residues 19 to 31, may bind to the active site of PKC and inhibit its activity. Activation of the enzyme by phospholipids would be expected to cause a conformational change that removed the pseudosubstrate sequence from the active site and allowed access to protein substrates. There are now several precedents for the regulation of protein kinases by pseudosubstrate structures. The inhibitor protein of the cAMP-dependent protein kinase is the most thoroughly studied example (see above). The "hinge" regions in the regulatory subunits of cAMP-dependent protein kinase are also believed to inhibit by binding to the active site of the catalytic subunit (*16*). Recently, we reported that the calmodulin-binding domain of the smooth muscle myosin light chain kinase contains a pseudosubstrate structure that may be responsible for maintaining the enzyme in the inactive form in the absence of calmodulin (*17*). Synthetic peptides corresponding to this region were shown to act as potent substrate antagonists.

The proposed role of the pseudosubstrate prototope present in the PKC regulatory domain provides an important focus for protein engineering studies to test the consequences of deletions and point mutations in this region of the enzyme. In view of the results obtained here and those with the cAMP-dependent protein kinase and the myosin light chain kinase, it would appear that pseudosubstrate prototopes may occur widely in the regulation of protein kinases and perhaps other enzymes.

REFERENCES AND NOTES

1. Y. Nishizuka, *Science* **233**, 305 (1986).
2. Y. Takai *et al.*, *J. Biol. Chem.* **252**, 7603 (1977).
3. P. Parker *et al.*, *Science* **233**, 853 (1986).
4. L. Coussens *et al.*, *ibid.*, p. 859; J. L. Knopf *et al.*, *Cell* **46**, 491 (1986); Y. Ono *et al.*, *FEBS Lett.* **203**, 111 (1986); S. Ohno *et al.*, *Nature (London)* **325**, 161 (1987).
5. The term prototope is defined as a functional segment of a protein that can be mimicked with a synthetic peptide usually not more than 30 residues in length. It is a general term to cover ligand-protein, peptide-protein, or protein-protein interactions where the protein's functional segment is dependent on the structure dictated by a short segment of the primary sequence. Thus linear epitopes recognized by antibodies represent a specific subgroup of prototopes.
6. D. Blumenthal *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3187 (1985).
7. B. M. Dutia, M. C. Frame, J. H. Subak-Sharpe, W. N. Clark, H. S. Marsden, *Nature (London)* **321**, 439 (1986); E. A. Cohen, P. Gandreau, P. Brazeau, Y. Langelier, *ibid.*, p. 441.
8. A. R. Neurath, S. B. H. Kent, N. Strick, K. Parker, *Cell* **46**, 429 (1986).
9. J. G. Sutcliffe, T. M. Shinnick, N. Green, R. A. Lerner, *Science* **219**, 660 (1983).
10. H. C. Cheng *et al.*, *Biochem. J.* **231**, 655 (1985); J. D. Scott, E. H. Fischer, J. G. Demaille, E. G. Krebs, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4379 (1985); H. C. Cheng *et al.*, *J. Biol. Chem.* **261**, 989 (1986).
11. C. A. O'Brian *et al.*, *Biochem. Biophys. Res. Commun.* **124**, 296 (1984); R. S. Turner *et al.*, *J. Biol. Chem.* **260**, 11503 (1985); S. Ferrari *et al.*, *FEBS Lett.* **184**, 72 (1985); J. R. Woodgett and T. Hunter, *Eur. J. Biochem.* **161**, 177 (1986).
12. C. House *et al.*, *J. Biol. Chem.* **262**, 772 (1987).
13. D. B. Glass and E. G. Krebs, *ibid.* **257**, 1196 (1982); B. E. Kemp *et al.*, *ibid.* **252**, 4888 (1977); T. K. Eckols *et al.*, *Eur. J. Biochem.* **124**, 249 (1983); B. E. Kemp, R. B. Pearson, C. House, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7471 (1983).
14. D. Mochly-Rosen and D. E. Koshland, Jr., *J. Biol. Chem.* **262**, 2291 (1987).
15. M. H. Lee and R. M. Bell, *ibid.* **261**, 14867 (1986).
16. K. Titani *et al.*, *Biochemistry* **23**, 4193 (1984); K. Takio *et al.*, *ibid.*, p. 4200; K. Takio *et al.*, *ibid.*, p. 4207.
17. B. E. Kemp *et al.*, *J. Biol. Chem.* **262**, 2542 (1987).
18. A. Y. Jeng *et al.*, *Cancer Res.* **46**, 1966 (1986).

19. This work was supported by grants from the Anti-Cancer Council of Victoria and the National Health and Medical Research Council. Equipment grants were provided by the Ian Potter Foundation, the Clive and Vera Ramaciotti Foundation, the Sunshine Trust, and the Buckland Foundation.

