls and panic responders had similar rates of brain Lac/NAA rise in response to lactate infusion, paired t tests were used to compare the averaged baseline lactate level to subsequent scans within diagnostic

groups (Table 2). Control subjects demonstrated significantly elevated Lac/NAA on the last infusion scan and on both postinfusion scans compared with baseline. In contrast, panic responders demonstrated significant Lac/NAA elevations during the first lactate infusion scan and on all subsequent scans compared with baseline. When t tests were corrected for multiple comparisons using the Bonferroni adjustment ($P < .01$ for significance), Lac/NAA rise did not reach significance on the first infusion and first postinfusion scan for panic responders. Even with this correction, panic responders demonstrated earlier brain Lac/NAA increases compared with controls.

To investigate whether brain Lac/NAA response was lateralized, ROIs in each hemisphere were pooled (lateralization effect) (Figure 1). Consistent with whole brain analyses, significant effects of group ($F_{1,37} = 10.94, P = .002$), scan ($F_{7,259} = 13.12, P < .001$; $\epsilon = 0.48, GG-P < .001$), and group \times scan ($F_{7,259} = 2.68, P = .01, GG-P = .04$) were shown for this subset of combined brain regions. However, no significant lateralization effect ($F_{1,37} = 0.02, P = .90$) or interactions of lateralization by group or scan were demonstrated (all F values $< 1.02, P$ values $> .20$).

To evaluate regional differences (region) in brain Lac/NAA response, RM-ANOVA was conducted on all ROIs,

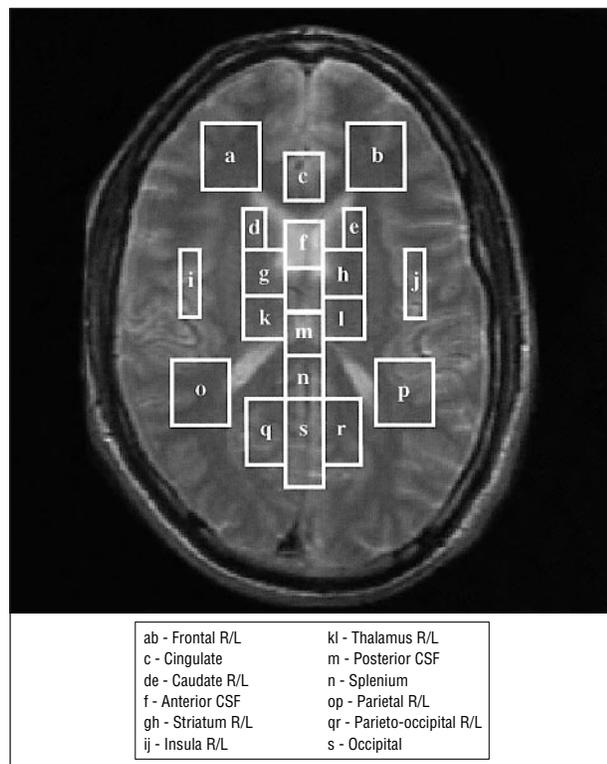


Figure 1. High-resolution prescription image showing region-of-interest selection for regional analysis of metabolic response. R/L indicates right/left; CSF, cerebrospinal fluid.

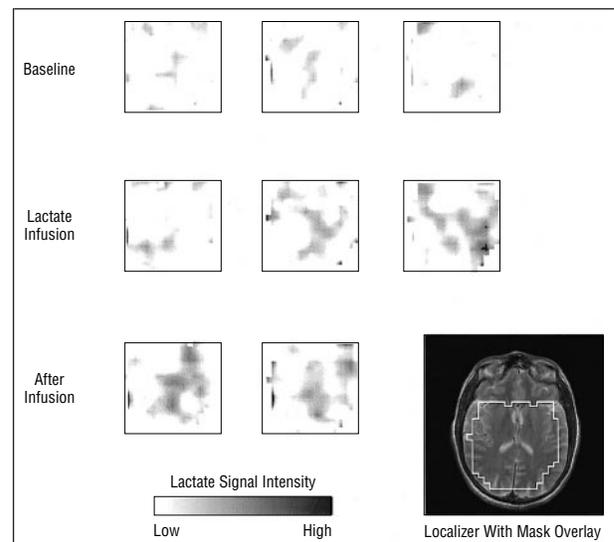


Figure 2. Lactate metabolic proton echo-planar spectroscopic images for a subject with panic disorder, acquired every 6½ minutes at baseline (3 scans, top), during lactate infusion (3 scans, middle), and after infusion (2 scans, bottom). This scan series illustrates the generalized increase in brain lactate levels associated with a lactate-induced panic response. The inset image demonstrates the “mask” that defines valid spectra across all scans.

