fect was found on photic responses or cell motility, the latter becoming impaired at $5 \times 10^{-3}M$ carbon monoxide. Carbon monoxide binds to cytochrome a_3 (*9*), but not to cytochrome a (*12*).

Sodium azide at concentrations as high as $5 \times 10^{-3}M$ had no effect on ring formation even though motility of the cells was significantly impaired at this concentration. While some investigators believe that azide binds to the oxidized form of cytochrome a_3 (*10, 13*), others maintain that azide binds to the reduced form of cytochrome a (*14*) (Table 1).

Sodium cyanide and carbon monoxide inhibit the chemosensory response of *Euglena* toward oxygen at concentrations where neither the movement of the cells, nor their ability to respond to photosensory stimuli are impaired. One might argue that these inhibitors affect respiration rather than chemoaccumulation, and that they act by inhibiting oxygen consumption and thus the formation within the sample chamber of the oxygen concentration gradient which triggers the chemophobic responses in the first place. That this is not the case at the concentrations used by us is evident from the fact that the cells move normally, and thus have available their usual source of metabolic energy. In addition, previous studies in vivo under experimental conditions very similar to our own have shown that cellular respiration of intact *Euglena gracilis* is only marginally inhibited by carbon monoxide (*15*), while cyanide even at $10^{-3}M$ inhibits oxygen consumption of whole cells by no more than 57 percent (*9*). However, the isolated a-type cytochrome is completely inhibited by $10^{-3}M$ cyanide (*9*), indicating that, while cytochrome oxidase does get inactivated by cyanide (which is in agreement with our hypothesis), in intact cells of *Euglena gracilis* there is an alternative pathway for the reoxidation of cytochrome b, and thus for the generation of metabolic energy. This explains the retention of motility in our cells at high inhibitor concentrations.

Since the photosensory responses of *Euglena* were unaffected by carbon monoxide and sodium cyanide at the concentrations studied by us, the effects we are observing do not reflect a generalized inhibition of sensory transduction in *Euglena* at the level of signal processing or effector mechanisms (*11*). We conclude that these results implicate the cytochrome a_3 moiety of cytochrome c oxidase as the receptor molecule for oxygen-mediated chemosensory responses in *Euglena*. In addition, it might be argued that our data support the contention (*14*) that azide, which is ineffective behaviorally even at a concentration at which motility of the cells is inhibited, inhibits cytochrome c oxidase by binding to cytochrome a rather than to cytochrome a_3. The main interest of this inference is that it is based entirely on studies of the behavior of *Euglena* rather than on direct observation of biochemical changes within the cell.

Table 1. Effects of inhibitors (sodium cyanide, carbon monoxide, and sodium azide) of cytochrome c oxidase on chemoaccumulation toward oxygen and motility of *Euglena gracilis*.

Concentration (*M*)	Effect on	
	Ring patterns	Motility (% of control)
	Sodium cyanide	
10^{-7}	None	None
10^{-6}	Inhibited	None
10^{-5}	Inhibited	None
10^{-4}	Inhibited	None
10^{-3}	Inhibited	40
	Carbon monoxide	
5×10^{-6}	None	None
5×10^{-5}	Inhibited	None
5×10^{-4}	Inhibited	None
5×10^{-3}	Inhibited	40
	Sodium azide	
5×10^{-3}	None	50

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13 October 1977; revised 28 December 1977

Ellipsosomes: Organelles Containing a Cytochrome-Like Pigment in the Retinal Cones of Certain Fishes

Abstract. *Ellipsosomes are dense spherical bodies containing a very large concentration of a heme pigment spectroscopically resembling pure cyctochrome c. They are located at the outer ends of the inner segments of the cones of certain fishes. Although, superficially, they resemble the similarly located oil droplets in the cones of birds and reptiles, their ultrastructure and staining properties resemble those of the neighboring mitochondria. However, like the oil droplets, they may serve as intracellular color filters.*

The outer part of the inner segment of the cone cells of the vertebrate retina is commonly known as the ellipsoid. This region is packed with large mitochondria and, in some avian and reptilian species, contains a large, clear or strongly colored oil droplet immediately in front of the light-absorbing outer segment (*1*). This droplet acts as a filter which prevents the shorter wavelengths of light from reaching the photosensitive pigment. Structures resembling the oil droplets of birds and reptiles have been described in a variety of fishes (*1, 2*). However, as previously pointed out (*3*), these droplets do not stain with oil-soluble dyes and electron micrographs show that they have an organized structure (which the oil droplets do not) reminiscent of an unusually large mitochondrion (Fig. 1). To prevent their being confused with true oil droplets we call them ellipsosomes. We have found that they have a spectral absorbance that is characteristic of a dense heme pigment, different from that of hemoglobin, and similar to that of reduced cytochrome c (*4*). They also stain positively for some other components of the respiratory chain. Furthermore, they contain no trace of carotenoid pigments (*5, 6*).

