A Route to Late Cenozoic Temperature History?

The recent report by Bada *et al.* (*1*) shows that racemization of the amino acid isoleucine to alloisoleucine occurs in fossil material in marine sediments from near the crest of the Mid-Atlantic Ridge and that the degree of racemization can be used to calculate late Cenozoic dates and sedimentation rates for the enclosing sediments, provided that a temperature history is known. They also show, however, that the first-order rate constant for interconversion of isoleucine is strongly temperature-dependent, with the result that sediment dates and sedimentation rates determined by this method for their example are in agreement with results from paleomagnetic or radioactive nuclide decay techniques only if an average temperature history of 2°C is assumed. The rate of racemization of isoleucine increases by about 20 percent per degree in the range of 0° to 4°C. As a general method applicable to other late Cenozoic examples, would it not be profitable to accept paleomagnetic, paleontologic, or radioactive nuclide decay dates as given and then, on that basis, to derive information about the temperature history of ancient sediments from the amount of racemization of various amino acids remaining in the contained fossils?

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The suggestion of McKenna that the temperature history of ancient sediments and fossils might be determined from the amount of racemization of various amino acids if the age had been determined by another method is an interesting possibility and one that we have, in fact, been investigating. In collaboration with M. Lewis and A. Lerman of the Canada Centre for Inland Waters, one of us (J.L.B.) has been studying the racemization of several amino acids in a 16-m piston core taken from Lake Ontario. By determining the radiocarbon ages of the sediments, we hope to use the racemization kinetics to estimate changes in the bottom water temperatures of the lake during the last 10,000 to 30,000 years.

It is doubtful that this method could be applied to exposed sediments and fossils. These specimens have been subjected to large temperature fluctuations between day and night and also from season to season. These large temperature fluctuations would complicate the racemization kinetics of the amino acids and make any interpretation difficult.

There may also be some problems in using the method to estimate temperature fluctuations in oceanic bottom waters where the temperature changes between glacial and interglacial ages may have amounted to only a few degrees. The age of the sediment would have to be determined with an uncertainty of less than ±20 percent in order to observe temperature fluctuations of 1° or 2°C. For isoleucine an uncertainty of ±25 percent in an age of a million years would yield an average temperature with an uncertainty of ±1°C. Therefore, it may be difficult to determine whether fluctuations of 1° or 2°C were real or just artifacts arising from the uncertainty of the age estimate. Temperature fluctuations of ~5°C should be readily distinguishable.

Another possibility that we are now investigating is the use of the amino acid racemization reaction to determine heat flows in sediments. In the 5-m core used in our investigations of the dating of marine sediments with the isoleucine racemization reaction (*1*, *2*), the increase in temperature from the top to the bottom of the core amounted to only a few tenths of a degree. However, in much longer cores or in cores taken from areas of high heat flow, the temperature increase should have a significant effect on the racemization kinetics. The slowly increasing temperature with depth in the sedimentary column would cause a corresponding slow increase in the racemization rates of the various amino acids. By determining the age of the sediments by another method, the racemization rates in the sedimentary column could be used to calculate the in situ temperature of the sediment and, therefore, the thermal gradient.

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References and Notes

1. J. L. Bada, B. P. Luyendyk, J. B. Maynard, *Science* **170**, 730 (1970).
2. We are aware of the fact that the reaction involving isoleucine is not racemization but rather epimerization. We use the term racemization to describe the reaction of amino acids which involves a change in configuration only at the α-carbon.
3. We thank H. Craig for helpful discussions. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research.

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Inactivation of Potassium Conductance in Slow Postsynaptic Excitation

Weight and Votava (*1*) propose that the slow excitatory postsynaptic potential (EPSP) of frog sympathetic ganglion cells is generated by an inactivation of resting potassium conductance of the membrane. In addition to confirming observations (*2*, *3*) that membrane resistance may be increased during the slow EPSP and that conditioning hyperpolarization can reverse the polarity of some of the slow EPSP, Weight and Votava (*1*) reported that the reversal potential of the slow EPSP was similar to the estimated potassium equilibrium potential (E_k). I would like to raise two difficulties, based on direct observations, that are not accounted for by their proposal.

First, the so-called "reversal" of the slow EPSP during conditioning hyperpolarization does not have the characteristics appropriate to their proposal. Kobayashi and Libet (*3*) noted that the phase of "reversed" polarity had a much shorter duration than that of the usual depolarizing slow EPSP; this difference appears also to be visible in figure 1C of Weight and Votava (*1*). Kobayashi and Libet also observed that the response of the conditioned hyperpolarized cell still showed a depolarizing phase, lasting 20 seconds or more after the brief phase of reversed polarity, and that the "reversed" hyperpolarizing phase seemed to have a distinctly shorter latency than the slow EPSP of the unconditioned cell (*3*). If the slow EPSP were generated by a change in potassium conductance the whole response should reverse in a mirror image

fashion when the membrane potential is electrically hyperpolarized beyond the value of E_k (*4*).

Indeed, the shorter latency and relatively brief duration of the "reversed" phase of the slow EPSP response suggests an alternative explanation, namely that the "reversal" is due to the appearance of another synaptic response, the slow inhibitory postsynaptic potential (IPSP), which does have these characteristics (*5, 6*). The slow IPSP has been detected only in C neurons of frog ganglia, not in the B neurons (*6*) which have been used in the present studies of slow EPSP (*1–3*). However, conditioning hyperpolarization does augment the slow IPSP (*2*). It is therefore possible that a very small or latent slow IPSP in a B neuron becomes larger and more manifest during conditioning hyperpolarization; the simultaneous reduction of the opposing depolarizing response by suppression (though not reversal) of the slow EPSP (*2, 3*) would also favor the manifestation of any slow IPSP.