13 July 1987; accepted 22 October 1987

Technical Comments

The Cerebellum and Memory Storage

R. F. Thompson (*1*) presents an overview of the neurobiology of learning and memory and includes a discussion of the role of the cerebellum in motor learning. Specifically he argues that "recent evidence . . . overwhelmingly favors an essential role for the cerebellum in both learning and memory of discrete, adaptive behavioral responses to aversive events, thus supporting the. . . role of the cerebellum in motor learning." The climbing fiber projection to the cerebellum is described as playing a major role in this learning process. I would like to point out that these views were expressed without adequate discussion of either the experimental findings that are inconsistent with this hypothesis or the shortcomings of the existing data obtained by using the conditioning paradigms.

The work of Thompson and his colleagues, as well as the studies of Yeo *et al.* (*2*), indicates that pathways involving the cerebellum are necessary for the acquisition of the classically conditioned eye-blink reflex in the rabbit. However, this demonstration that the cerebellum is required for an adaptive or associative process is not unique, as it has been shown previously that portions of the cerebellum are required for adaptation of the vestibuloocular reflex (*3*). The most pertinent issue is whether these data prove that the learning process actually occurs within the cerebellum itself.

In arguing that the climbing fiber system plays a key role in establishing memory traces in the cerebellum, two lines of evidence are cited: (i) Thompson's own studies implicating the climbing fiber system as an unconditioned stimulus (UCS) in the conditioned eye-blink paradigm, and (ii) the studies of Ito *et al.* (*4*) illustrating that the climbing fiber input to Purkinje cells can produce a long-term depression of Purkinje cell excitability. In my view, these experiments provide only indirect support for his arguments, particularly in the context of published results favoring other views of climbing fiber function. I also have concerns about the interpretation of Thompson's data.

The studies supporting UCS and conditioned stimulus (CS) roles for the climbing and mossy fiber systems, respectively, are difficult to evaluate because some of the cited articles and abstracts are "in press." On the basis of information that is available, there are specific problems and controversies regarding these experimental data. For example, whether stimulation of the inferior olive can evoke motor behavior is still an open question (*5*), as other investigators have not been able to demonstrate this phenomenon. Regrettably information regarding stimulus parameters essential for the evaluation of these data is not included. Second, arguments favoring the mossy fiber input from the pons as the CS cite as supportive evidence the immediate transfer occurring when the stimulus location was changed from one side of the pontine nuclei to the other. This finding may have little to do with the conditioning paradigm; rather it may reflect the fact that both stimulus sites are activating pontine projections to both sides of the cerebellum, as pontine neurons from one side predominantly cross and project through the pontine nuclei on the opposite side on their way to the middle peduncle. However, even if one assumes that olivary stimuli can act as UCS, this observation is not sufficient to differentiate between two viable interpretations: (i) that the climbing fibers are establishing a memory trace in the cerebellum and (ii) that the climbing fibers are essential for a cerebellar

operation required for inducing the plastic change *elsewhere* in the central nervous system. In addition, Thompson's argument that the climbing fiber system is involved in establishing the plastic changes underlying motor learning through the mechanisms proposed by Ito is not consistent with some of his own data. His findings clearly imply that the permanent memory trace required for nictitating membrane reflex conditioning cannot occur in the cerebellar cortex, since ablation of this structure does not permanently abolish the conditioned response. This would not be expected if the synapses modified in long-term depression, namely those on the dendrites of Purkinje cells, were responsible for the storage of the memory trace underlying this conditioned behavior.

Thompson discusses long-term depression as an example of a potential synaptic mechanism of plasticity, but several critical and controversial features of the experiments pertaining to this issue are not mentioned. To date, no one has challenged the precise set of observations reported by Ito and his colleagues (*4*). However, because long-term depression has been observed only when a technique referred to as conjunctive stimulation is employed, the functional relevance of these findings can be challenged. This technique employs coincident stimulation of mossy and climbing fiber inputs to the cerebellar cortex. In the published applications of this technique, the climbing fiber input is activated at a higher rate over a more prolonged stimulus period than occurs under behavioral conditions.

Studies from our laboratory support a considerably different view of climbing fiber function. These experiments did not employ electrical stimulation to activate climbing fibers at a specified rate over a specified duration. Rather they examined the effects of spontaneously occurring or naturally evoked climbing fiber inputs, including those evoked by stimuli applied during locomotion in high decerebrate animals. Our findings showed that the effect of the climbing fiber input on Purkinje cell simple spike discharge can be described as a short-term enhancement of the Purkinje cell's response to the peripheral event rather than a prolonged suppression, as implied by the data from the conjunctive stimulation experiments. At the very least these findings indicate that it is premature to conclude that long-term depression characterizes the functional action of the climbing fiber system and that this mechanism is responsible for establishing memory traces in the cerebellum.