Eyes were removed from guppies (*Poecilia reticulata*), mollies (*Poecilia latipinna*), killifish (*Fundulus heteroclitus*), and platies (*Xiphophorus maculatus*). The retinas were dissected out in calcium-free physiological solution [to facilitate retinal detachment and dissociation (*7*)] either in bright light or under infrared illumination. After 45 minutes in calcium-free solution, small pieces of retina were teased apart in a drop of the same solution on a large cover slip, ringed with silicone oil (DC 702 diffusion pump oil), and covered with another cover slip. They were then exam-

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ined in a microspectrophotometer by means of infrared television or an infrared image converter for viewing. Two different instruments were used, the dichroic microspectrophotometer (DMSP) (*8*) and the photon counting microspectrophotometer (PMSP) (*9*). Both of these are single-beam instruments that scan the spectrum repetitively once every second and average the photocurrents (DMSP), or photoelectron counts (PMSP), in a computer memory. A reference measurement (blank) is first made through a clear area of the preparation, then a measurement is made through the region of interest (sample). The DMSP, which uses a rotating polarizer, computes, calculates, and displays absorptance (fraction of the light absorbed) of two orthogonally polarized components as a function of wavelength. It was used to determine whether the elipsosomes show linear dichroism. None was found. The PMSP calculates and displays optical density as a function of frequency (velocity of light on wavelength) for a single polarized component. The measurements with this instrument reported herein were made with a light beam passing tranversely through the receptors and polarized perpendicular to their long axes. Rectangular spots smaller than the organelles being measured were used for measuring ellipsosomes and outer segments.

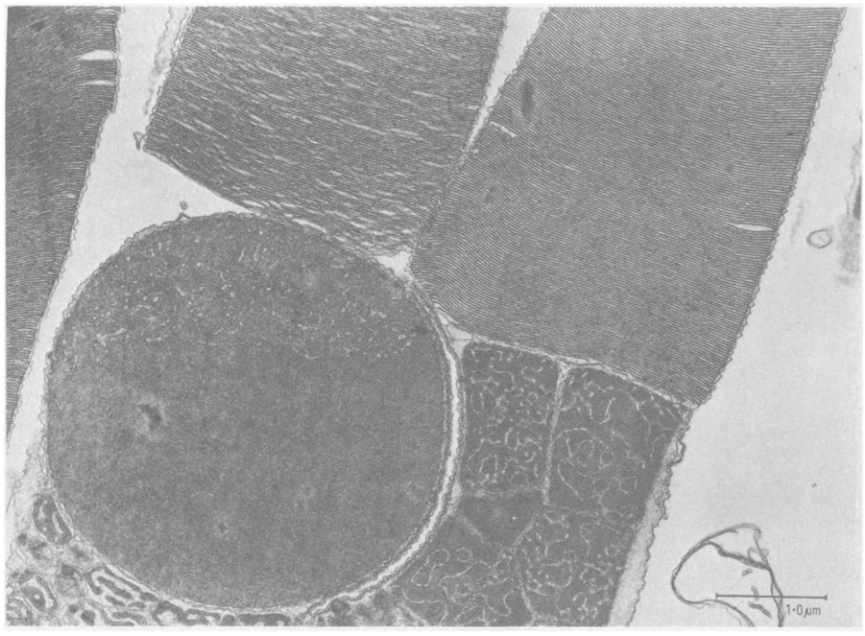
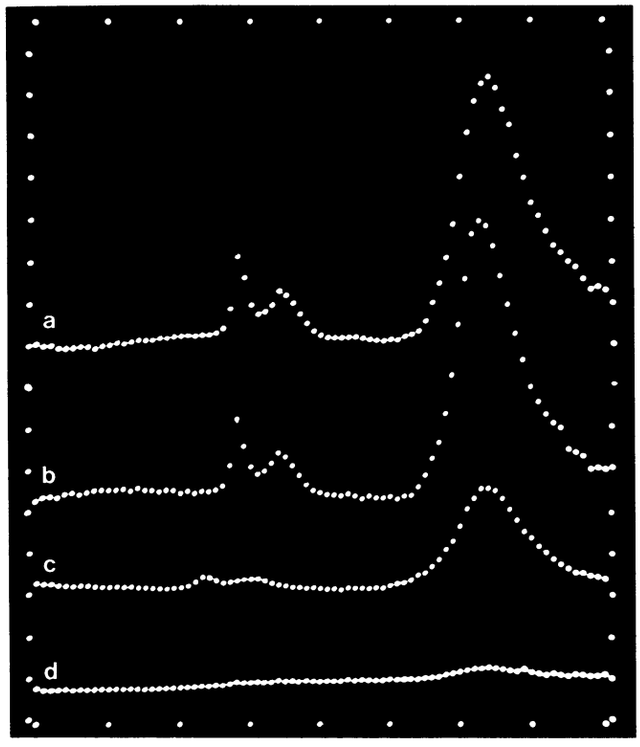


