out of six responded to peptide A1 and one out of three to peptide B1. Since T cell response is under HLA control it is not expected that all individuals respond to any given T cell epitope (*24*). The epitopes identified here interact with HLA class II molecules DQ (peptide A1) and HLA DR-6 (peptide B1, Table 4), as shown by the effect of monoclonal antibodies to DQ, DR, and DP in a T cell stimulation assay.

It is evident that for the design of a vaccine information is required not only on B and T cell epitopes but also on relevant HLA class II molecules within a population.

REFERENCES AND NOTES

1. R. Hall *et al.*, *Nature* **311**, 379 (1984).
2. L. H. Perrin *et al.*, *J. Exp. Med.* **160**, 441 (1984).
3. W. A. Siddiqui *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3014 (1987).
4. A. A. Holder and R. R. Freeman, *Nature* **294**, 361 (1981).
5. N. Epstein *et al.*, *J. Immunol.* **127**, 212 (1981).
6. J. S. McBride, C. I. Newbold, R. Anand, *J. Exp. Med.* **161**, 160 (1985).
7. E. M. Patarroyo *et al.*, *Nature* **328**, 629 (1987).
8. A. Cheung *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8328 (1986).
9. J. H. L. Playfair *et al.*, *Clin. Exp. Immunol.* **62**, 19 (1985).
10. M. Mackay *et al.*, *EMBO J.* **4**, 3823 (1985).
11. F. Sinigaglia *et al.*, *J. Immunol.*, in press.
12. R. Gentz *et al.*, *EMBO J.* **7**, 222 (1988).
13. The coding sequence of gp190 between the unique Pvu II and Hae III sites (bp 316–964) henceforth called F2, was fused to Bam HI (5′ end) and Hind III (3′ end) linkers and cloned into the polylinker of the expression vector pUH130,RBSII a derivative of pDS5 (*25*). Upon induction by IPTG fusion proteins containing the gp190 portion at the NH_2-terminus of CAT were obtained. After cleavage of the plasmid DNA at the Bam HI site the F2-specific sequence was shortened from the 5′ end by exonuclease III (40, 60, 80, 100, and 120 seconds) and then treated with nuclease S1 and Klenow enzyme. Bam HI-linkers were ligated to the blunted ends and after digestion with Bam HI and Hind III fragments of the desired length were isolated from polyacrylamide gels and recloned into pUH130,RBSII. The progressively shortened fusion proteins (2.1–2.12) differing from each other by four to ten amino acids were expressed in *E. coli*.
14. P. G. H. Gell and B. Benaceraf, *Immunology* **2**, 64 (1959).
15. R. J. Lamb *et al.*, *Nature* **300**, 66 (1982).
16. Cultures were harvested after exposure to [^{3}H]thymidine for 12 to 18 hours on day 3 of coculture of 2×10^4 responder cells with 5×10^5 autologous irradiated (3000 rad) peripheral blood mononuclear cells (PBMC) as source of antigen presenting cells (APC). The fragments were added as crude 6*M* urea lysates (10 μl/ml) of induced *E. coli* cultures (10^{10} cells per milliliter).
17. I. Clark-Lewis *et al.*, *Science* **231**, 134 (1986).
18. C. De Lisi and J. A. Berzofsky, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7048 (1985).
19. J. B. Rothbard, *Ann. Inst. Pasteur* **137E**, 518 (1986).
20. T cells were stimulated with 10 μl of *P. falciparum*–infected red blood cells. Packed erythrocytes of asynchronous cultures with 3 to 5% parasitemia were washed in RPMI medium and resuspended to a final concentration of 5%. Upon one cycle of freezing and thawing the material was used for T cell stimulation.
21. K. Tanabe *et al.*, *J. Mol. Biol.* **195**, 273 (1987).
22. J. L. Grun and W. P. Weidanz, *Nature* **290**, 143 (1981).
23. B. J. Cottrell, J. H. L. Playfair, J. B. DeSouza, *Clin. Exp. Immunol.* **34**, 147 (1978).
24. B. Benaceraf and N. R. Germain, *Immunol. Rev.* **38**, 71 (1987).
25. H. Bujard *et al.*, *Methods Enzymol.* **155**, 416 (1987).
26. A. Ziegler and C. Milstein, *Nature* **279**, 243 (1979).
27. A. J. Watson *et al.*, *ibid.* **304**, 358 (1983).
28. M. M. Trucco, G. Garotta, J. W. Stocker, R. Ceppellini, *Immunol. Rev.* **47**, 219 (1979).

