after antidepressant treatment. The ability of *cis*-vaccenic acid or colchicine to increase Gpp(NH)p activation of cyclase in membranes from control but not treated animals is consistent with the idea that G unit–catalytic moiety interaction is already enhanced in the latter.

The possibility that such an enhanced interaction is associated with antidepressant action is suggested by the finding that the effect is induced by typical and atypical tricyclic antidepressants as well as by ECT. Moreover, long-term treatment is required for the effect to become manifest.

Since a facilitated G unit–catalytic moiety interaction is generally associated with enhanced hormone responsiveness (*16*), the present results appear to be inconsistent with the observation that ECT or tricyclic antidepressants decrease norepinephrine-stimulated accumulation of cyclic AMP in brain slices (*4, 5*). However, the α_2-adrenergic receptor system is regulated by guanyl nucleotides (*22*) and may inhibit adenylate cyclase in a GTP-dependent manner (*23*). Thus, enhanced G unit–catalytic moiety interaction might augment α_2-adrenergic inhibition of cyclase by norepinephrine and thereby reduce the net stimulation of cyclic AMP accumulation in brain slices by norepinephrine (*24*).

Repeated ECT markedly enhances the stimulation of cyclic AMP levels in cortical slices by norepinephrine in the presence of adenosine (*25*). Similarly, recent electrophysiological and behavioral studies have shown enhanced α-adrenergic (*26*), serotonergic (*27*), and dopaminergic (*28*) responsiveness in the brain after long-term antidepressant treatment. It would be of particular interest to examine the role of G unit alterations in these effects as a means of integrating data on receptor binding with data on the physiological and behavioral effects of antidepressant treatment.

DAVID B. MENKES
MARK M. RASENICK*
Departments of Pharmacology and *Pathology, Yale University School of Medicine, New Haven, Connecticut 06510*
MARCIA A. WHEELER
MARK W. BITENSKY
Division of Life Sciences, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