Fig. 1. Electron micrograph of a portion of a pair of twin cones of *Poecilia reticulata*. The left member of the pair contains an ellipsosome, whereas the right member contains the large mitochondria ordinarily found in the inner segments of cones. [Courtesy A. Yacob, University College, Dublin, Ireland]

Figure 2 shows measurements, plotted on the same absolute density scale, of el-



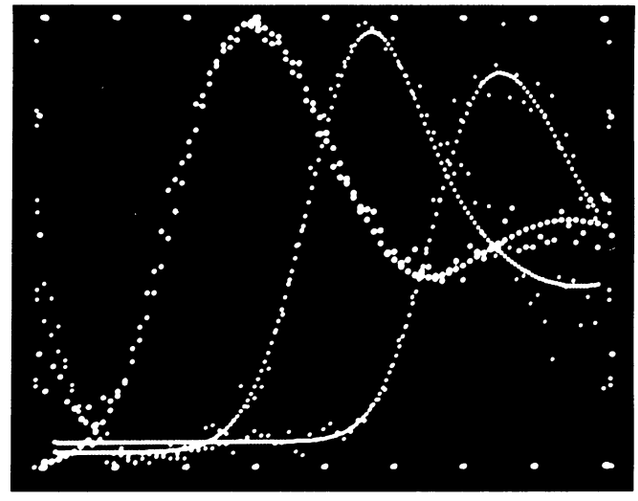


Fig. 2 (left). Microspectrophotometric comparison of ellipsosomes, erythroctyes, and an ellipsoid containing only mitochondria. (a) *Fundulus* ellipsosome (single measurement of 20 scans). (b) *Poecilia* ellipsosome (20 scans). (c) Average of five *Poecilia* erythrocytes (20 scans each). (d) *Poecilia* ellipsoid containing only mitochondria (20 scans). All recordings have the same absolute density scale. The spacing between dots on the left and right margins of the figure indicates an absorbance difference of 0.1. Dots at upper and lower margins of the figure indicate frequency in fresnels from 405 to 805 in 50 fresnel steps from left to right, corresponding to wavelengths of 741, 659, 594, 541, 496, 458, 425, 397, and 373 nm; 1 fresnel = 10^{12} hertz and λ (nm) = 300,000/ν (fresnels). The α, β, and γ bands of the heme pigments are in order from left to right [see (*10*) for wavelengths of maximum absorption]. Fig. 3 (right). Averages of a number of measurements of pigment absorption spectra of the three cone types in *Poecilia reticulata*: blue-absorbing inner cones, and red- and green-absorbing twin cones. The experimental data are fitted by eye to three template curves each generated from the sum of three Gaussian functions and adjusted on the oscilloscope by an interactive computer program. Template maxima are (from left to right) 548.9, 635.3, and 734.2 fresnels, corresponding to 546.5, 468.5, and 408.5 nm. Since the cones were measured transversely the absorption of the ellipsosomes is not reflected in the measurements. The frequency scale is the same as in Fig. 2. The absorbances are not plotted to the same scale and thus indicate only relative density as a function of the frequency of the light.

lipsosomes from two species of fishes together with an average curve for five erythrocytes of one species, and an ellipsoid containing only densely packed mitochondria. Although the γ (Soret) bands of ellipsosomes and erythrocytes resemble each other very closely it is evident that the peaks of the α and β bands of the ellipsosomes are both larger in proportion to the γ peak, and occur at a higher frequency (shorter wavelength), and that the ratio of the α peak to the β peak is considerably greater than in the erythrocyte spectrum. This is characteristic of reduced cytochrome c (*10*). Thus, the ellipsosome spectrum cannot be an artifact caused by contamination with hemoglobin.