29. We thank A. Treciak and D. Gillessen for help in peptide synthesis, K. Früh for discussions, and B. Janssen for technical assistance. This work was supported by grants of the Bundesministerium für Forschung und Technologie and by the Fond der Chemischen Industrie Deutschlands.

19 January 1988; accepted 12 April 1988

Lactate-Supported Synaptic Function in the Rat Hippocampal Slice Preparation

AVITAL SCHURR,* CATHERINE A. WEST, BENJAMIN M. RIGOR

The present study was undertaken to examine the possibility that cerebral energy metabolism can be fueled by lactate. As a sole energy substrate, lactate supported normal synaptic function in rat hippocampal slices for hours without any sign of deterioration. Slices that were synaptically silent as a result of glucose depletion could be reactivated with lactate to show normal synaptic function. When slices were exposed to the glycolytic inhibitor iodoacetic acid, lactate-supported synaptic function was unaffected, whereas that supported by glucose was completely abolished. This indicated that lactate was metabolized directly via pyruvate to enter the tricarboxylic acid cycle. Thus, under conditions that lead to lactate accumulation (cerebral ischemia) this "end product" may be a useful alternative as a substrate for energy metabolism.

GLUCOSE IS GENERALLY ACCEPTED to be the primary substrate in both aerobic and anaerobic brain energy metabolism (*1*), whereas lactate is the end product of such metabolism in the absence of oxygen (*1*). The oxidation of other substrates by different brain tissue preparations has been reported (*2*). However, the physiological significance of such capability in vivo has not been determined. Alternative substrates to glucose could be important in hypoglycemia or hypoxia, and especially during cerebral ischemia, which appears to cause increased damage under hyperglycemic conditions (*3, 4*). Lactic acidosis has been suggested as a major detrimental factor under these conditions (*4*). Our studies in vitro have indicated a beneficial effect of hyperglycemic levels of glucose on synaptic function recovery from hypoxia (*5*). Moreover, lactic acidosis had no adverse effect on hypoxic brain tissue in vitro (*5, 6*). We report here that synaptic function in vitro can be supported by lactate as the sole substrate for energy metabolism.

One possible explanation for the lack of adverse effects from lactic acidosis in brain tissue is rapid lactate metabolism. Is this metabolism simply a way to reduce lactic acid to safe levels or is it an important alternative as an energy substrate under certain circumstances? We used the rat hippocampal slice preparation to answer these questions.

We performed all experiments in a dual, linear-flow incubation chamber (*7*) using the rat hippocampal slice preparation and electrophysiological methods (*5–9*). Extracellular recording of evoked population responses (population spikes) from the stra-

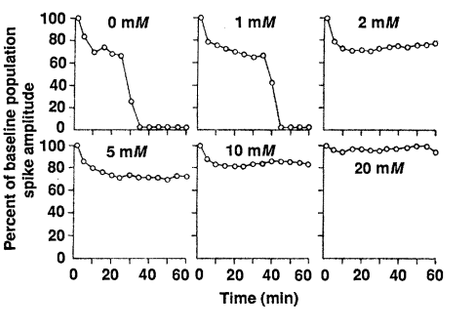

Fig. 1. The effect of a 60-min perfusion of rat hippocampal slices with the concentrations shown of lactate in ACSF on the evoked population spike amplitude at 34° ± 0.5°C. Slices were prepared, maintained, and stimulated orthodromically (Schaffer collaterals) as described (*5–9*); recordings (once per minute) were from the CA1 stratum pyramidale layer. Data show percent of baseline population spike amplitude at 5-min intervals. Lactate at 2 m*M* or higher could maintain synaptic function in hippocampal tissue in vitro as well as, or better than, 2 m*M* glucose (*9*). A dual chamber was used and control and experimental slices originated from one rat brain for any given experiment. Records were stored and analyzed later (*5–9*). Lactate (Sigma; 30% L-lactic acid solution) was neutralized with NaOH before use. Osmolarity was kept constant in the lactate ACSF solution by adjusting the concentration of NaCl.

