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Lactate rise detected by ^{1}H NMR in human visual cortex during physiologic stimulation

(brain/vision/neurochemistry)

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ABSTRACT **Brain lactate concentration is usually assumed to be stable except when pathologic conditions cause a mismatch between glycolysis and respiration. Using newly developed ^{1}H NMR spectroscopic techniques that allow measurement of lactate *in vivo*, we detected lactate elevations of 0.3–0.9 mM in human visual cortex during physiologic photic stimulation. The maximum rise appeared in the first few minutes; thereafter lactate concentration declined while stimulation continued. The results are consistent with a transient excess of glycolysis over respiration in the visual cortex, occurring as a normal response to stimulation in the physiologic range.**

Glucose and oxygen—the principal substrates of brain energy metabolism—are consumed by that organ at matched rates that ordinarily maintain stable lactate concentrations. Brain lactate elevations due to lack of oxygen or increased energy demand to the degree of status epilepticus are well-known phenomena, and extensive research on them has created a general impression that brain lactate elevation always reflects pathologic conditions. However, several recent reports suggest that brain activity within the physiologic range may cause brain lactate to rise. In an earlier study using nuclear magnetic resonance spectroscopy (MRS) *in vivo*, we found that lactate rose in posterior cerebral cortex of rabbits when electric shocks were delivered to the optic nerves (1). Ueki *et al.* (2) demonstrated lactate elevation in rat somatosensory cortex due to forepaw stimulation. In humans studied by positron emission tomography (PET), Fox *et al.* (3) showed that visual stimulation caused 30–50% increases in blood flow and glucose uptake of visual cortex, whereas oxygen extraction rose no more than 5%. Newly developed MRS techniques permit repeated noninvasive detection of lactate in a few cc of human brain (4–6). We have used such techniques to show that photic stimulation does indeed cause a clear, although transient, elevation of lactate in human visual cortex; a preliminary report has appeared (7).

METHODS

An Oxford Research Systems/Bruker 2.1-T Biospec spectrometer was used, with a 6-cm single-turn surface coil situated beneath the occiput of a supine subject positioned so that the orbito-meatal line was vertical. For ^{1}H spectroscopy, an inversion recovery gradient refocus image guided positioning of a 13-cc image-selected *in vivo* spectroscopy (ISIS)-selected sensitive volume (8) so as to include dorsal portions of both calcarine cortices while excluding bone marrow. Localized spectra were obtained using three-dimensional ISIS with 8-msec phase swept hyperbolic secant inversion pulses (μ = 5, 2000-Hz bandwidth, 1% truncation) (9). The acquisition sequence was a 6-msec seven-lobe sinc excitation pulse in a gradient perpendicular to the plane of the coil, followed by a spin echo sequence with a semiselective 2-2 refocusing pulse (10) and rephasing gradients in the refocusing period. Additional suppression of extracerebral signals was accomplished by a $\Theta/3$ depth pulse (5, 11) followed by dephasing gradients and a surface spoiler gradient (12); in some experiments noise pulses in two dimensions were also used (13). Localized shimming using the acquisition pulse sequence without the ISIS inversions achieved a water line width <6 Hz in all experiments. Spectra were made from 128 free induction decays acquired in 6 or 8 min. Processing was by a 5-Hz Lorentzian to Gaussian filter. Relative changes in lactate concentration were determined from the summed amplitudes of the methyl doublet resonance centered at 1.34 ppm with reference to the *N*-acetylaspartate resonance at 2.023 ppm (14). Absolute lactate concentrations were estimated from the ratio of the integrated intensities of a 24-Hz bandwidth centered at 1.34 ppm and the creatine resonance at 3.22 ppm in spectra optimized to correct for differences in excitation profile. The creatine resonance was assumed to represent a concentration of 9.6 mM (15). Subjects were healthy males 22–55 years old. All gave their consent according to procedures approved by the Yale Human Investigation Committee. Photic stimulation was given with a Grass S10 stimulator. Subjects wore goggles that excluded light, save that from 5 × 6 grids of red light-emitting diodes before each eye. Flashing of the grids at 16 Hz constituted the stimulus. Subjects lay quietly in the magnet with their eyes open throughout the experiment.

RESULTS

Fig. 1*A* shows ^{1}H spectra obtained from visual cortex before, during, and after a 48-min period of photic stimulation. The lactate resonance with its characteristic 7-Hz splitting is clearly visible, as is its increase in intensity during stimulation and decline thereafter. Fig. 1*B* shows the full time course of lactate changes in that experiment. Since the maximum lactate rise appeared to occur soon after stimulus onset, we used stimulation periods of 12 or 18 min in later experiments to minimize possible effects of subject fatigue.

Fig. 2*A* shows results from five subjects before, during, and after photic stimulation. For each subject, lactate intensity is normalized to its mean in control spectra. An immediate lactate elevation following onset of stimulation is evident in all, and in four, the maximum elevation was at the

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Abbreviations: MRS, magnetic resonance spectroscopy; PET, positron emission tomography.