Finally, I would like to raise some additional questions concerning the role of the cerebellum in VOR adaptation. Although the studies of Miles *et al.* (*6*) are cited by Thompson, the extent to which they challenge the view that plasticity occurs in the cerebellum is not fully addressed. These investigators recorded from Purkinje cells in the flocculus of animals whose VOR had been adapted by wearing prisms. The changes in the response characteristics of these cells and the latency of their responses were not as predicted from hypotheses proposing that these neurons are localized to the site at which the adaptation occurred. Furthermore, Demer and Robinson's experiments (*7*) strongly arguing against a "teaching" role for the climbing fibers in VOR adaptation are not discussed. In my view these are critical points in an overview of this issue.

In conclusion, I feel that at this time there is no direct proof that the cerebellum is a storage site for motor memory traces. The data only support the likelihood that the cerebellum is a component in a pathway necessary for the conditioning of the nictitating membrane reflex. Given the interest of neurobiologists in the broad issue of motor learning, it is imperative that this issue remain open and that hypotheses regarding the mechanisms underlying this process take all pertinent data into account.

JAMES R. BLOEDEL
Division of Neurobiology,
Barrow Neurological Institute,
Phoenix, AZ 36013

REFERENCES

1. R. F. Thompson, *Science* **233**, 941 (1986).
2. C. H. Yeo, M. J. Hardiman, M. Glickstein, *Exp. Brain Res.* **60**, 87 (1985).
3. D. A. Robinson, *J. Neurophysiol.* **39**, 954 (1976).
4. M. Ito, M. Sakurai, P. Tongroach, *J. Physiol. London* **234**, 113 (1982).
5. R. Gellman, A. R. Gibson, J. C. Houk, *J. Neurophysiol.* **54**, 40 (1985).
6. F. A. Miles, D. J. Braitman, B. M. Dow, *ibid.* **43**, 1477 (1980).
7. J. L. Demer and D. A. Robinson, *ibid.* **47**, 1084 (1982).

17 November 1986; accepted 8 May 1987

Response: When we began our search for the memory traces for basic associative learning (using classical conditioning of discrete behavioral responses) 17 years ago, we had no idea it would lead us to the cerebellum. We have been forced there by our findings, beginning in 1980, and into the midst of a long-standing and apparently sometimes heated controversy regarding the cerebellum and motor learning. Several eminent neuroscientists (for example, Eccles and Ito) favor the hypothesis that the memory traces for learned movements are stored in the cerebellum. Others (for example, Llinas) take the opposite position. Our findings to date are consistent with, and supportive of, the former view.

Bloedel's comment focuses on limited aspects of the field and of our work. It does not address much of the evidence presented in my article (*1*) favoring the hypothesis (which I clearly state is not yet proved) that memory traces for associative learning of discrete, behavioral responses are localized to the cerebellum. Our earlier electrophysiological recording data, together with lesion and stimulation data, provided strong evidence that the traces are formed in the cerebellum or in structures afferent to the cerebellum for which the cerebellum is a mandatory efferent, that is, the traces are not formed efferent from the cerebellum. This evidence is sketched briefly in my article and at greater length in earlier publications (*2*).

In brief, our findings are as follows.

1) Lesions (of cerebellum) completely, selectively, and permanently abolish the learned response in trained animals and completely prevent learning in naive animals, but have no effect on the reflex unconditioned response and do not cause sensory or motor impairments relative to the behavioral response.

2) Within trials, over the course of training, neurons in localized regions of the interpositus nucleus and cerebellar cortex develop changes in frequency of discharge (increases in interpositus) that "model" the behavioral conditioned response (CR), but not the reflex response, that is the increase in discharge frequency precedes the onset of the behavioral CR (by as much as 60 milliseconds), predicts the amplitude-time course of the behavioral CR within trials, and predicts its development over the trials of training.

3) Appropriate lesions [of the dorsal accessory olive (DAO)] in trained animals result in experimental *extinction* of the behavioral CR with continued paired conditioned stimulus (CS)–unconditioned stimulus (US) training. To our knowledge this result, which ought to be so if the essential US pathway is destroyed (it ought to be like removing the US in ordinary behavioral extinction training), is new.

4) By appropriate microstimulation of mossy fibers as the CS and climbing fibers as the US, the two types of direct input to the cerebellum, normal behavioral associative learning of virtually any phasic, coordinated skeletal muscle response can be established. Further, these learned responses (and the unlearned responses evoked by climbing fiber stimulation) are abolished by destruction of the interpositus nucleus.

5) Initial evidence suggests that the central CS pathway beyond the primary sensory (auditory) system involves the lateral pontine nuclear region and mossy fibers.

Bloedel, in his comment, selects limited