The second difficulty is that the slow EPSP has been found to be especially and selectively sensitive to inhibitors of metabolic energy supply in ganglia of rabbit (*2, 7*) and apparently of frog (*8*). Such a selective sensitivity of the response indicates that an active electrogenic mechanism is responsible for the slow EPSP (*2, 7*), rather than a simple change in membrane conductance for potassium.

Further testing of Weight and Votava's proposal thus appears to be required, both to account for the points of difficulty presented here and to develop more positive supporting evidence (*9*), before their hypothesis can be regarded as a seriously tenable one.

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References and Notes

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2. H. Kobayashi and B. Libet, *Proc. Nat. Acad. Sci. U.S.* **60**, 1304 (1968).
3. ———, *J. Physiol.* **208**, 353 (1970).
4. J. C. Eccles, *The Physiology of Synapses* (Springer-Verlag, Berlin, 1964).
5. B. Libet, *J. Neurophysiol.* **30**, 494 (1967); ———, S. Chichibu, T. Tosaka, *ibid.* **31**, 383 (1968).
6. T. Tosaka, S. Chichibu, B. Libet, *ibid.* **33**, 396 (1970).
7. B. Libet, *Fed. Proc.* **29**, 1945 (1970).
8. H. Kobayashi and B. Libet, unpublished data.
9. Such testing should obviously include a study of the effects of changes in external potassium concentration on the slow EPSP, while the resting membrane potential is held at a constant level by electrical polarization. (It may be necessary to perform such a test on the muscarinic response of the ganglion cell to acetylcholine directly, in order to avoid confusing the results by the effects of changing the potassium concentration on presynaptic function.)
10. Our work was supported in part by PHS research grant NS00884.

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Libet's first point rests on the observation that Kobayashi and Libet (*1*) did not see a mirror reversal of the slow EPSP. We have, however, recorded such mirror reversals (*2*). Although in some cells, the reversed slow EPSP does repolarize more rapidly, this is apparently due to contamination by a late component (*3*) that has properties similar to the nicotinic fast EPSP and is not completely blocked by nicotine. The possibility of an underlying slow IPSP seems unlikely for the following reasons. The size of the slow EPSP is a continuous inverse function of membrane potential, not only being decreased by hyperpolarizing current and reversing near E_k, but also increasing in size with increasing depolarizing current [figure 1E in (*4*)]. Libet's proposal of a masked underlying slow IPSP does not explain the voltage dependence of the slow EPSP. Furthermore, Libet has reported that the slow IPSP in type C and rabbit cells (*5*) is not associated with a change in membrane conductance; therefore, his proposal that such a slow IPSP underlies the slow EPSP in frog type B cells does not explain the decrease in membrane conductance associated with the slow EPSP [figure 1B in (*4*)]. In short, Libet's proposal that there is an underlying slow IPSP does not explain the essential and unique features of the slow EPSP, namely voltage dependence and decreased conductance. On the other hand, an EPSP generated by an inactivation of potassium conductance would have the following properties: (i) voltage dependence, being an inverse function of membrane potential; (ii) reversal to a hyperpolarizing potential at E_k; and (iii) a decrease in membrane conductance. Thus, there is no need to postulate a separate underlying slow IPSP, since the proposal that the slow EPSP is generated by an inactivation of potassium conductance satisfactorily explains not only the reversal near E_k but also the other unique properties of the slow EPSP.

Recently, we have been investigating the effect of changing extracellular potassium (K_o) on the reversal potential of the slow EPSP. The preliminary results indicate that increasing K_o decreases the reversal potential of the slow ACh response, further supporting our proposal that the slow EPSP is generated by a synaptic inactivation of potassium conductance.

Libet's second point is based on gross indirect recording of a late-negative (LN) wave from the whole sympathetic ganglion (*5*). No direct evidence from ganglion cells has been presented to exclude nonspecific effects of the metabolic poisons cyanide and dinitrophenol on membrane conductance, membrane potential, or synaptic transmitter release. It is not clear what Libet means by "active electrogenic mechanism." If by that phrase he means some type of electrogenic pump, it is clear that the most extensively studied electrogenic Na pump is not involved for the following reasons: (i) The Na pump is not significantly voltage dependent, (ii) the activity of the Na pump is usually not associated with a significant decrease in membrane conductance, (iii) synaptic activation of the Na pump would hyperpolarize the membrane, and (iv) the slow EPSP is not blocked by ouabain or Ringer solution free of K (*5, 6*). Nor are there other electrogenic pumps known which have the properties of the slow EPSP. However, if it can be demonstrated that metabolic inhibitors specifically and selectively block the slow EPSP of type B cells, then it is possible that metabolic energy might be necessary for a synaptic inactivation of potassium conductance. We are currently investigating this possibility.

In summary, our proposal that the slow EPSP is generated by a synaptic inactivation of potassium conductance provides a satisfactory explanation of the properties of the slow EPSP. On the other hand, Libet's proposals of an underlying slow IPSP and an "active electrogenic mechanism" do not explain the properties of the slow EPSP and should not, therefore, necessitate any modification of our proposal.

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