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.

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References and Notes

1. T. H. Goldsmith, in *Handbook of Sensory Physiology*, H. J. A. Dartnall, Ed. (Springer, Heidelberg, 1972), vol. 7, part 1, p. 685; A. Knowles and H. J. A. Dartnall, in *The Eye*, H. Davson, Ed. (Academic Press, New York, ed. 2, 1977), vol. 2B, p. 519; K. Hamdorf, in *Handbook of Sensory Physiology*, H. Autrum, Ed. (Springer, Heidelberg, 1979), vol. 7, part 6A, p. 151.
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).
4. H. J. A. Dartnall, *Br. Med. Bull.* **9**, 24 (1953); T. G. Ebrey and B. Honig, *Vision Res.* **17**, 147 (1977). Vertebrate rhodopsins have similar absorbance spectra when plotted against wave number. I find that the absorbance spectrum of butterfly rhodopsin R530 is the same as that of a vertebrate rhodopsin R530. The term density units refers to the integrated, longitudinal optical density (base 10) of either rhodopsin or metarhodopsin, at the wavelength for maximal absorbance (λ_{max}) for only a single pass through the rhabdom.
5. For details of this analysis, see G. D. Bernard, *Biophys. Struct. Mechanism*, in press.
6. I thank T. W. Cronin, T. H. Goldsmith, S. B. Laughlin, W. H. Miller, D. G. Stavenga, and the reviewers for their suggestions. Supported by grants EYO1140 and EY00785 from the National Eye Institute, by the Connecticut Lions Eye Research Foundation, and by Research to Prevent Blindness, Inc.

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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

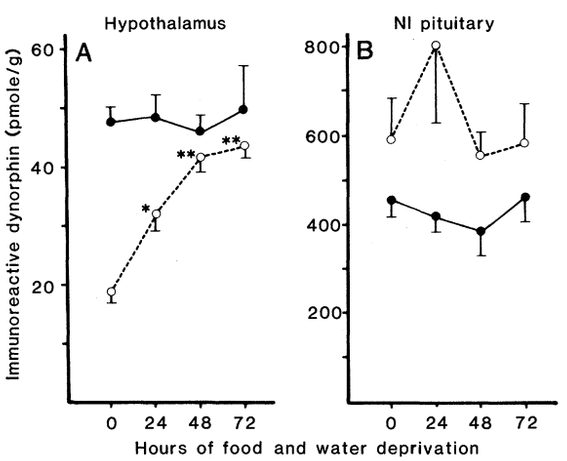

Fig. 1. Mean immunoreactive dynorphin concentration (expressed as picomoles per gram of wet tissue) in (A) the hypothalamus and (B) the neurointermediate lobe of the pituitary (NI pituitary) during the day (○) and during the night (●) as a function of hours of food and water deprivation. Analysis of 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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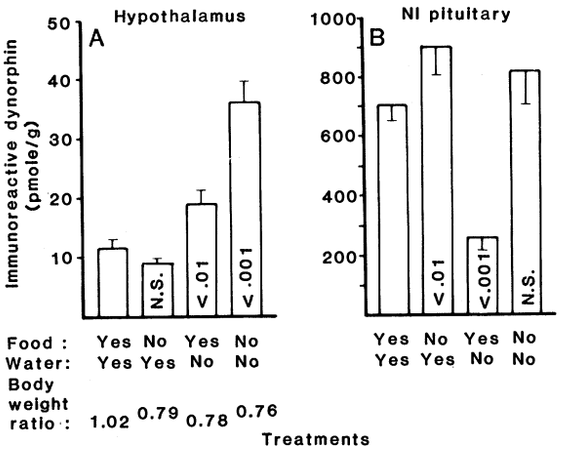

Fig. 2. Means (± S.E.M.) of immunoreactive dynorphin concentration (picomoles per gram of wet tissue) in (A) the hypothalamus and (B) the NI pituitary during a 72-hour period of free access to food and water, deprivation of water, deprivation of food, and deprivation of both food and water. Analysis of variance of the data in (A) yielded $F(3, 32) = 46.03$ ($P < .001$) and of the data in (B) yielded $F(3, 32) = 18.98$ ($P < .001$). Values in the bars are P values from Dunnett's test for comparing means to a control value; *N.S.*, not significant. The body weight ratio is the ratio of the mean weight of the rats just after the 72-hour treatment period to their mean weight just before the treatment period.

standard large plastic cages, in groups of ten rats per cage (*4*). Rats were handled daily, and their body weights were measured twice daily until they were habituated to both the living conditions and handling (*5*).