Department of Anesthesiology, University of Louisville School of Medicine, Louisville, KY 40292.

*To whom correspondence should be addressed.

tum pyramidale of the CA1 region were used as a measurement of synaptic function. A slice in which a response could not be evoked was considered to be synaptically inactive. The glucose concentration in the standard artificial cerebrospinal fluid (ACSF) perfusing the slices was 10 m*M* (*5–9*). When the perfusion medium was changed to ACSF without glucose (0 m*M*), the synaptic function gradually diminished (decreased amplitude of population spike) until responses could not be evoked 30 to 40 min after the beginning of such perfusion (Fig. 1, 0 m*M*). However, when slices were perfused with glucose-free ACSF containing 2 to 20 m*M* lactate, synaptic function could be maintained for at least 60 min (Fig. 1). Changes in the amplitude of evoked responses were determined continuously in two slices, control and experimental, one in each compartment of the dual chamber during any given experiment (Fig. 1). The remaining 10 to 15 slices in each compart ment were tested for the presence of synaptic function by stimulating them orthodromically at the end of the experiment. Any slice showing an evoked response (population spike) of an amplitude 3 mV or larger was counted as synaptically active (Table 1).

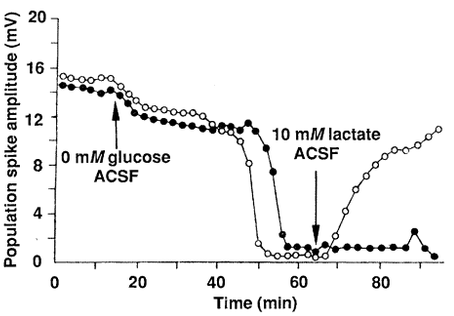

Fig. 2. The effect of lactate supplement on the synaptic function of rat hippocampal slices depleted of glucose. Slices in both compartments of the dual chamber were perfused with standard ACSF (10 m*M* glucose) for 15 min. Thereafter, glucose-depleted ACSF was passed through the chamber (95 min). At 65 min, 10 m*M* lactate was added to the glucose-depleted ACSF in one compartment (○). Synaptic function was recorded as described in Fig. 1. The slice supplemented with lactate recovered its synaptic function after glucose deprivation, whereas the control slice (●) did not.

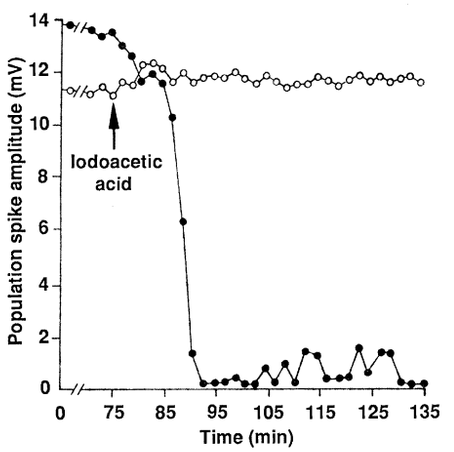

Fig. 3. The effect of the glycolytic inhibitor iodoacetic acid on synaptic function of rat hippocampal slices supplemented with lactate (○) or glucose (●) as a sole energy substrate. The slices in both compartments of the dual chamber were perfused with standard ACSF (10 m*M* glucose) for 15 min (baseline); the medium in one compartment (○) was then replaced with 20 m*M* lactate in ACSF without glucose; 60 min later 0.2 m*M* iodoacetic acid–supplemented medium was introduced to both compartments for an additional hour of perfusion. Data points of 2-min intervals are shown; however, stimulating and recording procedures were as described in Fig. 1.