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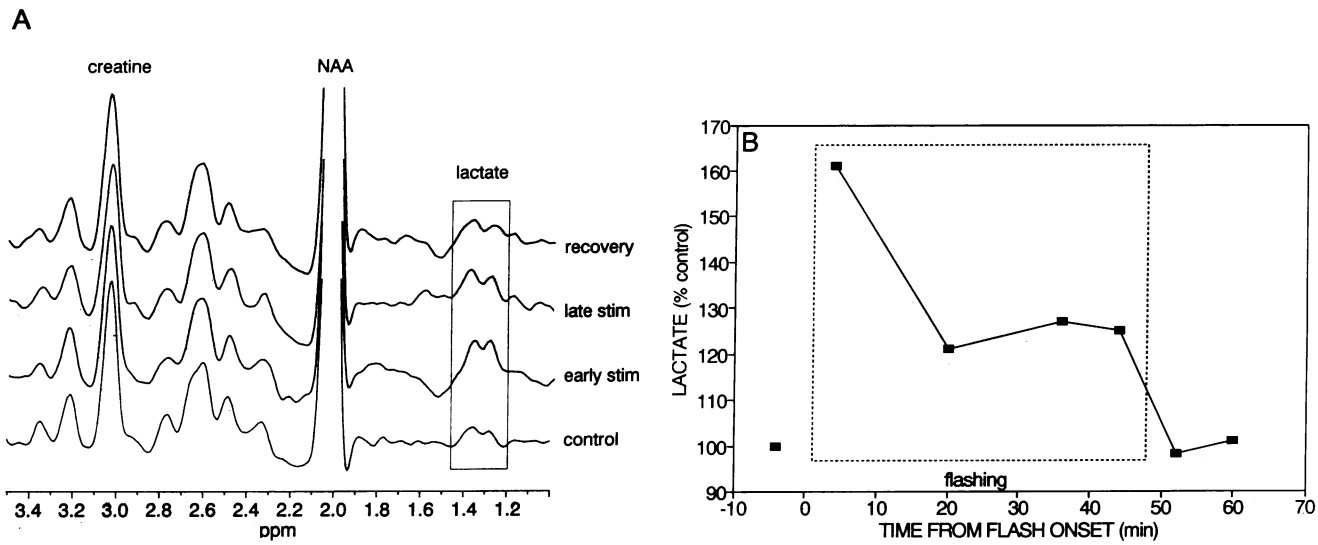

FIG. 1. (*A*) ^1H spectra from 13 cc of human visual cortex before, during, and after photic stimulation (stim) by red dot grids before each eye flashing at 16 Hz. The frequency axis is in ppm and was set from the prominent resonance of *N*-acetylaspartate (NAA) at 2.02 ppm. The shaded area highlights the lactate methyl proton resonance at 1.33 ppm, with its characteristic 7-Hz splitting. The creatine resonance at 3.04 ppm is the total signal from methyl protons of phosphocreatine and creatine. (*B*) Time course of the experiment illustrated in *A*, with intensities of the lactate resonance plotted as a percentage of its control intensity.

earliest time point obtained. All showed a subsequent time course of decline qualitatively similar to that in Fig. 1*B*. The mean difference between the last point before onset of stimulation and the first point thereafter was 54%, with a 99 percent confidence interval from 8.8% to 100% (degrees of freedom 4, *t* statistic 4.6) (16).

Absolute lactate concentrations in these five subjects were estimated as described above. The mean in the control period was 0.71 mM, with a range from 0.53 mM to 1.0 mM, which agrees well with our earlier measurement of resting cerebral lactate concentration of 0.6 mM, based on lactate signals obtained by homonuclear editing (5). Increases in lactate concentration during stimulation were 0.3–0.9 mM.

Fig. 2*B* shows data from five experiments on the same subject. Again, an immediate initial rise in lactate after onset of photic stimulation and a subsequent decline similar to that in Fig. 1*B* are evident. The mean difference between the last point before onset of stimulation and the first point thereafter was 57%, with a 99 percent confidence interval from 3.3% to 90% (degrees of freedom 4, *t* statistic 4.6) (16). Two of the experiments were done on successive days, and the others were several months apart; in these limited data, no effect of prior experience was detectable.

Hyperventilation was not the cause of the lactate elevation. In four subjects—2 of them naive to the photic stimulus and ignorant of the nature of the experiment at the time of testing—end-tidal CO_2 measured outside the magnet with a Hewlet–Packard Capnometer did not change at any time before, during, or after a 10-min period of photic stimulation identical to that used in the spectroscopic experiments. The same monitoring was done twice in the magnet; the photically induced rise in brain lactate occurred without any change in end-tidal CO_2.