Table 1. Clinical Response to Lactate Infusion

Clinical Rating (Scale)	Time Point	Mean \pm SD Score		Independent t , Controls vs Subjects With Panic Disorder
		Controls (n = 10)	Subjects With Panic Disorder (n = 15)	
Acute Panic Inventory score (0-51)	Baseline	1.5 \pm 1.8	8.9 \pm 8.2	$t_{23} = 2.76, P < .01$
	Maximum	6.7 \pm 4.8	21.2 \pm 6.7	$t_{23} = 7.33, P < .001$
Panic severity (0-10)	Baseline	0.2 \pm 0.4	2.4 \pm 3.0	$t_{23} = 2.33, P < .03$
	Maximum	1.4 \pm 1.9	5.3 \pm 2.7	$t_{23} = 3.76, P < .001$
Anxiety severity (0-4)	Baseline	0.5 \pm 0.9	2.1 \pm 1.3	$t_{23} = 3.61, P < .001$
	Maximum	0.7 \pm 0.8	2.9 \pm 1.2	$t_{23} = 5.15, P < .001$

excluding CSF regions, as shown in Figure 1. No significant region effects were demonstrated (all F values <1.28 , P values $>.22$). To maximize signal-to-noise ratio, scans were combined to provide an average baseline, average lactate infusion, and average postinfusion measure. Consistent with 2-dimensional axial brain results, significant group, scan, and group \times scan effects were demonstrated (all F values >11.14 , P values $<.001$). Although no region ($F_{16,252}=1.35$, $P=.17$) or group \times region ($F_{16,252}=0.92$, $P=.55$) effects were shown, a region \times scan interaction was demonstrated ($F_{32,504}=1.49$, $P=.04$; $\epsilon=0.81$, $GG-P=.06$) as shown in **Figure 6**. One-way ANOVA comparing region \times scan showed a significant baseline effect ($F_{16,360}=3.14$, $P<.001$), accounted for by elevated lactate in the left frontal region ($P<.05$, Bonferroni corrected). Although, there was a significant infusion effect ($F_{16,338}=1.72$, $P=.04$), no individual ROIs reached significance. There was no postinfusion effect by region ($F_{16,320}=0.84$, $P=.63$).

We further compared CSF with other brain regions. Consistent with metabolite ratio analyses for averaged time points, group, scan, and group \times scan ef-

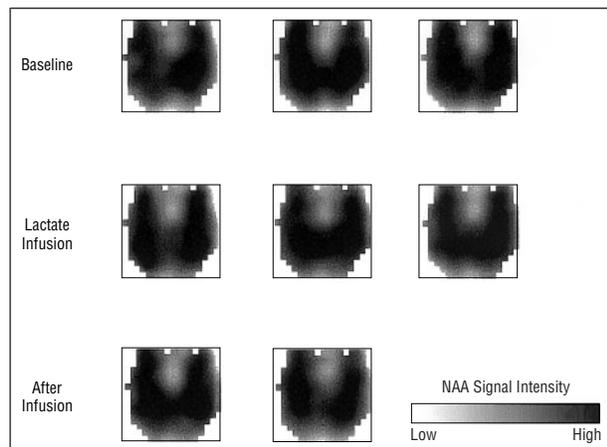


Figure 3. Corresponding N-acetylaspartate (NAA) metabolic proton echo-planar spectroscopic images for the same subject in Figure 2, acquired every 6½ minutes at baseline (3 scans, top), during lactate infusion (3 scans, middle), and after infusion (2 scans, bottom). These images demonstrate the stability of NAA levels across the experimental protocol.

fects were again demonstrated using absolute lactate signal intensity (all F values >9.71 , P values $<.001$). Consistent with regional results using metabolite ratios, no region ($F_{18,273}=1.18$, $P=.27$) or group \times region ($F_{18,273}=0.60$, $P=.90$) effects were shown but there was a consistent region \times scan interaction ($F_{36,546}=1.45$, $P=.05$; $\epsilon=0.82$, $GG-P=.06$). One-way ANOVA revealed baseline differences ($F_{18,392}=1.68$, $P=.04$) that remained significant for the left frontal region ($P<.05$, Bonferroni corrected), but not regional differences during infusion ($F_{18,369}=0.95$, $P=.52$) or after infusion ($F_{18,348}=1.18$, $P=.27$), as shown in **Figure 7**.