During a specific time period, one-fourth of the rats continued to have food and water available continuously, as before, while the others were separated into groups that were deprived of food and water for 24, 48, or 72 hours by the end of the period. Half of the rats began the starvation regimen at the start of the light period, and the other half at the start of the dark period. At the end of the deprivation periods, nine rats from each group were killed, and the hypothalamus and the NI pituitary were removed for radioimmunoassay. Data were obtained on each kind of tissue to conform to a 2 by 4 factorial design, with one factor associated with time of day (day or night) and the other associated with four deprivation levels.

As the tissue was obtained, it was placed in plastic tubes, weighed, and frozen. Peptides were then extracted, and radioimmunoassays were performed that were similar to those described (*3*, *6*). Data from the gamma scintillation counter were analyzed with computer program NIHRIA (*7*). After the weight of the original tissue was taken into account, the results were expressed as picomoles of immunoreactive material per gram of fresh tissue. These data, for each kind of tissue, were then submitted to analysis of variance in a 2 by 4 factorial design.

Immunoreactive dynorphin was increased in the hypothalamus when tissue was taken during the night and increased in the NI pituitary when tissue was taken during the day (Fig. 1). Food and water deprivation did not markedly affect immunoreactive dynorphin levels in the NI pituitary or the hypothalamus when tissue was taken during the night. Deprivation, however, resulted in orderly changes in daytime levels of hypothalamic immunoreactive dynorphin, with starvation shifting the typically low daytime levels to the high levels of nighttime.

In another experiment, rats were treated similarly to those of the first experiment, except they were deprived of water only, food only, both food and water, or were not deprived for 72 hours. Although deprivation of food only or water only led to similar weight losses (Fig. 2A), deprivation of each had characteristic features. The tissue for the radioimmunoassays (nine rats for each treatment) of the different kinds of deprivation were taken during the light period of the daily light-dark cycle.

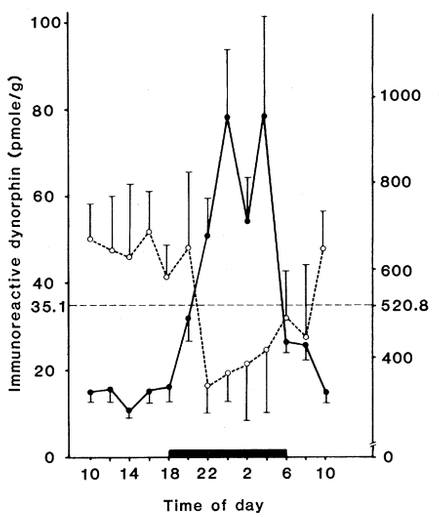

Fig. 3. Mean immunoreactive dynorphin concentration (picomoles per gram of wet tissue) in (●) the hypothalamus and (○) the NI pituitary tissue taken from rats every 2 hours for 24 hours. The horizontal line represents the mean of all values across the 24-hour period. Values on the left ordinate are those for the hypothalamus and those on the right ordinate are for the NI pituitary. Lines extending from data points are S.E.M.'s ($N = 6$).

The finding in the first experiment that daytime levels of hypothalamic immunoreactive dynorphin are higher in food- and water-deprived rats than in nondeprived rats (Fig. 1) was confirmed with the second set of measurements (Fig. 2A). Under conditions of water-only deprivation, there were increases of immunoreactive dynorphin in the hypothalamus and decreases in the NI pituitary, in comparison with the nondeprived condition. With food-only deprivation, values of immunoreactive dynorphin were not significantly different from those of controls, but the shifts in levels were in the direction opposite those resulting from water deprivation. The values of food-only deprivation were significantly different from those of water-only deprivation ($P < .01$).