References and Notes

1. I. Oswald, V. Brezinova, D. L. F. Dunleavy, *Br. J. Psychiatry* **120**, 673 (1972); D. V. Klein, R. Gittelman, F. Quitkin, A. Rifkin, *Diagnosis and Drug Treatment of Psychiatric Disorders: Adults and Children* (Williams & Wilkins, Baltimore, ed. 2, 1980).
2. F. Sulser, J. Vetulani, P. L. Mobley, *Biochem. Pharmacol.* **27**, 257 (1978); D. S. Charney, D. B. Menkes, G. R. Heninger, *Arch. Gen. Psychiatry* **38**, 1160 (1981).
3. M. F. Sugrue, *Pharmacol. Ther.* **13**, 219 (1981).
4. A. Frazer, G. Pandey, J. Mendels, S. Neeleg, M. Kane, M. E. Hess, *Neuropharmacology* **13**, 1131 (1974).
5. J. Vetulani, R. J. Stawarz, J. V. Dingell, F. Sulser, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **293**, 109 (1976).
6. F. E. Bloom, *Rev. Physiol. Biochem. Pharmacol.* **74**, 1 (1975); P. Greengard, *Nature (London)* **260**, 101 (1976).
7. Adenylate cyclase is composed of at least three plasma membrane–associated proteins: the hormone or neurotransmitter receptor, the guanyl nucleotide–binding regulatory subunit (G unit), and the catalytic moiety. The enzyme is activated when hormone, bound to the receptor, causes the G unit to bind GTP and subsequently activates the catalytic moiety (*8*).
8. M. Rodbell, *Nature (London)* **284**, 117 (1980).
9. S. P. Banerjee, L. S. Kung, S. J. Riggi, S. K. Chanda, *ibid.* **268**, 455 (1977); B. S. Wolfe, T. K. Harden, J. R. Sporn, P. B. Molinoff, *J. Pharmacol. Exp. Ther.* **207**, 446 (1978); R. Mishra, A. Janowsky, F. Sulser, *Neuropharmacology* **19**, 983 (1980).
10. E. A. Swinyard, in *Experimental Models of Epilepsy*, D. Purpura *et al.*, Eds. (Raven, New York, 1972), pp. 434–458.
11. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
12. M. Turck, W. F. Schocker, A. M. Fathy, J. E. Schultz, *Arch. Pharm. (Weinheim, Ger.)* **313**, 768 (1980).
13. M. M. Rasenick and M. W. Bitensky, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4628 (1980).
14. E. Ross and A. G. Gilman, *Annu. Rev. Biochem.* **49**, 533 (1980).
15. M. M. Rasenick, P. J. Stein, M. W. Bitensky, *Nature (London)* **294**, 560 (1981).
16. J. Orly and M. Schramm, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3433 (1975); G. Rimon, E. Hanski, S. Braun, A. Levitzki, *Nature (London)* **276**, 295 (1978).
17. Although colchicine, vinblastine, and *cis*-isomers of unsaturated fatty acids are capable of enhancing Gpp(NH)p or NaF activation of adenylate cyclase in cortical synaptic membranes, there is a mechanistic distinction between the microtubule-disrupting agents and the fatty acids. The fatty acids increase synaptic membrane fluidity and may consequently enhance G unit–catalytic moiety interaction. Colchicine and vinblastine, however, do not appear to increase overall membrane fluidity but rather may liberate a constraint on the G unit, increasing its interaction with the catalytic moiety (*15*).
18. M. Shinitzky and Y. Barenholz, *J. Biol. Chem.* **249**, 2652 (1974).
19. Only the cortex shows increased Gpp(NH)p-activated adenylate cyclase activity after long-term ECT. Conversely, only the hypothalamus displays increased adenylate cyclase activity after long-term iprindole treatment. A possible explanation for this disparity is the heterogeneity of various brain regions. Hypothalamic and cortical adenylate cyclase varies in the "coupled" response to agonist as well as to fluoride [(*15, 29*); J. W. Daly, *Cyclic Nucleotides in the Nervous System* (Plenum, New York, 1977)].
20. M. A. Wheeler and M. M. Rasenick, unpublished observations.
21. G. C. Palmer, D. J. Jones, M. A. Medina, W. B. Stavinoha, *Neuropharmacology* **16**, 435 (1977).
22. D. C. U'Prichard and S. H. Snyder, *J. Biol. Chem.* **253**, 3444 (1978); D. J. Kahn, J. C. Mitrius, D. C. U'Prichard. *Mol. Pharmacol.* **21**, 17 (1982).
23. W. Saur and G. Scholtz, *FEBS Lett.* **85**, 167 (1978).
24. P. L. Mobley and F. Sulser, *Eur. J. Pharmacol.* **60**, 221 (1979).
25. A Sattin, in *Chemisms of the Brain*, S. Stahl and R. Rodnight Eds. (Churchill Livingstone, Edinburgh, Scotland, 1981).
26. D. B. Menkes, G. K. Aghajanian, R. B. McCall, *Life Sci.* **27**, 45 (1980); D. B. Menkes and G. K. Aghajanian, *Eur. J. Pharmacol.* **74**, 47 (1981); J. Maj, E. Mogilnicka, V. Klimek, *J. Neural Transm.* **44**, 221 (1979); J. Maj, E. Mogilnicka, A. Kordecka-Magiera, *Pharmacol. Biochem. Behav.* **13**, 153 (1981).
27. C. de Montigny and G. K. Aghajanian, *Science* **202**, 1303 (1978); R. Y. Wang and G. K. Aghajanian, *Commun. Psychopharmacol.* **4**, 83 (1980); E. Friedman and A. Dallob, *ibid.* **3**, 89 (1979); D. W. Costain *et al.*, *Psychopharmacology* **61**, 167 (1979).
28. A. R. Green, D. J. Heal, D. G. Grahame-Smith, *Psychopharmacology* **52**, 195 (1977); C. Spyraki and H. C. Fibiger, *Eur. J. Pharmacol.* **74**, 195 (1981).
29. M. Wheeler, M. Tishler, M. W. Bitensky, *Brain Res.* **231**, 387 (1982).
30. B. Brown, R. Elkins, J. Albano, *Adv. Cyclic Nucleotide Res.* **2**, 25 (1972).
31. M. Bradford, *Anal. Biochem.* **72**, 248 (1976).
32. We thank G. Aghajanian and D. U'Prichard for helpful discussions. Supported by NIH grants AM 20179, AM 06287, and GM 07205.
* Correspondence should be addressed to the Department of Neurology, Yale University School of Medicine, New Haven, Conn. 06510.