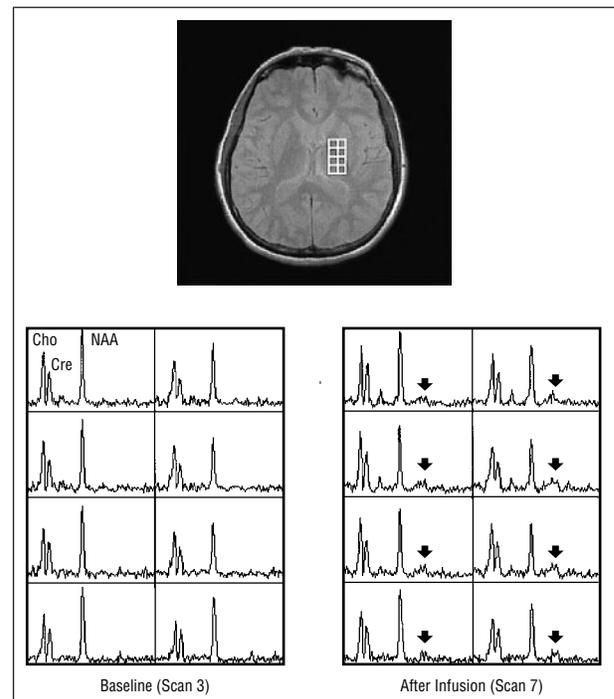


Figure 4. A subset of characteristic spectra, acquired using proton echo-planar spectroscopic imaging, for a 3×3 voxel array from the left insular cortex region of a subject with panic disorder at baseline (left) and at completion of lactate infusion (right), which demonstrates the increase in lactate resonance (arrows). NAA indicates N-acetylaspartate; Cho, choline; and Cre, creatine.

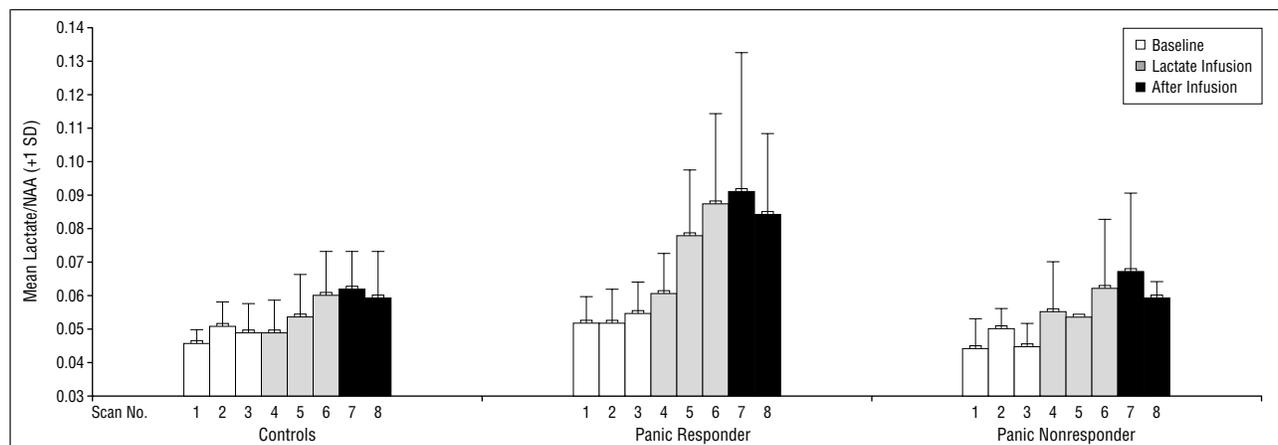


Figure 5. Mean \pm SD global brain lactate/N-acetylaspartate (NAA) metabolite ratio response for control subjects ($n=10$), panic responders (subjects with panic disorder who had lactate-induced panic) ($n=12$), and panic nonresponders (subjects with panic disorder who did not have lactate-induced panic) ($n=3$). Partial data sets are included for 1 control, 2 panic responders, and 1 panic nonresponder due to system failure during lactate infusion. In addition, 1 panic responder discontinued the study following the first infusion scan.

The relationship between psychiatric symptom severity (API, panic, and anxiety scales) and whole brain Lac/NAA was evaluated at baseline and for maximal response to lactate infusion across all subjects with panic disorder and for panic responders without evidence of significant correlation (all r values < 0.33 , P values $> .20$). To address whether left frontal region Lac/NAA elevations at baseline corresponded to baseline anxiety levels, we further evaluated this relationship for the above-noted symptom scales across all subjects and for subjects with panic disorder without evidence of significant correlation (all r values < 0.15 , P values $> .96$).

COMMENT

Lactate elevations in control subjects and the greater lactate elevations exhibited by subjects with panic disorder in response to lactate infusion are widely distributed across a 2-dimensional axial brain section. This dynamic spectroscopic imaging study, by demonstrating the generalized nature of abnormal brain lactate increases among subjects with panic disorder, greatly extends our prior single-voxel MRS studies of lactate infusion.⁵⁻⁷ There did not appear to be any hemispheric

lateralization, nor a discrete anatomical locus for metabolic response to lactate-induced panic. Furthermore, comparison of ventricles with other brain regions shows a similar time course for response, suggesting that brain lactate increases do not differentially reflect the CSF compartment. Consistent with results of prior single-voxel MRS lactate studies,^{6,7} subjects with panic disorder are well able to tolerate the combined stress of magnet confinement and lactate infusion and panic provocation.

