their brains were removed, and cortical as well as striatal tissue was dissected, weighed, and frozen at −70°C until assayed.
18. Cortical tissue from individual rats was used for preparation of the P2 synaptosomal membrane fraction, which was used in radioreceptor binding studies at 0.2 mg of protein per milliliter of incubation solution. Porcine (p) [^{3}H]-propyonyl-NPY (Amersham) was used in seven concentrations ranging from 0.25 to 25 nM. Specific binding was defined as that displaced by excess (1 μM) unlabeled NPY. Incubations were for 180 min at room temperature in a final volume of 250 μl. All conditions were tested in triplicate. Total [^{3}H]NPY binding, representing a sum of Y1- and Y2-type receptors, was obtained with no masking agent present. Determination of Y1 sites and Y2 binding was done as for [^{125}I]PYY (*13*). Saturation curves, constructed separately from each rat with the use of computerized nonlinear least squares regression (Accufit and Accucomp, Lundon Software, Chagrin Falls, OH), yielded individual estimates of binding site numbers (B_{max}) and binding affinities (K_d). Group averages of these parameters were compared.
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GABA-Activated Chloride Channels in Secretory Nerve Endings

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Neurotransmitters acting on presynaptic terminals regulate synaptic transmission and plasticity. Because of the difficulty of direct electrophysiological recording from small presynaptic terminals, little is known about the ion channels that mediate these actions or about the mechanisms by which transmitter secretion is altered. The patch-clamp technique is used to show that the predominant inhibitory presynaptic neurotransmitter, γ-aminobutyric acid (GABA), activates a $GABA_A$ receptor and gates a chloride channel in the membranes of peptidergic nerve terminals of the posterior pituitary. The opening of a chloride channel by GABA weakly depolarizes the nerve terminal membrane and blocks action potentials. In this way, GABA limits secretion by retarding the spread of excitation into the terminal arborization.

The neurotransmitter GABA inhibits the release of transmitters from nerve terminals throughout the nervous system. This action has been demonstrated in preparations as diverse as the arthropod neuromuscular junction, fish retina, and mammalian spinal cord and hippocampus (*1–5*). In contrast to its actions at postsynaptic targets, the actions of GABA at presynaptic targets are poorly understood; the nature of the channels gated by GABA at presynaptic sites is not known, and the evaluation of the pharmacological properties of presynaptic GABA receptors relies heavily on extrapolations from studies of postsynaptic receptors. The situation is the same for virtually all substances that act at presynaptic targets. Little is known of the ionic mechanisms by which neurotransmitters alter the release of neurotransmitters from nerve endings.

The posterior pituitary is very rich in secretory nerve terminals, some of which are larger than 10 μm in diameter. Patch-clamp recordings from posterior pituitary neurosecretosomes (*6, 7*) and slices (*8, 9*) have revealed some of the basic membrane mechanisms that govern the excitability of nerve terminals. Using thin slices of the posterior pituitary, we studied the membrane mechanisms that underly the responses of nerve endings to chemical signals (*10*). Under whole cell voltage clamp, the application of GABA elicited a membrane current in 85 of 95 recordings (89%) (Fig. 1A). With Cl^- concentration nearly equal on both sides of the membrane and a holding potential of −70 mV, the mean GABA-activated current was 92 ± 8 pA (mean ± SEM; $n = 30$). The current varied linearly with voltage, reversing at 0.1 ± 1.4 mV ($n = 11$) (Fig. 1B). The reversal potential was close to the Cl^- Nernst potential of 0.9 mV (Fig. 1C). With fixed extracellular Cl^- concentration, the reversal potential varied linearly with the logarithm of the intracellular Cl^- concentration with a slope of 63.7 mV per tenfold change. These data indicate that GABA gates a Cl^--selective ion channel in the membranes of posterior pituitary nerve endings.

GABA application to outside-out patches produced single-channel currents (Fig. 2A). Individual patches had as many as three channels; about one-third of the patches had none. At a holding potential of −70 mV, the average single-channel current was 1.8 pA. Amplitude distributions revealed only one open conductance level (Fig. 2B, inset) and no subconductance states. In contrast, GABA-activated channels in nerve cell bodies exhibit abundant subconductance state activity (*11*), reflecting a subtle difference in channel properties. Single-channel current varied linearly with voltage (Fig. 2B) and reversed at 0.0 ± 1.1 mV ($n = 4$), a value again near the Nernst potential for Cl^-. The slope from the best fitting line furnished an average single-channel conductance of 26.3 ± 0.9 pS ($n = 4$). This conductance is similar to the conductance of GABA-activated channels in nerve cell bodies (*11, 12*), endocrine secretory cells (*13*), and glia (*14*).