Only 21% (41/196) of the slices exposed for 60 min to glucose-free ACSF displayed synaptic function, whereas a dose-dependent increase in this rate was found with increasing lactate concentration in the ACSF, reaching 100% at 20 m*M* (Table 1). In these experiments lactate gradually and completely replaced glucose in the bathing medium, yet, synaptic function was not completely diminished (as long as lactate concentration was ≥2 m*M*). In addition, lactate was capable of resuscitating synaptically silent slices that had been exposed to 45 min of glucose-free ACSF (Fig. 2). When we compared the resuscitating ability of lactate to that of standard ACSF (10 m*M* glucose), 79% (38/48) of the lactate-treated slices recovered their synaptic function after 30 min of resuscitation and 87% (41/47) of the glucose-treated slices recovered theirs (insignificant difference with chi-square test). Hence, both glucose and lactate are capable of restoring synaptic activity to synaptically silent, glucose-depleted hippocampal slices. Lactate at 10 m*M* appeared to be less efficient as an energy substrate than 10 m*M* glucose. Evidence of this was the ~20% fall in the amplitude of the evoked population spike when lactate was substituted for glucose in the perfusion medium (Fig. 1). However, at low concentration lactate seemed to be a more efficient energy source than glucose: whereas 2 m*M* lactate could maintain synaptic function for at least 60 min (Fig. 1), 2 m*M* glucose could not (*9*).

We also tested whether lactate utilization as an energy substrate proceeds directly via lactate dehydrogenase and pyruvate into the tricarboxylic acid cycle or via gluconeogenesis and glycolysis. Slices were maintained with lactate-ACSF or standard glucose-ACSF for 120 min. Exactly at half time the respective substrates were supplemented (for the remainder of the experiment) with 0.2 m*M* iodoacetic acid (Fig. 3). Of the lactate-perfused slices, 97% (28/29) were not affected by the glycolytic inhibitor since they continued to display normal synaptic function. However, synaptic function in all (n = 31) control, glucose-supplied slices was completely abolished. We concluded that lactate utilization as an energy substrate by rat hippocampal slices proceeds via pyruvate, bypassing glycolysis.

Table 1. Percentage of rat hippocampal slices showing synaptic function after 60 min of perfusion with glucose-depleted ACSF containing lactate at the molar concentrations shown. Each slice in a given experiment (10 to 15 slices per compartment of the dual chamber per experiment) was tested at the end of the 60-min perfusion period for presence of a population spike (>3 mV) by stimulating it orthodromically and recording from the CA1 stratum pyramidale layer. For other details see Fig. 1.

Lactate (m*M*)	Number of slices	Recovered (%)
0	41/196	21
1	19/94	20
2	78/91	86
5	73/81	90
10	77/80	96
20	48/48	100

Our present and previous studies (*5, 6*) suggest that lactate is not strictly an end product of energy metabolism but rather a secondary energy substrate which, under certain circumstances (hypoglycemia, ischemia) can become the principal energy source. It has been argued that because there are concomitant increases in glucose, the depressed levels of lactate during the first 12 hours of ischemic resuscitation are due to hypometabolism (*10*). Our results suggest that the decrease in lactate is due to an increase in its metabolism; this would explain the decrease in glucose utilization during the initial hours of resuscitation. Apparently, the conversion of lactate to pyruvate, the substrate for the tricarboxylic acid cycle, is preferred thermodynamically over the conversion of glucose to pyruvate, since the latter requires the investment of adenosine triphosphate while the former does not.

REFERENCES

1. L. Sokoloff, in *Basic Neurochemistry*, G. J. Siegel, R. W. Albers, B. W. Agranoff, R. Katzman, Eds. (Little, Brown, Boston, ed. 3, 1981), pp. 471–495.
2. K. A. C. Elliott, in *Neurochemistry, The Chemical Dynamics of Brain and Nerve*, K. A. C. Elliott, H. I. Page, J. H. Quastel, Eds. (Thomas, Springfield, IL, 1955), pp. 53–93; H. E. Himwich, *Brain Metabolism and Cerebral Disorders* (Williams & Wilkins, Baltimore, 1951), pp. 9–25; H. McIlwain, *Biochemistry and the Central Nervous System* (Churchill, London, ed. 3, 1966), pp. 49–77; T. Ide, J. Steinke, G. F. Cahill, Jr., *Am. J. Physiol.* **217**, 784 (1969); J. A. Zivin and J. F. Snarr, *J. Appl. Physiol.* **32**, 664 (1972); S. R. Cohen, *J. Neurochem.* **35**, 1008 (1980); *Brain Res.* **205**, 157 (1981); *J. Neurochem.* **44**, 455 (1985).
3. R. E. Myers and S. Yamaguchi, *Arch. Neurol.* **34**, 65