Regional brain lactate increases can be detected in response to neuronal activation from sensory stimulation,^{12,26} and are thought to reflect decoupling of neuronal blood flow and energy requirements.²⁷ There also is evidence of regional reductions of cerebral blood flow in the context of lactate-induced panic²⁸ or a spontaneously occurring panic attack.²⁹ On this basis, we might expect regionally specific magnitude differences in brain lactate elevation in response to lactate-induced panic. Although power issues may have limited our ability to detect regional differences, similar lactate rises in all regions indicate the diffuse nature of this effect. It is conceivable that administration of a large lactate load obscured small regionally specific lactate increases arising from neuronal activation related to a panic response. The 1-cm³ spatial reso-

Table 2. Time Course of Brain Lactate Response

Time Point	Scan No.	Controls, Mean (SD) Lac/NAA	Controls % Change From Averaged Baseline (Paired t)	Panic Responders, Mean (SD) Lac/NAA	Panic Responders % Change From Averaged Baseline (Paired t)	Independent t , Controls vs Panic Responders
Baseline	1	0.045 (0.005)	...	0.052 (.008)	...	$t_{20} = 2.37, P = .01$
	2	0.050 (0.007)	...	0.052 (.009)	...	$t_{20} = .50, P = .63$
	3	0.049 (0.009)	...	0.053 (.009)	...	$t_{20} = 1.11, P = .28$
Lactate infusion	4	0.052 (0.007)	8 ($t_8 = 1.56, P = .16$)	0.063 (.012)	21 ($t_{10} = 2.06, P = .03$)	$t_{18} = 2.33, P = .02$
	5	0.053 (0.013)	10 ($t_9 = 1.47, P = .18$)	0.077 (.007)	48 ($t_9 = 3.20, P = .006$)	$t_{17} = 3.07, P = .004$
	6	0.060 (0.013)	25 ($t_8 = 3.36, P = .005$)	0.087 (.026)	57 ($t_8 = 3.77, P = .002$)	$t_{17} = 2.85, P = .006$
After infusion	7	0.062 (0.012)	29 ($t_8 = 4.53, P = .001$)	0.091 (.042)	75 ($t_8 = 2.56, P = .02$)	$t_{17} = 2.04, P = .03$
	8	0.059 (0.014)	23 ($t_8 = 3.05, P = .008$)	0.084 (.025)	52 ($t_8 = 3.61, P = .004$)	$t_{17} = 2.58, P = .010$

*Lac/NAA indicates lactate/N-acetylaspartate (metabolite ratio); ellipses, not applicable.