3 August 1982

Parathyroid Hormone: Effects of the 3–34 Fragment in vivo and in vitro

Abstract. *The biologically active fragment of parathyroid hormone, consisting of residues 1–34, and its in vitro antagonist, fragment 3–34, were administered separately or in combination to chronically thyroparathyroidectomized dogs. These fragments were also studied in vitro with dog renal cortical membranes. Fragment 3–34 inhibited the stimulation of adenylate cyclase by fragment 1–34 in vitro, but had no agonist or antagonistic effects on renal phosphate transport in vivo.*

The native bovine parathyroid hormone (bPTH) molecule is a single-chain polypeptide of 84 amino acids. Many of its biological effects, however, can be reproduced by the first 34 residues at the amino terminus, bPTH-(1–34) (*1, 2*). The 1–34 fragment can thus increase urinary phosphate excretion (*1*), increase the excretion of adenosine 3′,5′-monophosphate (cyclic AMP) (*1*), mobilize calcium from bone (*1, 2*), and stimulate the adenylate cyclase system in vitro in both rat renal cortex and calvaria (*1, 2*). Furthermore, its potency is equal to that of the native molecule (on a molar basis) in most bioassays of parathyroid hormone action. Removal of the first two amino acid residues, to produce bPTH-(3–34), not only results in the loss of these agonist properties of the molecule, but produces a fragment that is antagonistic in vitro to both the 1–34 and 1–84 hormone molecule. This inhibitory effect has been demonstrated in the rat renal cortex adenylate cyclase system of the rat renal cortex (*3, 4*). Modification of the 3–34 fragment, [Nle-8, Nle-18, Tyr-34]bPTH-(3–34)-amide [sub-bPTH-(3–34)], produces a more potent and more stable antagonist (*4*).

0036-8075/83/0107-0067$01.00/0

In the studies described here we observed the action of the 3–34 fragment in vivo. Groups of chronically thyroparathyroidectomized (TPTX) dogs were treated with the 1–34 compound alone, the 3–34 compound alone, or a combination of the two in various concentration ratios. Urinary phosphate excretion was followed as a measure of biological activity. Because this animal represents a new test system for the 3–34 fragment, we also studied the effects of the fragments on dog renal cortex preparations in vitro.

Clearance studies were performed in female mongrel dogs that had undergone thyroparathyroidectomy at least 48 hours and up to 1 week before the experiment. The dogs were accepted for study only if the concentration of calcium in their serum had decreased by at least 25 percent after parathyroid ablation, and if they had not received parathyroid extract for the treatment of tetany for at least 48 hours before the experiment. The dogs were first given priming doses of inulin and *p*-aminohippurate (PAH) and were then infused with these substances. After an equilibration period of 1 hour, samples of blood and urine were collected. The urine samples were collected prior to the administration of the hormone fragments so that each animal could serve as its own control. Blood was again obtained at the end of the control period. Three experiments were then performed in which the hormones were given as a bolus injection and then infused in the same quantity per hour for 2 hours. (i) Four dogs received bPTH-(1–34), as previously described (*1*), in a dose of 0.1 μg/kg, and another four dogs received sub-bPTH-(3–34) (*5*). The data from these two sets of dogs were almost identical and were therefore pooled. (ii) Six dogs received different amounts of sub-bPTH-(3–34). One dog received 0.1 μg/kg, three received 1.0 μg/kg, and two were given 10 μg/kg. The results from these dogs were also the same and were therefore combined. (iii) Eight dogs received both the 1–34 and 3–34 hormone fragments. Unsubstituted bPTH-(1–34) was given in a dose of 0.1 μg/kg. Two dogs received sub-bPTH-(3–34) in a dose of 1.0 μg/kg, and the other six received between 5.0 and 10.0 μg/kg. Thus, the ratio of 3–34 to 1–34 ranged from 10:1 to 100:1. The data from the three experiments and for the tests in vitro were analyzed by Student's *t*-test.

