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

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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,

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with no noticeable bleaching (*1*). I now present evidence that butterfly rhabdoms can be bleached and that metarhodopsin content of the rhabdoms is not stable in the dark.

Both partial and total bleaches of rhabdoms are demonstrated by the following experiments (*2*) performed in vivo on a 2-month-old *Vanessa cardui*. After the butterfly was dark-adapted in the microspectrophotometer for 12 hours, the reference spectrum (inset in Fig. 1) was measured (*3*). Then an actinic orange flash was delivered, and after 112 minutes, difference spectrum A of Fig. 1 was measured. This spectrum is well fitted by a Dartnall nomogram with a wavelength for maximal absorption (λ_{max}) of 530 nm and density of 0.11 unit (*4*). After spectrum A was measured, another flash was delivered, and after 66 minutes in the dark, difference spectrum B was measured. This procedure was continued with larger actinic doses to obtain spectra C and D. The solid curves in Fig. 1 are least-square fits [standard error (S.E.) < .02] of the Dartnall nomogram R530 to each of the experimental spectra. My interpretation of these data is that the rhabdoms in the eye of *Vanessa* contain a rhodopsin with λ_{max} at 530 nm and that this rhodopsin can be bleached from the rhabdoms.

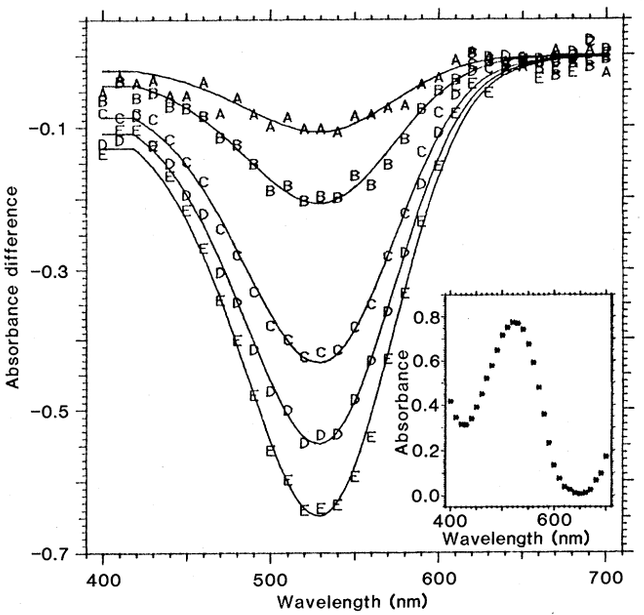

Fig. 1. Difference spectra for a series of partial bleaches. The inset shows the normalized reference spectrum taken after 12 hours of dark adaptation. A 3-second orange flash was delivered, and after a dark period of 112 minutes, the data for difference spectrum A were measured. Another 3-second flash was delivered, and after 66 minutes, spectrum B was measured. Then five flashes (3 seconds each at 60-second intervals) were delivered, and after 85 minutes, spectrum C was measured. Five more flashes were delivered, and after 66 minutes, spectrum D was measured. Finally, 21 orange flashes (1 second at 60-second intervals) were delivered, and after 35 minutes, spectrum E was measured. The solid lines are least-squares fits of nomogram spectra (R530) to experimental spectra A through E. The peak densities for the nomogram spectra are 0.11, 0.21, 0.43, 0.55, and 0.65, respectively.

No metarhodopsin was detectable 1 hour after actinic treatment, although metarhodopsin was present just after actinic treatment. What were the kinetics of its disappearance? Orange irradiation converts R530 to M490. In subsequent darkness, M490 disappears exponentially without passing through a colored intermediate. In the dark, R530 regenerates more slowly than M490 decays and does so with more complicated kinetics. By the time M490 has disappeared, R530 has only partially regenerated.

These conclusions are based on the following argument. A series of spectra (one approximately every 3 minutes) were measured after each actinic treatment and subjected to numerical analysis (*5*). Each difference spectrum can be well fitted (S.E. < .02) by

$$\{D_{M490}(t)\alpha_{M490}(\lambda) - [D_{R530}(t_0) - D_{R530}(t)]\alpha_{R530}(\lambda)\}$$

where λ is the wavelength, α_{M490} (λ) and α_{R530} (λ) are normalized absorbance spectra taken from the Dartnall nomogram, $D_{M490}(t)$ is the density of metarhodopsin M490 present at time t, $D_{R530}(t_0)$ is the density of rhodopsin R530 at time t_0 when the reference spectrum was measured, and $D_{R530}(t)$ is the density of rhodopsin R530 present at time t. There is no evidence for a photochemical intermediate, other than metarhodopsin, having a lifetime greater than 2 minutes at 23°C and having measurable absorbance within 400 to 750 nm. The density of M490 decayed exponentially with a time constant of 18 minutes at 23°C (Fig. 2). Thus the metarhodopsin content of the rhabdom is not stable; more than 50 percent of M490 was lost within 13 minutes after the first flash.