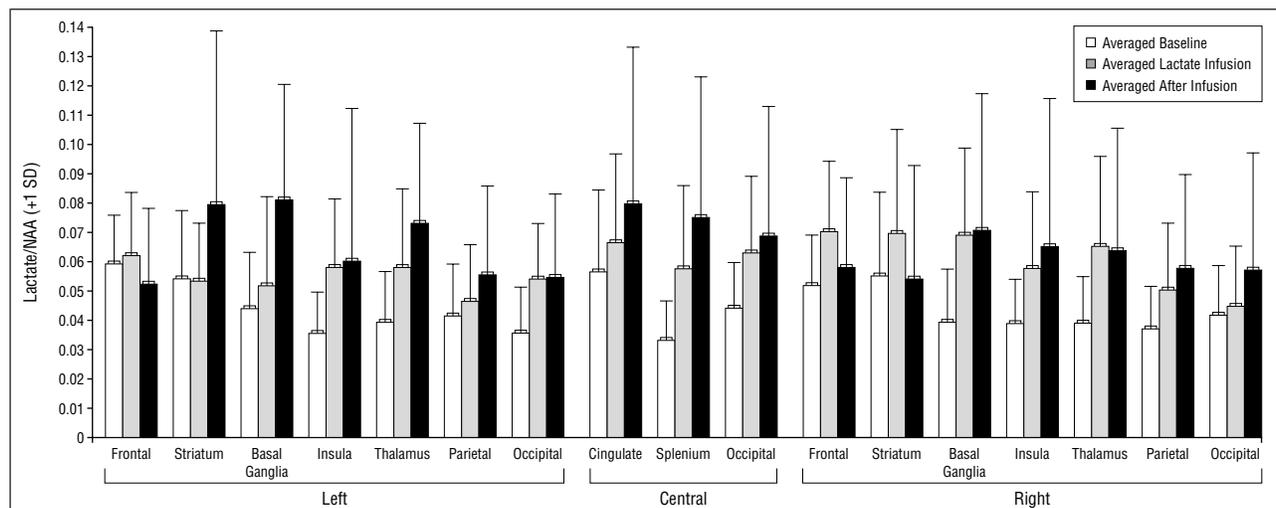


Figure 6. Regional analysis of lactate/N-acetylaspartate (NAA) response within discrete brain regions (mean \pm SD) across averaged baseline, infusion, and postinfusion periods. Both subjects with panic disorder and control subjects are included as no differential group response by region was shown. For the majority of regions sampled, there was a rise in lactate level during and after lactate infusion. Some regions (ie, frontal lobe and cingulate) had reduced numbers of subjects with valid spectra due to lipid contamination and inhomogeneity effects.

lution obtained in this study also may have obscured more limited regions of activation, although this same PEPSI technique, used to study metabolic effects of caffeine, was able to demonstrate regional brain lactate differences.³⁰ At baseline, lactate level in the left frontal region was elevated compared with other regions; however, there was no significant correlation with anxiety or arousal. As the frontal regions, in particular, suffer from susceptibility artifacts when using spectroscopic imaging techniques, this finding from the left frontal region must be viewed with caution until confirmed by further studies.

A lack of correlation between (1) API symptoms, (2) anxiety, panic severity, and brain lactate measurements at baseline, or (3) in response to lactate infusion suggests that brain lactate elevations are not directly related to symptom severity, although we are unable to account for possible threshold effects. There were insufficient numbers of panic nonresponders to show differences from controls or panic responders, although lactate insensitivity among these few panic-disordered subjects suggests clinical heterogeneity in the sample. As the study design did not include a placebo cell and no subjects panicked during the baseline period, we also are unable to address potential effects of generalized arousal on brain lactate findings. In this regard, prior work demonstrating greater brain lactate rises during controlled hyperventilation among subjects with panic disorder in remission who were previously lactate sensitive,⁸ and persistent higher brain lactate rises with reinfusion among a small group of fluoxetine-treated subjects with panic disorder who converted to a negative lactate response,⁷ indicates that symptom provocation, per se, is not a necessary requirement for abnormal brain lactate rises among subjects with panic disorder. Instead, a role for metabolic and/or neurovascular blood flow dysregulation is suggested by the widely distributed abnormal brain lactate rises observed in this study, that would be in keeping with results of prior reports of systemic lactate abnormalities associated with “anxiety neurosis” or panic disorder.³¹⁻³⁶

Although brain lactate and $P_{ET}CO_2$ levels were negatively correlated only in the postinfusion period, hyper-

ventilation, among all subjects, undoubtedly contributed to brain lactate rise in response to lactate infusion. In conjunction with the similar overall decrease in $P_{ET}CO_2$ for both panic-disorder and control subjects during lactate infusion, hypocapnia can decrease the cerebral perivascular hydrogen concentration, resulting in cerebral vasoconstriction.^{37,38} Vasoconstriction, in addition to its effects on lactate clearance, may increase lactate levels in 1 of 2 ways: first, by stimulating the release of the vasodilatory chemicals that normally counteract excessive vasoconstriction,³⁹⁻⁴³ and second, as a result of anaerobic metabolism of glucose (when excessive vasoconstriction causes areas of relative ischemia).^{44,45} As lactate itself is one of the chemical metabolic vasodilators released to antagonize cerebral vasoconstriction,³⁹ if other vasodilatory mechanisms are more active in controls, they will antagonize hypocapnia-induced vasoconstriction and little stimulus for lactate release will be present. On the other hand, greater adrenergic nerve activity in subjects with panic disorder and increased cellular release of endothelin, endothelial-derived constricting factor, prostaglandins, thromboxanes, and leukotrienes, would accentuate vasoconstriction with resultant greater anaerobic metabolism and increased lactate production.^{44,45}

We were unable to measure T_2 relaxation times (ie, spin-spin relaxation or transverse relaxation time) for lactate due to its low concentration at baseline and rapidly changing concentration during and after lactate infusion. It is conceivable that T_2 relaxation time differences between NAA and lactate, within the context of diverse metabolic effects, such as pH changes during lactate infusion, could have affected our measurements using metabolic ratios. But, this is unlikely to have differentially affected measurements between diagnostic groups. Furthermore, assessment of absolute lactate signal also differentiated subjects with panic disorder from control subjects and demonstrated the same pattern of metabolic response to lactate infusion. It is possible that only a portion of brain lactate is being measured due to a bound component that is “magnetic resonance invisible,”⁴⁶ but this