The ellipsosomes from dark-adapted retinas were not significantly different in density or spectrum from those that had been thoroughly light-adapted. If they are indeed functional mitochondria we would expect that other respiratory enzymes would be present. We therefore stained the retinas with nitro blue tetrazolium (NBT). This stain, which is commonly used as an indicator of succinate dehydrogenase activity (E.C. 1.3.99.1), deposits a blue diformazan precipitate when incubated in the presence of succinate (*11*). Dark-adapted retinas were dissected in calcium-free solution (*4*) in room light and kept in the dark for 45 minutes as before. They were then transferred in room light to a complete physiological solution containing glucose and sucrose. They were placed in the NTB incubation medium (0.05 g/10 ml) and kept in an incubator at 37°C for 10 minutes, and were then fixed in isosmotic buffer containing 10 percent formalin at room temperature in darkness for 10 minutes. Small pieces were then teased apart, mounted as for microspectrophotometry, and examined in a Zeiss Nomarski microscope. Photographs were made with Polaroid No. 108 color film.

As we expected, inner segments of all types of cones and the rods stained intensely, whereas the outer segments did not. The ellipsosomes stained as intensely, but no more so, than the adjacent mitochondria. Thus, the succinic dehydrogenase system appears to be present in the ellipsosomes as well as in the mitochondria. However, the mitochondria in ellipsoids contain much less heme pigment than the ellipsosomes, the Soret band being barely visible when displayed on the same density scale (Fig. 2, curve d).

We made a single unsuccessful attempt to oxygenate the presumed cytochrome c by exposing the retina to oxygen while incubating it in an inhibitor of the electron transport chain. Lacking a more suitable inhibitor such as seconal or antimycin a, we exposed the live fish and subsequently the isolated retina to Veronal, a slow-acting barbiturate, in oxygenated media. Subsequent measurements of the ellipsosomes showed a considerable decrease in the density of all three peaks but no shifts in either their frequencies or relative densities that would be characteristic of the oxidized state (*12*). This experiment should be repeated with a more suitable inhibitor.

Thus we have confirmed that the "oil droplets" in the cones of three species of fishes resemble mitochondria in that they contain not only an unusually large amount of pigment closely resembling cytochrome c in its spectral absorbance, but also the succinic dehydrogenase system. If other light-absorbing components of the respiratory chain were present in the same concentrations as in normal mitochondria (*12*) they would be masked by this large quantity of a single pigment; this is evident if one compares curve d with curves a and b in Fig. 2.

Whether ellipsosomes are functional mitochondria remains to be determined. From their anatomical position it is clear that light must pass through them to reach the photosensitive outer segments. With a peak density in the γ band greater than 0.5 (some of our recordings have shown densities > 1.0) they will prevent at least 60 percent of a narrow band of blue light between 400 and 430 nm from reaching the outer segments. In fact, the ellipsosomes of *Fundulus* are so dense that they look definitely pink under the microscope, while visual pigments in outer segments of the same thickness (4 μm) do not appear to have an appreciable color. Thus the ellipsosomes undoubtedly have an appreciable filtering effect, though a smaller one than that of the colored oil droplets of birds and reptiles. Ellipsosomes of guppy cones whose receptor pigments were also measured were invariably associated with the intermediate wavelength-absorbing (green) member of a pair of twin cones (*13*). However, in mollies the ellipsosomes were found not only in green cones but also in both members of red-red twins. The guppy cone pigments have absorption maxima at about 410, 470, and 545 nm (rod pigment 502). Like rhodopsin and other vertebrate visual pigments, both green- and red-absorbing cones have at least 20 percent of maximum absorption in the spectral range between the main peak and the *cis* peak in the ultraviolet, as shown in Fig. 3. With the γ band as a filter this absorption would be cut by half or more, and the stimulation of the green cones (and red cones in mollies) relative to the blue cones would be considerably decreased; this would then increase the contrast in the blue-violet region of the spectrum. This could have a functional significance. Wolbarsht (*14*) has postulated a similar function for the colored oil droplets of birds and reptiles.