The kinetics for recovery of R530 were not the same as for the disappearance of M490. The half-time for recovery of R530 was 70 minutes for the first flash,

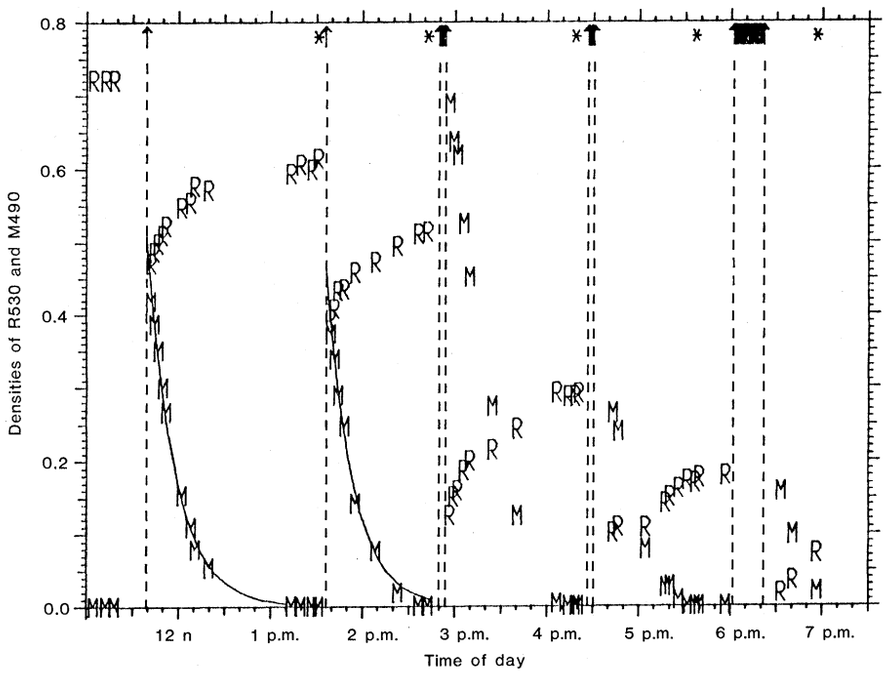

Fig. 2. Kinetics for decay of metarhodopsin M490 and for recovery of rhodopsin R530 for the experiment of Fig. 1. $D_{M490}(t)$ is plotted with symbol *M*, and $D_{R530}(t)$ is plotted with symbol *R*. The arrows and dashed lines show times of actinic illumination. The solid lines are least-square fits to simple exponential decay (rate constant 0.055 per minute) of M490. The asterisks show the times at which the difference spectra of Fig. 1 were measured.

112 minutes for the second flash, and > 240 minutes for subsequent flashes. Recovery of rhodopsin was therefore considerably slower than decay of metarhodopsin.

After spectrum D was measured, a total bleach was approximated by illuminating the eye with a series of 21 1-second flashes. Spectrum E was measured 35 minutes after the last flash and was less than 0.07 unit from a total bleach. Evidence for this interpretation was obtained by delivering five flashes (lasting 3 seconds each at 1-minute intervals) of bright blue light (*3*). The blue flashes caused < 0.1 unit change in absorbance, indicating the absence of M490. Finally, a 1-second white flash with ultraviolet cutoff filter L42 caused < 0.1 unit change, indicating the absence of R530. Thus $D_{R530}(t_0) \sim 0.72$. The eye recovered from this total bleach. After 14 hours, about 60 percent of R530 had returned. I fed the butterfly, conducted three more days of experiments on the same eye region, then left it in the dark for 64 hours. The titer of rhodopsin returned to within 0.03 unit of the initial level.

Vanessa cardui is not the only species that has unstable metarhodopsin. I have observed bleaching of rhabdoms in more than 22 butterfly species. Furthermore, green receptors are not the only spectral class of photoreceptor having bleachable photoreceptor membranes. The rhabdomeres of ultraviolet, blue, and red receptors can also be bleached.

Now that it is known that rhodopsins can be bleached from photoreceptor membranes of butterflies, the next step is to understand the molecular mechanisms. Two reasonable possibilities are hydrolysis of the metarhodopsin chromophore in situ and removal of metarhodopsin associated with membrane turnover. The precise experimental control of the concentrations of rhodopsin and metarhodopsin in vivo makes the butterfly a useful preparation for the study of membrane turnover and the visual pigment cycle.