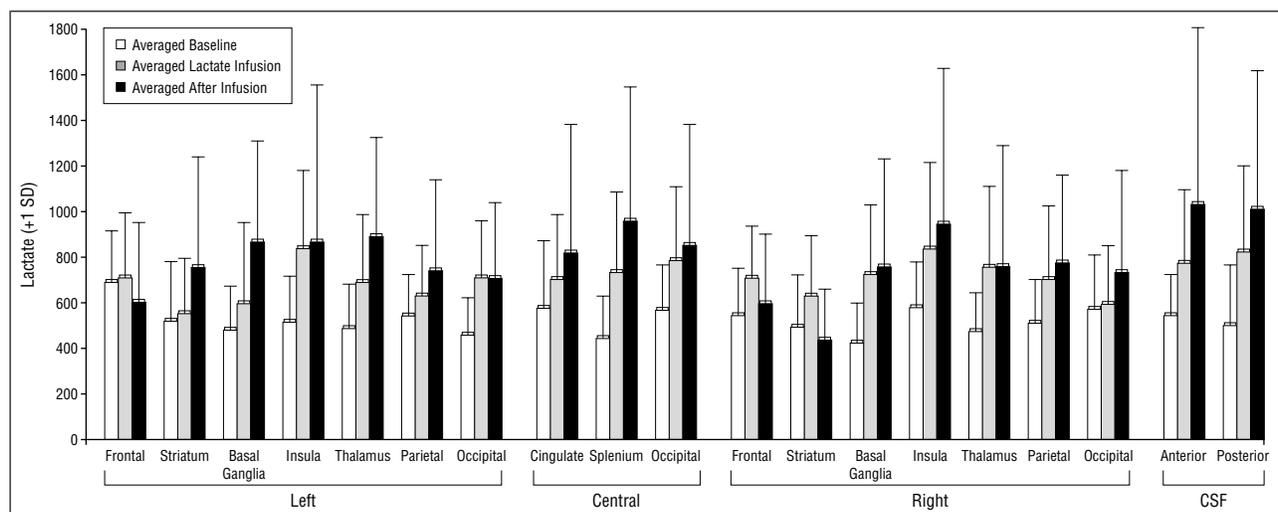


Figure 7. Regional analysis of lactate signal amplitude changes within discrete brain regions (mean \pm SD) across averaged baseline, infusion, and postinfusion periods. The use of lactate signal allowed assessment of cerebrospinal fluid (CSF) regions, shown on the right-hand side of the graph. Both subjects with panic disorder and control subjects are included as no differential group response by region was shown. Lactate in CSF demonstrates a similar rise as other brain regions in response to lactate infusion.

should only affect the relative signal-to-noise ratio for lactate measurement among all subjects and not affect the measurement of changes between groups over time.

Fast, spectroscopic imaging offers a powerful new tool for serially measuring brain chemistry using the intrinsic hydrogen signal of brain regions sampled.^{12,13} Our application using PEPSI to map the brain distribution of changing metabolic conditions during lactate precipitation of panic indicates that generalized or diffuse brain lactate elevations occur, with a time course and magnitude of rise between diagnostic groups consistent with findings of earlier single-voxel studies. In the present study, no temporal relationship between lactate changes and discrete neuroanatomical loci was found. Regional brain lactate measurements demonstrate that the brain lactate abnormality associated with lactate-induced panic is diffuse, opening up new directions of inquiry for understanding pathological mechanisms underlying panic disorder.

Accepted for publication September 22, 1998.

This work was supported by grant RO1 MH50579 from the National Institute of Mental Health, Rockville, Md (Dr Dager).

The authors thank Marie Domsalla for subject coordination and administrative support and acknowledge our indebtedness to the Diagnostic Imaging Sciences Center, under the direction of James Nelson, MD, for its support and encouragement in conducting this research.

Reprints: Stephen R. Dager, MD, University of Washington, Box 354694, 4225 Roosevelt Way NE, Suite 306-C, Seattle, WA 98105-6099 (e-mail: srd@u.washington.edu).