We conclude only that the ellipsosome is an unusual organelle, possibly derived from a mitochondrion during the process of evolution, whose function could be either metabolic or optical. The biological significance of the ellipsosomes remains to be determined.

Note added in proof: Contrary to our previous statement, ellipsosomes from light-adapted retinas have been found to be 25 to 50 percent denser (γ peak) than those kept in the dark. In contrast, electron micrographs of dark-adapted ellipsosomes stained more strongly with osmium.

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4. The curves we obtain are the same as the curve in figure 4 of D. Keilin and E. C. Slater [*Br. Med. Bull.* **9**, 89 (1953)] which shows the spectral extinction of pure cytochrome c.
5. The oil droplets of reptiles and birds have been studied in detail by pigment extraction and chromatography, and by microspectrophotometry, and found to contain very dense carotenoid pigments. For comprehensive reviews see F. Crescitelli, in *Handbook of Sensory Physiology*, H. J. A. Dartnall, Ed. (Springer-Verlag, New

York, 1972) vol. 7, pt. 1, p. 245; P. A. Liebman, in *ibid.*, p. 481; W. R. A. Muntz, in *ibid.*, p. 529; and Stell and Hárosi (*6*). We have recently examined turtle cone oil droplets and found no trace of a pigment similar to that of ellipsosomes even in the colorless (ultraviolet absorbing) droplets.
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7. The calcium-free and complete saline solutions contained the following:

Chemical	Concentration (m*M*)	
	Calcium-free	Complete
NaCl	133.5	100.0
$NaHCO_3$	11.9	5.0
NaH_2PO_4	3.3	0.5
KCl	3.4	2.5
$MgCl_2$	21.0	1.0
$CaCl_2$	0	1.5
Glucose	11.1	10.0
Sucrose	0	90.0
Adjusted *p*H	7.4	7.0

In all these experiments 10 m*M* $MgCl_2$ was inadvertently used in the complete medium without apparent ill effects.
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10. Frequencies and wavelength of α, β, and γ peaks were as follows:

Source	Frequency	Wavelength (nm)	Source
	α Peak		
Xipophorus	545.9	549.6	Ellipsosomes
Poecilia reticulata	546.2	549.2	Ellipsosomes
Fundulus	546.0	549.0	Ellipsosomes
Poecilia reticulata	521.5	575.2	Erythrocytes
Reduced cytochrome c	545.5	550.0	Mitochondria
	β Peak		
Xipophorus	575.6	521.2	Ellipsosomes
Poecilia reticulata	576.5	520.4	Ellipsosomes
Fundulus	575.0	522.0	Ellipsosomes
Poecilia reticulata	555.8	540.0	Erythrocytes
Reduced cytochrome c	576.0	521.0	Mitochondria
	γ Peak		
Xipophorus	720.7	416.3	Ellipsosomes
Poecilia reticulata	721.6	415.7	Ellipsosomes
Fundulus	720.0	417.0	Ellipsosomes
Poecilia reticulata	724.0	414.0	Erythrocytes
Reduced cytochrome c	723.0	415.0	Mitochrondria

The data for mitochondria are from H. R. Mahler and E. H. Cordes, *Biological Chemistry* (Harper & Row, New York, ed. 1, 1966), table 14–10. In this table the extinction per millimole is given for the γ band as 125. The average peak density of *Xiphophorus* cones was more than 0.7, indicating a concentration of more than 11 m*M*.
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19 December 1977; revised 24 February 1978

Stimulation of Dopamine Synthesis in Caudate Nucleus by Intrastriatal Enkephalins and Antagonism by Naloxone