GARY D. BERNARD
Yale University, Department of Ophthalmology and Visual Science, Post Office Box 3333, New Haven, Connecticut 06510

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2. Each butterfly rhabdom is a wave guide that is optically terminated by a tapetal reflector that creates colored eyeshine [W. H. Miller and G. D. Bernard, *J. Ultrastruct. Res.* **24**, 286 (1968); W. A. Ribi, *Z. Naturforsch.* **34c**, 284 (1979)]. By focusing an incident-light microspectrophotometer on the center of the deep pseudopupil it is possible to measure the light that has survived a double pass through the rhabdom, with superb discrimination against stray light. Thus, the absorbance of the rhabdom is proportional to one-half the common logarithm of the measured reflectance [N. Franceschini and K. Kirschfeld, *Kybernetik* **9**, 159 (1971); D. G. Stavenga, *Nature (London)* **254**, 435 (1975); in *Photoreceptor Optics*, A. W. Snyder and R. Menzel, Eds. (Springer, Heidelberg, 1975), p. 290; G. D. Bernard, *J. Opt. Soc. Am.* **67**, 1362 (1977); *Science* **203**, 1125 (1979)].
3. The butterfly was obtained from Carolina Biological Supply, Burlington, North Carolina. Twenty ommatidia in the medioventral region (azimuth, 20°; elevation, −15°) of the eye were illuminated with a 0.2-mm beam of numerical aperture 0.07. The reflectance spectrum was measured with dim monochromatic flashes (< 10^{10} photons per ommatidium per flash) that neither triggered a pupillary response nor altered the photochemical state. Photochemical conversions were created by a 0.23-mm beam of numerical aperture 0.07 that originated from a 45-W tungsten lamp covered by a 3-mm heat filter (Schott KG3) plus a color filter. The filter for "orange" flashes was a 3-mm Schott 0G590, which created a beam of intensity 0.12 lux at the eye. The filters for "blue" flashes were a cutoff filter (Hoya L42) and a 450-nm wideband (40-nm half bandwidth) interference filter (Ditric). For a detailed description of the apparatus, see G. D. Bernard and D. G. Stavenga, *J. Comp. Physiol.* **134**, 95 (1979).
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5. For details of this analysis, see G. D. Bernard, *Biophys. Struct. Mechanism*, in press.
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The Opioid Peptide Dynorphin, Circadian Rhythms, and Starvation

Abstract. *Dynorphin, an opioid peptide whose functions are unknown, is found in brain, pituitary, and peripheral organs. Specific radioimmunoassays were used to measure dynorphin in the hypothalamus and pituitary, during the day and at night, as a function of food and water deprivation. Immunoreactive dynorphin was increased in the hypothalamus and decreased in the pituitary at night. Water deprivation led to more than 50 percent reduction in daytime levels of pituitary dynorphin and concomitant increases in hypothalamic dynorphin.*

Dynorphin is a recently characterized peptide occurring widely in the central nervous system, pituitary, and gut (*1*). It has potent opioid-like activity in bioassays on guinea pig ileum (*1*). An immunohistochemical study has shown dynorphin-containing cells in the hypothalamus, with fibers projecting to the neurointermediate lobe of the pituitary (NI pituitary) (*2*). We now report that immunoreactive dynorphin in the hypothalamus and NI pituitary shift with circadian rhythms and water deprivation.

A specific and sensitive radioimmunoassay for dynorphin has been developed using an antiserum having little cross-reactivity to enkephalins, β-endorphin, α-neoendorphin, and other peptides (*3*).

Eighty male albino rats were housed in

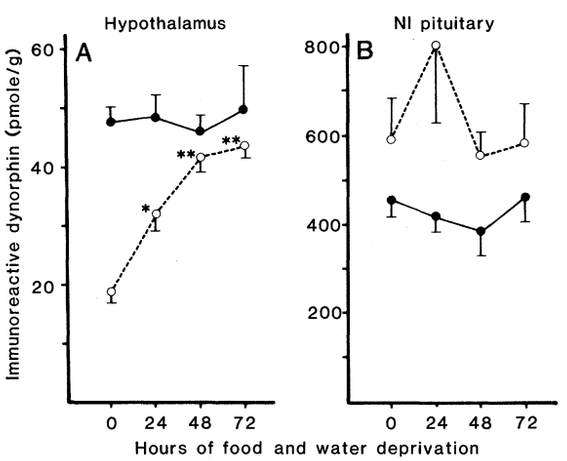

Fig. 1. Mean immunoreactive dynorphin concentration (expressed as picomoles per gram of wet tissue) in (A) the hypothalamus and (B) the neurointermediate lobe of the pituitary (NI pituitary) during the day (○) and during the night (●) as a function of hours of food and water deprivation. Analysis of variance of the data in (A) yielded $F(1, 59) = 30.51$ ($P < .001$) for the day-night factor, $F(3, 59) = 3.98$ ($P < .02$) for hours of deprivation, and $F(3, 59) = 4.46$ ($P < .01$) for the interaction. All daytime deprivation levels differed reliably (*$P < .05$; **$P < .005$) from the control (0 hours of deprivation), with Dunnett's test used for making comparisons. Analysis of variance of the data in (B) yielded $F(1, 64) = 10.00$ ($P < .002$) for the day-night factor, other factors not being reliable sources of variance. Standard errors (S.E.M.'s) are shown.

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