DISCUSSION

These experiments demonstrate that photic stimulation within the physiologic range raises the lactate concentration of human visual cortex. The effect is maximum within the first 6 min of stimulation in most cases and declines thereafter. We believe that these results reflect a transient excess of glycolysis over respiration in the visual cortex, occurring as a normal response to physiologic stimulation. This interpretation is consistent with that of Fox *et al.* (3) concerning their PET data. However, the transiency and modest degree of the lactate rise we observed suggest that glycolysis and respiration did not remain unmatched very long. The mis-

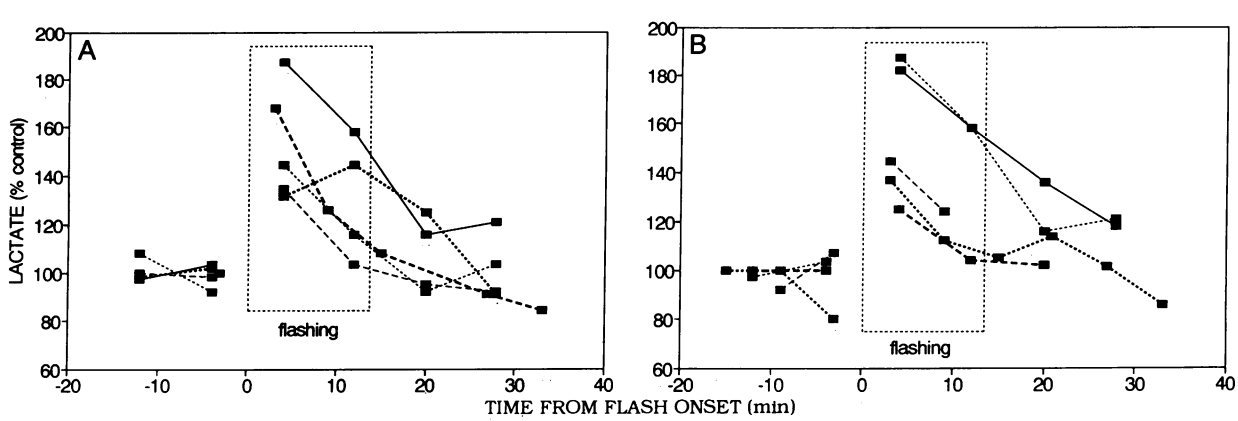

FIG. 2. (*A*) Photic stimulation of five different subjects. Lactate resonance intensities are plotted as a percentage of the mean of all control points. (*B*) Photic stimulation of the same subject on five different occasions, plotted as in *A*.

match between glucose uptake and oxidation in the Fox study would cause lactate to rise at a rate of 0.3 mM/min if lactate transport remained constant. The peak lactate elevations we observed were 0.3–0.9 mM, which implies that a net mismatch equivalent to 0.3 mM lactate per min was present for no more than 1–3 min. The oxygen extraction measurements in the Fox study were made within the first minute after the onset of stimulation (P. T. Fox, personal communication). Our data suggest that oxygen extraction measured later would show a rise, unless the mismatch is due to a burst of increased glycolysis lasting only a few minutes. This distinction is quite important for determining function-related energy cost, which would be much less in the latter case. It can be made by existing PET and MRS techniques.

The generality of our result—which has been confirmed in the human visual system by another group (17)—remains to be tested by examination of auditory, somatosensory, and motor systems. As MRS methods are quite capable of detecting lactate elevations in the parts of the brain associated with those functions, relevant data should soon be available. In any system where lactate elevation accompanies activation, functional metabolic mapping by ^{1}H spectroscopic imaging of lactate (18) may prove useful.

An excess of glycolysis over respiration occurring in the course of *normal* brain activity is of considerable interest for theories of how biochemical events are related to brain function. One basic question about brain biochemical organization emerges immediately: Does the excess occur in most brain cells affected by the stimulus, or does the stimulation call into action cells that are by enzymatic endowment principally glycolytic? Muscle cells are known to vary with respect to relative capacity for glycolysis and respiration, and the differences are of physiologic importance (19). Recent work suggests that cellular distribution of glycolytic and respiratory enzymes in the brain is also heterogeneous (20). A second basic question: Might lactate have some direct effect on neural function? The possibility is not wholly speculative. In susceptible humans, lactate infusion causes panic attacks associated with localized abnormalities of cerebral blood flow (21), possibly due to a central action of lactate (22). Lactate suppresses depolarization-induced release of acetylcholine by *Torpedo* electroplaques (23). Whether lactate exerts any such direct effect in the mammalian brain remains to be elucidated.

In conclusion, lactate elevation occurs in the course of normal brain function, most probably due to a transient excess of glycolysis over respiration. The noninvasiveness of PET and MRS and the chemical specificity of the latter were necessary to detect the phenomenon, which can be investigated further by MRS studies of cerebral lactate (24) and glutamate (25) turnover following [1-^{13}C]glucose infusion. Methods that resolve events at the cellular level will be necessary to establish its full biological significance.

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