As a further study of the relation of immunoreactive dynorphin levels to time of day, six rats were killed every 2 hours during a 24-hour period. These adult male albino rats had been habituated for 21 days to a cycle of 12 hours of light and 12 hours of darkness (lights on at 0600) while living in groups of six to eight in large cages with food and water always available. The results confirm the marked differences in immunoreactive dynorphin as a function of time of day and kind of tissue (Fig. 3). The hypothalamic data are fitted well by a sinusoidal curve with a period of 24 hours (significant 24-hour component with no other prominent phase in the distribution of power magnitudes of a Fourier analysis). The calculated 24-hour component accounts for 50 percent of the total variance of the data, clearly indicating 24-hour rhythmicity. The maximum of the curve occurs at 1:24 hours ± 40 minutes. A significant 24-hour component is also seen in the NI pituitary data, but it accounts for only 17 percent of the total variance. The maximum of the phase of the calculated curve for the NI pituitary data is 13:58 hours ± 60 minutes, which is roughly 12 hours after the peak for the hypothalamus.

These results show that (i) immunoreactive dynorphin levels in the hypothalamus and in the NI pituitary are different during the day from their values at night, immunoreactive dynorphin being lower

in the hypothalamus and higher in the NI pituitary during the day; and (ii) deprivation of both food and water, or of water only, increases daytime levels of hypothalamic immunoreactive dynorphin, whereas deprivation of water alone decreases NI pituitary immunoreactive dynorphin.

In view of the relatively rapid catabolism of dynorphin (*1*), increased levels of immunoreactive dynorphin probably reflect increased synthesis and storage, and decreased levels in the NI pituitary probably reflect rapid utilization. There seems to be a reciprocal relation between the levels of immunoreactive dynorphin in the hypothalamus and the NI pituitary, one being high when the other is low.

These results, showing a relation between circadian rhythm and immunoreactive dynorphin, lead to a number of hypotheses concerning the role of dynorphin, including those involving such variables as sleep and general activity, and the possibility that the endorphins may be integral to a biological clock. An immunohistochemical study (*2*) indicates the presence of dynorphin cells in the region of the suprachiasmatic nuclei of the hypothalamus, an area whose destruction disrupts circadian rhythm (*8*). The changes of immunoreactive dynorphin with circadian rhythm suggest that dynorphin may be related to events sensitive to opioid antagonists showing circadian rhythms. Evidence indicating that dynorphin is an endogenous ligand for the κ-opioid receptor (*9*), together with our results, lead to the hypothesis that κ-opioid drugs would be potent in affecting behavioral regulation of ingestion or of circadian rhythms. The interaction among water balance, circadian rhythm, and immunoreactive dynorphin implicates dynorphin in behavioral maintenance of homeostasis.