The studies of bPTH-(1–34) and bPTH-(3–34) in vitro were performed with a particulate fraction of dog kidney cortex according to methodology described previously (*6*, *7*). Briefly, kidneys obtained from chronically TPTX dogs were harvested and plunged into ice-cold saline. The cortical tissue was weighed and homogenized in 50 volumes (weight to volume) of buffer containing 2 m*M* tris-HCl, *p*H 7.4, and 1 m*M* EDTA in a Polytron homogenizer. The homogenate was centrifuged at 25,000*g* for 10 minutes. The supernatant fraction was removed and the pellet homogenized again in 50 volumes of 2 m*M* tris-HCl buffer, *p*H 7.4, and centrifuged. The second pellet obtained was resuspended and centrifuged. The final pellet (containing the membrane preparation) was suspended in 25 volumes of 2 m*M* tris-HCl buffer, *p*H 7.4, and then adenylate cyclase activity in response to bPTH-(1–34), sub-bPTH-(3–34), or both, was measured. Protein was determined according to the method of Lowry *et al.* (*8*). The concentration of bPTH-(1–34) was $2.4 \times 10^{-8} M$ (0.01 μg per tube) and $2.5 \times 10^{-6} M$ (1.0 μg per tube) of 1–34 and 3–34, respectively.

Table 1. Effects of bPTH-(1–34) and bPTH-(3–34) on renal hemodynamics and phosphate excretion. The dogs in experiment 2 received between 0.1 and 10.0 μg of bPTH-(3–34) per

Clearance (ml/min)				Excretion of phosphate (%)	
Inulin		PAH			
Control	Experimental	Control	Experimental	Control	Experimental
Experiment 1. Dogs received bPTH-(1–34). N = 8					
40.9 ± 9.8	44.9 ± 8.1	142.2 ± 24.4	143.8 ± 21.1	5.38 ± 1.66	23.02 ± 3.95
N.S.		N.S.		$P < .005$	
Experiment 2. Dogs received bPTH-(3–34). N = 8					
38.6 ± 8.78	43.3 ± 8.49	150.9 ± 25.6	141.0 ± 25.8	4.19 ± 1.65	4.20 ± 1.54
N.S.		N.S.		N.S.	
Experiment 3. Dogs received bPTH-(1–34) and bPTH-(3–34). N = 6					
58.5 ± 6.34	56.2 ± 5.95	147.1 ± 16.4	149.5 ± 14.0	4.15 ± 1.29	21.96 ± 6.43
N.S.		N.S.		$P < .05$	

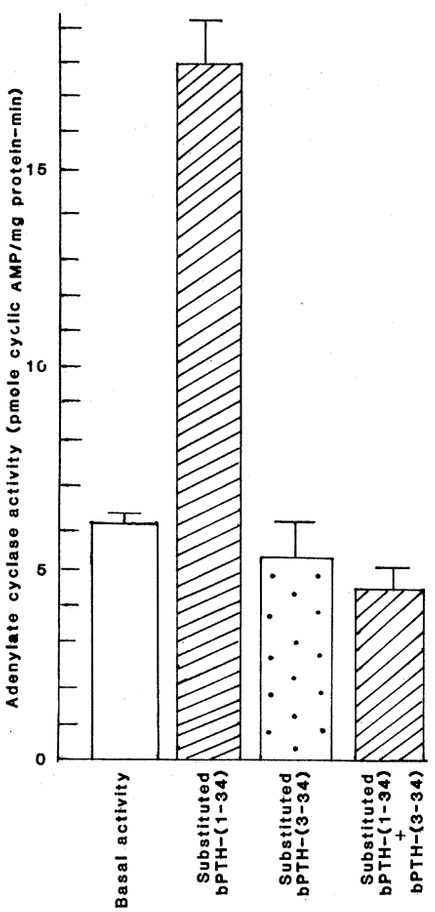

Fig. 1. Determinations of adenylate cyclase activity in vitro in response to bPTH-(1–34), bPTH-(3–34), or both. The 1–34 fragment was present in a concentration of 0.01 μg per tube, and the 3–34 fragment, 1.0 μg per tube.

Infusion of the 1–34 fragment (0.1 μg/kg) into chronically TPTX dogs regularly resulted in a phosphaturia. There was no alteration in renal hemodynamics. The increment in the mean percentage of filtered phosphate excreted was from 5.38 ± 1.66 to 23.02 ± 3.95 ($P < .005$) (Table 1, experiment 1). This was reflected in the greater than fourfold increase in absolute phosphate excretion (see Table 1). Serum phosphate declined, but the filtered load of phosphate was unaltered from the control to experimental phases of the study. Neither did the concentration of ultrafilterable serum calcium change significantly.