(1977); E. Siemkowicz and A. Hansen, *Acta Neurol. Scand.* **58**, 1 (1978); M. D. Ginsberg, F. A. Welsh, W. W. Budd, *Stroke* **11**, 347 (1980); F. A. Welsh, M. D. Ginsberg, W. Rieder, W. W. Budd, *ibid.*, p. 355; H. Kalimo, S. Rehncrona, B. Soderfeldt, Y. Olsson, B. K. Siesjo, *J. Cereb. Blood Flow Metab.* **1**, 313 (1981); S. Rehncrona, I. Rosen, B. K. Siesjo, *ibid.*, p. 297; F. A. Welsh, R. E. Sims, A. E. McKee, *ibid.* **3**, 486 (1983).
4. W. A. Pulsinelli, S. Waldman, D. Rawlinson, F. Plum, *Neurology* **32**, 1239 (1982).
5. A. Schurr *et al.*, *Brain Res.* **421**, 135 (1987).
6. A. Schurr, W.-Q. Dong, K. H. Reid, C. A. West, B. M. Rigor, *ibid.* **438**, 311 (1988).
7. A. Schurr, K. H. Reid, M. T. Tseng, H. L. Edmonds, Jr., B. M. Rigor, *Comp. Biochem. Physiol.* **82A**, 701 (1985).
8. A. Schurr *et al.*, *Brain Res.* **297**, 357 (1984); A. Schurr *et al.*, *Brain Res. Bull.* **16**, 299 (1986); A. Schurr, K. H. Reid, M. T. Tseng, C. A. West, B. M. Rigor, *Brain Res.* **373**, 244 (1986).
9. A. Schurr, C. A. West, M. T. Tseng, K. H. Reid, B. M. Rigor, in *Brain Slices: Fundamentals, Applications and Implications*, A. Schurr, T. J. Teyler, M. T. Tseng, Eds. (Karger, Basel, 1987), pp. 39–44.
10. W. D. Lust *et al.*, in *Cerebral Energy Metabolism and Metabolic Encephalopathy*, D. W. McCandless, Ed. (Plenum, New York, 1985), pp. 79–117.

28 January 1988; accepted 13 April 1988

Expression of c-*fos* Protein in Brain: Metabolic Mapping at the Cellular Level

S. M. SAGAR,* F. R. SHARP, T. CURRAN

The proto-oncogene c-*fos* is expressed in neurons in response to direct stimulation by growth factors and neurotransmitters. In order to determine whether the c-*fos* protein (Fos) and Fos-related proteins can be induced in response to polysynaptic activation, rat hindlimb motor/sensory cortex was stimulated electrically and Fos expression examined immunohistochemically. Three hours after the onset of stimulation, focal nuclear Fos staining was seen in motor and sensory thalamus, pontine nuclei, globus pallidus, and cerebellum. Moreover, 24-hour water deprivation resulted in Fos expression in paraventricular and supraoptic nuclei. Fos immunohistochemistry therefore provides a cellular method to label polysynaptically activated neurons and thereby map functional pathways.

THE C-*fos* GENE, THE NORMAL CELLULAR counterpart of the viral oncogene v-*fos* (*1*), is rapidly and transiently expressed in many tissues in response to growth factor stimulation (*2*). It encodes a nuclear phosphoprotein (Fos) that exhibits both nonspecific (*3–5*) and sequence-specific (*6*) DNA binding properties. A set of Fos-related antigens has been identified that also appear to be nuclear DNA binding proteins (*4, 7, 8*). Although the function of c-*fos* is not known, it has been suggested that it acts as a "third messenger" molecule in signal transduction systems, where it would couple short-term intracellular signals elicited by a variety of extracellular stimuli to long-term responses by altering gene expression (*9*).