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References and Notes

1. A. Goldstein, S. Tachibana, L. I. Lowney, M. Hunkapillar, L. Hood, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6666 (1979); A. Goldstein and V. E. Ghazarossian, *ibid.* **77**, 6207 (1980).
2. S. J. Watson, H. Akil, V. E. Ghazarossian, A. Goldstein, *ibid.* **78**, 1260 (1981).
3. V. E. Ghazarossian, C. H. Chavkin, A. Goldstein, *Life Sci.* **27**, 75 (1980); V. Höllt, I. Haarmann, B. R. Seizinger, A. Herz, *Neuroendocrinology* **33**, 333 (1981). Although the antiserum used in these studies is directed against synthetic dynorphin-(1–13), gel chromatography indicates that more than one dynorphin-like immunoreactive component is recognized by the antiserum. Further research is necessary to determine if the changes reported here are limited to one of the immunoreactive dynorphin-related peptides in the hypothalamus.
4. Standard cages of plastic and wire mesh (55 by 34 by 18 cm) were kept in a colony room at 24°C and 45 percent humidity. The lighting cycle was 12 hours of light and 12 hours of darkness, with lights on at 0800.
5. The goal of the procedures of habituation and handling was to minimize extraneous sources of stress, leaving only those associated with privation as the critical variable. Accordingly, rats were housed in groups to minimize isolation stress. Assays were carried out only after rats were gaining weight across days, indicating that they were habituated to the living conditions [see H. Steinberg, in *Factors Affecting the Action of Narcotics*, M. L. Adler, L. Manara, R. Samanin, Eds. (Raven, New York, 1979), pp. 595–611]. The rats were eating and drinking most of their food and water at night (> 82 percent of each), indicating that they were entrained to the lighting cycle. They were not defecating when being handled and weighed, indicating that they were accustomed to being handled. In addition, only nine rats of each group were killed, so that the last rat left in a cage (having an unusual potential for stress) was not part of the sample.
6. Peptides were extracted by placing tissue in 0.1*N* HCl at 95°C for 10 minutes, and then homogenized and centrifuged at 140,000*g* for 30 minutes at 4°C. The radioimmunoassay, conducted in triplicate, involved adding to plastic tubes (i) 50 μl of extract of the tissue, (ii) 300 μl of buffer, (iii) 100 μl of antiserum solution (dilution, 1:50,000), and (iv) 50 μl of iodinated tracer solution. After an incubation period of 20 hours, unbound ^{125}I-labeled tracer [dynorphin-(1–17)] was removed by charcoal separation.
7. D. Rodbard, J. Huston, Jr., P. J. Munson, *RIA Data Processing: Basic Programs* (Vanderbilt Medical Center, Nashville, Tenn., 1980).
8. F. K. Stephan and I. Zucker, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1583 (1972); A. N. Van den Pol and T. Powley, *Brain Res.* **160**, 307 (1979); G. E. Pickard and F. W. Turek, *Science* **215**, 1119 (1982).
9. J. P. Huidobro-Toro, K. Yoshimura, N. M. Lee, H. H. Loh, E. L. Way, *Eur. J. Pharmacol.* **72**, 265 (1981); M. Wüster, P. Rubini, R. Schulz, *Life Sci.* **29**, 1219 (1981); C. Chavkin, I. F. James, A. Goldstein, *Science* **215**, 413 (1982).
10. Supported by the Max-Planck-Institute. We thank T. Costa for critical discussion of the manuscript and H. Pfister and L. von Lindern of the Max-Planck-Institute's computer center for computing help. R. Przewłocki, A. M. Konecka, and L. D. Reid were or are visiting scientists at the Max-Planck-Institute. Portions of these data were presented at the International Narcotic Research Conference, Falmouth, Massachusetts on 17 June 1982.

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Relation Between Plasma and Cerebrospinal Fluid Levels of 3-Methoxy-4-Hydroxyphenylglycol

Abstract. *Concentrations of free 3-methoxy-4-hydroxyphenylglycol in the plasma and cerebrospinal fluid are highly correlated, but concentrations in the cerebrospinal fluid are always higher than those in plasma, even when large amounts of the catecholamine metabolite are derived from a tumor of the adrenal medulla. This is explained by considering the plasma and cerebrospinal fluid as a two-compartment system in which the rate constants for entry into and exit from the cerebrospinal fluid compartment are similar. 3-Methoxy-4-hydroxyphenylglycol that is synthesized, but not catabolized, in the central nervous system maintains cerebrospinal fluid levels at an increment over those in plasma. This increment can be used to provide the best available index of formation of 3-methoxy-4-hydroxyphenylglycol in the central nervous system.*

In the brain of most species, 3-methoxy-4-hydroxyphenylglycol (MHPG) is the major metabolite of norepinephrine (*1*). Although most MHPG is excreted as a conjugate, about one-third of the total MHPG in human plasma is unconjugated and almost all MHPG in cerebrospinal fluid (CSF) occurs in the free form (*2*). Measurement of CSF concentrations of MHPG has become a widely accepted means of assessing norepinephrine formation and utilization in the central nervous system of man and other primates (*3*). Although in humans (*4*) and monkeys (*5*) there is a highly significant correlation between CSF and plasma levels of MHPG, it has been presumed that both reflect central noradrenergic activity (*5*). From the results obtained in our present study it becomes clear that a substantial portion of free MHPG in human CSF is derived from plasma, that this is to be expected on the basis of kinetic considerations, and that CSF levels of MHPG can be interpreted as reflecting central nervous system norepinephrine metabolism only when the plasma MHPG contribution can be adequately assessed.

To determine the extent to which peripherally formed MHPG can affect CSF levels of this metabolite, we examined plasma and CSF levels of MHPG in patients with phaeochromocytoma, a tumor of the adrenal medulla which contains high concentrations of MHPG (*6*). These patients were expected to have relatively constant high levels of plasma MHPG. Normal subjects and patients with idiopathic orthostatic hypotension (known to have deficits in peripheral

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