In contrast, doses of bPTH-(3–34) as high as 10 μg/kg had no effect on either absolute or percentage phosphate excretion (Table 1, experiment 2). As with the 1–34 fragment, glomerular filtration rate, determined as the clearance of inulin, and effective renal plasma flow, estimated as the clearance of PAH, were unaltered by the infusion of bPTH-(3–34). None of the other parameters under study were changed by the infusion of bPTH-(3–34).

In experiment 3, the mean excretion of phosphate increased from 4.15 ± 1.29 to 21.96 ± 6.43 percent ($P < .05$) (Table 1). Therefore, even though given in

kilogram; those in experiment 3 received, per kilogram, 0.1 μg of bPTH-(1–34) and between 1.0 and 10.0 μg of bPTH-(3–34); N.S., not significant.

Absolute phosphate excretion (μmole/min)		Serum phosphate (μmole/ml)		Filtered phosphate load (μmole/min)	
Control	Experimental	Control	Experimental	Control	Experimental
Experiment 1. Dogs received bPTH-(1–34). N = 8					
3.02 ± 1.15	14.16 ± 3.15	1.96 ± 0.08	1.57 ± 0.09	79.11 ± 20.55	63.11 ± 11.67
$P < .01$		$P < .001$		N.S.	
Experiment 2. Dogs received bPTH-(3–34). N = 8					
1.97 ± 0.68	2.51 ± 1.30	1.51 ± 0.18	1.27 ± 0.13	56.5 ± 14.2	52.5 ± 9.69
N.S.		N.S.		N.S.	
Experiment 3. Dogs received bPTH-(1–34) and bPTH-(3–34). N = 6					
3.91 ± 1.32	11.25 ± 2.51	1.65 ± 0.076	1.16 ± 0.14	86.8 ± 8.08	61.5 ± 7.02
$P < .05$		$P < .01$		$P < .02$	

amounts of up to 100 times that of the 1–34 fragment, sub-bPTH-(3–34) did not block the phosphaturic effect of the bPTH-(1–34). As in the other two experiments, renal hemodynamics were not a factor. In experiment 3, a decrease in serum phosphate and the filtered phosphate load resulted from the phosphaturia.

The incubation of bPTH-(1–34) with the particulate fraction of dog renal cortical tissue resulted in a threefold increase in adenylate cyclase activity as determined by the production of cyclic AMP (Fig. 1). Basal activity was 6.3 ± 0.7 and increased to 18.5 ± 1.1 pmole per milligram of protein per minute ($P < .005$). Sub-bPTH-(3–34) had no agonist activity (mean, 5.3 ± 1.0 pmole/mg-min, $P > .50$) but did completely inhibit the response of the adenylate cyclase system to the 1–34 fragment (4.6 ± 0.6 pmole/mg-min, $P < .001$) (Fig. 1). These data represent the results of five determinations for each treatment group, with tissue obtained from a single dog kidney. Similar data were obtained in nine additional dogs.

These data verify that sub-bPTH-(3–34) inhibits the stimulation of adenylate cyclase by the 1–34 fragment in a membrane fraction obtained from dog renal cortical tissue, and are similar to data obtained previously with homogenized tissue (*6*). Nevertheless, when introduced in vivo, the 3–34 fragment did not prevent bPTH-(1–34) from having its usual biological effect, that is, a phosphaturia. Thus, the phosphate excretion of 21.96 percent in those dogs given both the 3–34 and 1–34 fragments was similar to that obtained in the animals given only bPTH-(1–34) (23.02 percent).

The discrepancy in the results obtained with bPTH-(3–34) in vitro and in vivo are difficult to explain, especially since the analog binds to canine renal receptor sites with an avidity equal to bPTH-(1–34) (*9*). There are two possible explanations for our results. The first is that bPTH-(3–34) may be metabolized or distributed in vivo in such a manner as to preclude the effects seen in broken cell preparations. Examples of drugs and hormones that act dissimilarly in vivo and in vitro are well known. Thus, the antagonistic interaction between the 3–34 and 1–34 fragments in vitro may be obviated by exposing the 3–34 fragment to the biological systems of the intact organism.