Abstract. *The intraventricular injection of methionine-enkephalin (50 to 100 micrograms) or [D-Ala²]-methionine-enkephalinamide (1.5 to 12 micrograms), a synthetic enkephalin analog resistant to enzyme degradation, caused a marked dose-dependent increase in dihydroxyphenylacetic acid and homovanillic acid concentrations in the rat striatum. The [D-Ala²] analog increased the accumulation of dopa in the striatum after aromatic amino acid decarboxylase inhibition, indicating that it increased dopamine synthesis. At the highest doses used both enkephalins failed to modify brain serotonin metabolism. The monolateral microinjection of the [D-Ala²] analog (3 to 6 micrograms) into the caudate nucleus increased the concentration of dihydroxyphenylacetic acid in the injected side, whereas bilateral injection increased the concentration of this compound in both caudate nuclei and caused catalepsy. The stimulant effect of the [D-Ala²] analog on dopamine synthesis in the striatum persisted after destruction of striatal postsynaptic dopamine receptors with kainic acid. The biochemical and behavioral effects of enkephalins were prevented by naloxone, a specific narcotic antagonist. The results indicate that enkephalins stimulate dopamine synthesis by an action on opioid receptors localized on dopaminergic nerve terminals.*

The central effects of morphine and opiate-like drugs are considered to be mediated by specific opiate receptors that have been mapped in the mammalian brain (*1*). Endogenous peptides with opiate agonist properties have been isolated from the mammalian brain and identified as the pentapeptides methionine-enkephalin and leucine-enkephalin (*2*).

However, the two enkephalins exert a weak morphine-like effect when injected intraventricularly or intracerebrally into rats because they are rapidly destroyed by peptidases in brain (*3*). An enkephalin analog, [D-Ala²]methionine-enkephalinamide, has been synthesized which has almost the same affinity for the opiate receptors as the endogenous peptides but is resistant to enzymatic degradation (*4*). Thus the [D-Ala²] analog induces profound and long-lasting analgesia and other opiate-like behavioral effects when injected intracerebrally (*4*), and therefore might be useful in the study of the mechanism of action of endogenous and exogenous opiates. Narcotic analgesics stimulate dopamine turnover (*5*, *5a*), but the mechanism of this effect is not clear. Yet this action has been correlated with several central effects of these compounds, such as the analgesic effect, catalepsy, motor stimulation, circling behavior, and tolerance (*6*).

We now report that methionine-enkephalin and, to a much greater degree, the [D-Ala²] analog stimulate striatal dopamine synthesis when injected intracerebrally. This effect is reversed by naloxone, a specific opiate antagonist, but persists after destruction of striatal dopamine receptors with kainic acid, suggesting that enkephalins stimulate dopamine synthesis by an action on opioid receptors localized on dopamine nerve terminals.

Male Sprague-Dawley rats (260 to 280 g) were implanted stereotaxically with

Table 1. Effect of the intraventricular injection of methionine-enkephalin and the (D-Ala²) analog on dopamine metabolism in the caudate nucleus. The [D-Ala²] analog and methionine-enkephalin were given intraventricularly 45 and 15 minutes, respectively, before the animals were killed. Naloxone (3 mg/kg, intraperitoneally) was given at the same time as the peptides. Each value is the mean ± standard error of six determinations. Plus and minus signs indicate degree of catalepsy.

Treatment	Amount (μg)	Dopamine (μg/g)	DOPAC (μg/g)	HVA (μg/g)	Catalepsy
Saline		10.61 ± 0.51	2.36 ± 0.01	1.73 ± 0.9	−
Methionine-enkephalin	50.0	10.05 ± 0.60	2.75 ± 0.01*	2.21 ± 0.01*	−
Methionine-enkephalin	100.0	12.85 ± 0.39*	2.96 ± 0.01†	2.75 ± 0.02†	+
[D-Ala²] analog	1.5	10.11 ± 0.46	2.77 ± 0.02†	2.45 ± 0.01†	−
[D-Ala²] analog	3.0	10.08 ± 0.51	3.02 ± 0.01†	2.75 ± 0.01†	++
[D-Ala²] analog	6.0	12.41 ± 0.38*	3.97 ± 0.01†	3.11 ± 0.02†	+++
[D-Ala²] analog	12.0	13.31 ± 0.61*	4.34 ± 0.02†	3.61 ± 0.01†	+++
Naloxone			2.32 ± 0.01	1.65 ± 0.09	−
Naloxone plus methionine-enkephalin	100.0		2.33 ± 0.02	1.90 ± 0.01	−
Naloxone plus [D-Ala²] analog	3.0		2.38 ± 0.02	1.88 ± 0.01	−

*$P < .01$ with respect to saline-injected rats. †$P < .001$ with respect to saline-injected rats.

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