Fos can be demonstrated immunohistochemically to be in the nuclei of neurons in normal and pathological circumstances (*10–12*). In vitro, c-*fos* RNA and Fos rapidly increase in pheochromocytoma (PC12) cells after depolarization or stimulation with cholinergic agonists (*13*). Fos is normally present in rodent brain, particularly cerebral cortex and hippocampus, and its expression increases transiently after generalized seizures (*11, 12*). Moreover, cutaneous stimulation induces Fos expression in neurons of the spinal cord dorsal horn (*10*).

This literature suggests that Fos is transiently expressed in neurons after synaptic stimulation. Since the basal expression of Fos is relatively low in most central nervous system (CNS) regions, Fos expression might provide an anatomic technique for metabolic mapping similar in some respects to 2-deoxyglucose autoradiography (*14*). Although lacking the quantitative potential of the 2-deoxyglucose method, Fos expression offers cellular resolution, since immunohistochemical Fos staining is localized to the cell nucleus.

Bipolar stimulating electrodes were implanted into the hindlimb motor/sensory cortex of adult rats that were under general anesthesia (*15*). Twenty-four hours later, electrical stimulation was carried out in four awake, restrained animals for 15 min or for 1 hour in one animal (*16*). Two sham-operated animals were restrained, but received no electrical stimulation. Two unoperated, unanesthetized animals were also examined. Three hours after the beginning of stimulation, rats were anesthetized with pentobarbital, 50 mg/kg intraperitoneally, and were perfused through the aorta with saline and then with 4% paraformaldehyde in 0.1*M* sodium phosphate buffer, *p*H 7.4 (PB). Brains were removed and immersed in the same fixative for 4 hours at 4°C and then washed in PB. Fifty-micrometer coronal sections were cut on a Vibratome and were stained immunohistochemically for Fos by the avidin-biotin-peroxidase (ABC) method (*17*). The primary antiserum is an affinity-purified antiserum raised to a synthetic peptide, the M peptide (*5, 7*), corresponding to residues 127 to 152, which are common to both c-*fos* and v-*fos*. This antiserum has been characterized (*5, 7*) and cross-reacts with several Fos-related antigens (*4, 5, 7, 8*).

In control brains into which electrodes were inserted but no current passed, there was staining of cell nuclei in a reproducible and symmetric pattern in cerebral cortex (predominantly layers 1 to 4), hippocampal formation, striatum, piriform cortex, amygdala, and basal forebrain. The cerebellum, brainstem, thalamus, and globus pallidus of control brains were mainly devoid of staining, except for scattered cells. This is in agreement with previous observations (*12*).

The brains of stimulated rats exhibited two major differences from control brains. First, all stimulated subjects had increased staining in the motor/sensory cortex compared to controls, though only in three of five was the staining asymmetrically increased on the stimulated side (Fig. 1A) compared to the contralateral side (Fig. 1B). Second, in all stimulated animals there were focal areas of asymmetric increased Fos staining corresponding to brain regions known to be targets of motor/sensory cortex output. These regions include ipsilateral thalamus in the ventrolateral, ventrobasal, centrolateral, mediodorsal, posterior, parafascicular, ventromedial, and reticular nuclei (Fig. 1, C and D). In one animal a few cells in the ipsilateral globus pallidus expressed Fos nuclear staining (*18*). Focal staining of cells in ipsilateral pontine nuclei was also seen (Fig. 2C).

Of particular note are areas of nuclear staining in the copula pyramidus of the cerebellum, most marked contralateral to the stimulation, but with less dense staining in the corresponding ipsilateral area as well (Fig. 2A). These regions appear to correspond to cerebellar microzones. These are zones 0.5 to 1 mm long in cerebellar cortex, which can be defined electrophysiologically, anatomically by afferent inputs, or anatomically by neurotransmitter distribution (*19*). They may represent regions in which different functional modalities are integrated within the cerebellum (*20*). The vast majority of stained nuclei in the cerebellum were

S. M. Sagar, Department of Neurology, University of California, and Neurology Service, Veterans Administration (VA) Medical Center, San Francisco, CA 94121.
F. R. Sharp, Departments of Neurology and Physiology, University of California, and Neurology Service, VA Medical Center, San Francisco, CA 94121.
T. Curran, Department of Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

*To whom correspondence should be addressed.