A second possibility is that the physiological effects of PTH are not all mediated by cyclic AMP (*10*). The original studies that showed an increase in urinary cyclic AMP excretion in response to bPTH involved pharmacological doses of the hormone (*11*) and were associated with proximal tubular changes in phosphate handling (*10, 12*). However, a recently reported series of studies demonstrated that physiological doses of bPTH can produce phosphaturia without either the participation of the superficial proximal tubule or alterations in cyclic AMP excretion or production (*10*). Therefore, the 3–34 fragment may have antagonist properties at higher doses or may affect PTH actions other than phosphaturia (*13*), or the phosphaturia may be mediated by mechanisms other than the adenylate cyclase system.

JOHN A. MCGOWAN
TAI C. CHEN
JORGE FRAGOLA
JULES B. PUSCHETT
Renal-Electrolyte Division,
University of Pittsburgh
School of Medicine,
Pittsburgh, Pennsylvania 15261
MICHAEL ROSENBLATT
Endocrine Unit,
Massachusetts General Hospital,
Boston 02114

References and Notes

1. J. T. Potts *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 63 (1971).
2. G. W. Tregear, J. Van Rietschoten, E. Greene, H. T. Keutmann, H. D. Niall, B. Reit, J. A. Parsons, J. T. Potts, *Endocrinology* **93**, 1349 (1973).
3. D. Goltzmann, A. Peytremann, E. Callahan, G. W. Tregear, J. T. Potts, *J. Biol. Chem.* **250**, 3199 (1975).
4. M. Rosenblatt, E. N. Callahan, J. E. Mahaffey, A. Pont, J. T. Potts, *ibid.* **252**, 5847 (1977).
5. M. Rosenblatt and J. T. Potts, *Endocrinol. Res. Commun.* **4**, 115 (1977).
6. T. C. Chen, M. Rosenblatt, J. B. Puschett, *Biochem. Biophys. Res. Commun.* **94**, 1227 (1980).
7. T. C. Chen and J. B. Puschett, *ibid.* **100**, 1471 (1981).
8. O. H. Lowry, N. G. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
9. G. V. Segre, M. Rosenblatt, B. L. Reiner, J. E. Mahaffey, J. T. Potts, *ibid.* **254**, 6980 (1979).
10. J. B. Puschett, J. Winaver, T. C. Chen, J. Fragola, D. B. Sylk, J. S. Robertson, *Miner. Electrolyte Metab.* **6**, 190 (1981).
11. C. R. Chase and G. D. Aurbach, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 518 (1967).
12. Z. S. Agus, J. B. Puschett, D. Senesky, M. Goldberg, *J. Clin. Invest.* **50**, 617 (1971).
13. G. V. Segre, G. Tully, M. Rosenblatt, J. Laugharn, B. Reit, J. T. Potts, *Calcif. Tissue Int.* **28**, 171 (1979).
14. Supported in part by grants from the Veterans Administration, the National Science Foundation (PCM-8021238), and the National Institutes of Health (AM-11714). We thank D. Puschett, J. Benson, and S. Gormley for technical assistance.

10 May 1982; revised 9 September 1982

Bleaching of Rhabdoms in Eyes of Intact Butterflies

Abstract. *The photochemistry of butterfly rhabdoms has properties that had been associated exclusively with the photoreceptor organelles of vertebrates. Noninvasive measurements of the absorbance spectra of rhabdoms in intact butterflies show that their rhodopsins are converted by light to metarhodopsins that decay from the rhabdom in the dark. A total bleach is possible because the first-order decay of metarhodopsin is considerably faster than the kinetically more complicated recovery of rhodopsin.*

Rhodopsins of vertebrates are converted by light to metarhodopsins that are unstable, decaying with the dissociation of chromophore from protein. Since the dissociated components of the metarhodopsin are colorless, a vertebrate rhodopsin is said to bleach after absorption of light. Rhodopsins of invertebrates are also converted by light to metarhodopsin, but work to date indicates that invertebrate metarhodopsins are thermally stable; the two stable states R (rhodopsin) and M (metarhodopsin) can be interconverted by light many times,

0036-8075/83/0107-0069$01.00/0