REFERENCES

- Cowley DS, Dager SR, Dunner DL. The lactate infusion challenge. In: Asnis GM, van Praag HM, eds. *Pathogenetic Mechanisms of Panic Disorder*. New York, NY: Brunner/Mazel; 1995.
- Dager SR, Rainey JM, Kenny MA, Artru AA, Metzger GD, Bowden DS. Central nervous system effects of lactate infusion in primates. *Biol Psychiatry*. 1990;27:193-204.
- Dager SR, Steen RG. Applications of magnetic resonance spectroscopy to the investigation of neuropsychiatric disorders. *Neuropsychopharmacology*. 1992;6:249-266.
- Dager SR, Marro KI, Metzger GD, Richards TL. Detection of whole brain lactate increases in rats during intravenous 1 M sodium L-lactate infusion by NMR spectroscopy. *Biol Psychiatry*. 1992;32:913-921.
- Dager SR, Marro KI, Richards RL, Metzger GD. Localized magnetic resonance spectroscopy measurement of brain lactate during intravenous 0.5 M sodium lactate infusion in healthy volunteers. *Life Sci*. 1992;51:973-985.
- Dager SR, Marro KI, Metzger GD, Richards TL. Preliminary applications of magnetic resonance spectroscopy to investigate lactate-induced panic. *Am J Psychiatry*. 1994;151:57-63.
- Dager SR, Richards T, Strauss WL, Artru A. Single-voxel ¹H MRS investigation of brain metabolic changes during lactate-induced panic. *Psychiatry Res*. 1997;76:89-99.
- Dager SR, Strauss WL, Marro KI, Richards TL, Metzger GD, Artru AA. Proton magnetic resonance spectroscopy investigation of hyperventilation in subjects with panic disorder and comparison subjects. *Am J Psychiatry*. 1995;152:666-672.
- Reiman EM, Raichle ME, Robins E, Mintun MA, Fusselman MJ, Fox PT, Price JL, Hackman KA. Neuroanatomical correlates of a lactate-induced anxiety attack. *Arch Gen Psychiatry*. 1989;46:493-500.
- Reiman EM, Fusselman MJ, Fox PT, Raichle ME. Neuroanatomical correlates of anticipatory anxiety. *Science*. 1989;243:1071-1074.
- Drevets WC, Videen TO, MacLeod AK, Haller JW, Raichle ME. PET images of blood flow changes during anxiety [correction]. *Science*. 1992;256:1696.
- Posse S, Muller-Gartner H-W, Dager SR. Functional magnetic resonance studies of brain activation. *Semin Clin Neuropsychiatry*. 1996;1:76-88.
- Posse S, Dager SR, Richards TL, Yuan C, Artru AA, Ogg R, Müller-Gartner HW, Hayes C. Measurement of regional brain metabolic response to hyperventilation using functional proton echo-planar spectroscopic imaging (PEPSI). *Magn Reson Med*. 1997;37:858-865.
- American Psychiatric Association. *Diagnostic and Statistical Manual Disorders, Fourth Edition*. Washington, DC: American Psychiatric Association; 1994.

- Pitts FN, McClure JN. Lactate metabolism in anxiety neurosis. *N Engl J Med*. 1967;277:1329-1336.
- Liebowitz MR, Fyer AJ, Gorman JM, Dillon D, Appleby IL, Levy G, Anderson S, Levitt M, Palij M, Davies SO, Klein DF. Lactate provocation of panic attacks, I: clinical and behavioral findings. *Arch Gen Psychiatry*. 1984;41:764-770.
- Dager SR, Cowley DS, Dunner DL. Biological markers in panic states: lactate-induced panic and mitral valve prolapse. *Biol Psychiatry*. 1987;22:339-359.
- Dillon DJ, Gorman JM, Liebowitz MR, Fyer AJ, Klein DF. Measurement of lactate-induced panic and anxiety. *Psychiatry Res*. 1987;20:97-105.
- Richards TL, Dager SR, Panagiotides HS, Hayes CE, Posse S, Serafini S, Nelson JA, Maravilla KR. Functional magnetic resonance spectroscopy during language activation: a preliminary study using proton echo-planar spectroscopic imaging. *Int Neuroradiol*. 1997;3:490-495.
- Tofts PS, Wray S. A critical assessment of methods of measuring metabolite concentrations by NMR spectroscopy. *NMR Biomed*. 1988;1:1-10.
- Williams SR, Proctor E, Allen K, Gadian DG, Crockard HA. Quantitative estimation of lactate in the brain by ¹H NMR. *Magn Reson Med*. 1988;7:425-431.
- Birken DL, Oldendorf WH. N-acetyl-L-aspartic acid: a literature review of a compound prominent in ¹H-NMR spectroscopic studies of brain. *Neurosci Biobehav Rev*. 1989;13:23-31.
- Damasio D, Damasio AR. *Lesion Analysis in Neuropsychology*. New York, NY: Oxford University Press; 1989.
- Geisser S, Greenhouse SW. An extension of Box's results on the use of the F distribution in multivariate analysis. *Ann Math Stat*. 1958;29:885-891.
- Dunn OJ. Multiple comparisons among means. *J Am Stat Assoc*. 1961;56:52-64.
- Pritchard J, Rothman D, Nowotny E, et al. Lactate rise detected by ¹H NMR in human visual cortex during physiologic stimulation. *Proc Natl Acad Sci U S A*. 1991;88:5829-5831.
- Dager SR, Swann AC. Advances in brain metabolism research: toward a moving picture of neural activity. *Biol Psychiatry*. 1996;39:231-233.
- Stewart RS, Devous MD, Rush AJ, Lane L, Bonte FJ. Cerebral blood flow changes during sodium lactate-induced panic attacks. *Am J Psychiatry*. 1988;145:442-449.
- Fischer H, Andersson JL, Furmark T, Fredrikson M. Brain correlates of an unexpected panic attack: a human positron emission tomographic study. *Neurosci Lett*. 1998;251:137-140.
- Dager SR, Layton ME, Strauss W, Richards TL, Heide A, Friedman SD, Artru AA, Hayes CE, Posse S. Human brain metabolic response to caffeine and the effects of tolerance. *Am J Psychiatry*. In press.
- Linko E. Lactic acid response to muscular exercise in neurocirculatory asthenia. *Ann Med Int Fenniae*. 1950;39:161-176.
- Holmgren A, Strom G. Blood lactate concentration in relation to absolute and relative work load in normal men and in mitral stenosis, atrial septal defect, and vasoregulatory asthenia. *Acta Med Scand*. 1959;163:185-193.
- Rainey JM, Frohman CE, Warner K, Bates S, Pohl R, Yeragani V. Panic anxiety and lactate metabolism. *Psychopharm Bull*. 1985;21:434-437.
- Liebowitz M, Gorman J, Fyer A, Levitt M, Dillon D, Levy G, Appleby IL, Anderson S, Palij M, Davies SO, Klein DF. Lactate provocation of panic attacks, II: biochemical and physiological findings. *Arch Gen Psychiatry*. 1985;42:709-719.
- Uhde TW, Boulenger JP. Caffeine model of panic. In: Lerer B, Gershon S, eds. *New Directions in Affective Disorders*. New York, NY: Springer-Verlag; 1989.
- Maddock RJ, Mateo-Bermudez J. Elevated serum lactate following hyperventilation during glucose infusion in panic disorder. *Biol Psychiatry*. 1990;27:411-418.
- Miller JD, Bell BA. Cerebral blood flow variations with perfusion pressure and metabolism. In: Wood JH, ed. *Cerebral Blood Flow: Physiologic and Clinical Aspects*. New York, NY: McGraw-Hill Book Co; 1987:119-130.
- Edvinsson L, MacKenzie ET, McCulloch J. Changes in arterial gas tensions. In: Edvinsson L, MacKenzie ET, McCulloch J, eds. *Cerebral Blood Flow and Metabolism*. New York, NY: Raven Press; 1993:524-552.
- Artru AA. Influence of anesthetic agents and techniques on intracranial hemodynamics and cerebral metabolism. In: Lam AM, ed. *Anesthetic Management of Acute Head Injury*. New York, NY: McGraw-Hill Inc; 1995:143-179.
- Edvinsson L, MacKenzie ET, McCulloch J. Acetylcholine. In: Edvinsson L, MacKenzie ET, McCulloch J, eds. *Cerebral Blood Flow and Metabolism*. New York, NY: Raven Press; 1993:285-312.
- Edvinsson L, MacKenzie ET, McCulloch J. Adenosine. In: Edvinsson L, MacKenzie ET, McCulloch J, eds. *Cerebral Blood Flow and Metabolism*. New York, NY: Raven Press; 1993:325-338.
- Edvinsson L, MacKenzie ET, McCulloch J. Histamine. In: Edvinsson L, MacKenzie ET, McCulloch J, eds. *Cerebral Blood Flow and Metabolism*. New York, NY: Raven Press; 1993:313-324.
- Edvinsson L, MacKenzie ET, McCulloch J. Perivascular nerve fibers in brain vessels. In: Edvinsson L, MacKenzie ET, McCulloch J, eds. *Cerebral Blood Flow and Metabolism*. New York, NY: Raven Press; 1993:57-91.
- Edvinsson L, MacKenzie ET, McCulloch J. Adrenergic mechanisms. In: Edvinsson L, MacKenzie ET, McCulloch J, eds. *Cerebral Blood Flow and Metabolism*. New York, NY: Raven Press; 1993:183-230.
- Edvinsson L, MacKenzie ET, McCulloch J. Prostaglandins and other eicosanoids. In: Edvinsson L, MacKenzie ET, McCulloch J, eds. *Cerebral Blood Flow and Metabolism*. New York, NY: Raven Press; 1993:339-377.
- Chang LH, Pereira BM, Weinstein PR. Comparison of lactate concentration determinations in ischemic and hypoxic rat brains by in vivo and in vitro ¹H NMR spectroscopy. *Magn Reson Med*. 1987;4:575-581.