A $GABA_A$ receptor mediated these responses. The responses were blocked more than 90% by the $GABA_A$ receptor antagonists bicuculline and picrotoxin in each of four nerve terminals tested with each drug (Fig. 3, A and B). Muscimol, a $GABA_A$ receptor agonist, produced responses that were similar in shape but were 28 ± 8% larger ($n = 4$; $P < 0.005$ of zero difference by z statistic) than the responses of the same nerve endings to GABA (Fig. 3C). The benzodiazepine chlordiazepoxide enhanced GABA responses 27 ± 8% ($n = 8$; $P < 0.005$) (Fig. 3D). These drug actions resemble those at $GABA_A$ receptors of cell bodies. The $GABA_B$ receptor agonist baclofen (100 μM), on the other hand, did not change the holding current when applied to five different nerve endings (*15*). In these experiments, voltage was varied in 20-mV increments from −70 to 30 mV. Because the K^+ Nernst potential was −90 mV, these experiments would have detected $GABA_B$ receptors if they were coupled to a K^+ channel (*16*). Furthermore, 200 μM baclofen had no effect on the maximum amplitude and the voltage depen-

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dence of inactivation of A currents (7–9) in five nerve terminals (15). Finally, 100 μM baclofen had no effect on Ca^{2+} currents activated by voltage pulses from −80 to −10 mV in nine nerve terminals (17). Thus, modulation of Ca^{2+} channels by GABA, an effect found in sensory nerve cell bodies (18), is absent from peptidergic nerve terminals.

It is not known whether presynaptic inhibition results from the limitation of action potential propagation into nerve terminals or from a reduction in the amount of release induced by a single, propagated action potential (1). To answer this question, we examined the effect of GABA on the excitability of posterior pituitary nerve terminals. The action of GABA depends on the number of Cl^- channels that are gated and the intracellular Cl^- concentration. The intracellular Cl^- concentration sets the Cl^- Nernst potential and determines whether GABA depolarizes or hyperpolarizes the membrane of a nerve terminal. Whole cell recording imposes a specified Cl^- concentration on the cell interior and, therefore, cannot be used to determine the physiological driving force for Cl^-. We addressed this question by recording currents through single K^+ channels in cell-attached patches.

The membranes of pituitary nerve terminals have a high density of K^+ channels (7–9); three types of K^+ channels have been identified and characterized (9). Cell-attached patches often contain K^+ channels, the single-channel current amplitudes of which serve as an indicator of membrane potential. Application of GABA, either to the bath or locally from a pressure pipette, reduced the amplitude of K^+ channel currents (a Ca^{2+}-activated K^+ channel in this case) in cell-attached patches with 140 mM KCl in the patch pipette (Fig. 4A). The shift in the current-voltage plot of a single K^+ channel (Fig. 4C) indicates that GABA depolarized the nerve terminals. From the conductance γ of the single K^+ channel and the change in single-channel current Δi, we estimated the mean depolarization induced by GABA ($\Delta V = \Delta i/\gamma$) to be 14.3 ± 2.2 mV ($n$ = 7). Muscimol (50 μM) produced a depolarization of 13.7 ± 1.9 mV (n = 5), which was similar to that produced by GABA (P = 0.85) (Fig. 4B). In contrast, baclofen (100 μM) produced no change in the amplitude of currents through single K^+ channels in six recordings. Thus, the depolarization results exclusively from the activation of $GABA_A$ receptors.

Assuming a resting membrane potential of −60 mV (resting potentials were measured in current-clamp experiments and from reversal potentials of K^+ channels in cell-attached patches), we used the Goldmann-Hodgkin-Katz equation to calculate a Cl^- Nernst potential of about −43 mV and an intracellular Cl^- concentration of 25 mM. In whole cell current-clamp recordings with this concentration of Cl^- in the recording pipette, GABA produced an average depolarization of 15.9 ± 1.3 mV (n = 5), which was not significantly different (P = 0.5) from the depolarization estimated above from GABA reduction of K^+ channel amplitudes (Fig. 4D). A depolarization of this magnitude would explain why GABA blocks hormone release induced by electrical stimulation in endocrine cells but stimulates release in the absence of stimulation (19).

We subsequently used patch pipette solutions with 25 mM Cl^- to produce the physiological Cl^- driving force and examine how GABA-induced depolarizations influence action potentials under current clamp. The generation of action potentials by current injection was prevented by GABA, provided that the membrane potential was allowed to depolarize in response to GABA (Fig. 4D). However, when GABA was applied and the holding current was adjusted to restore the mem-

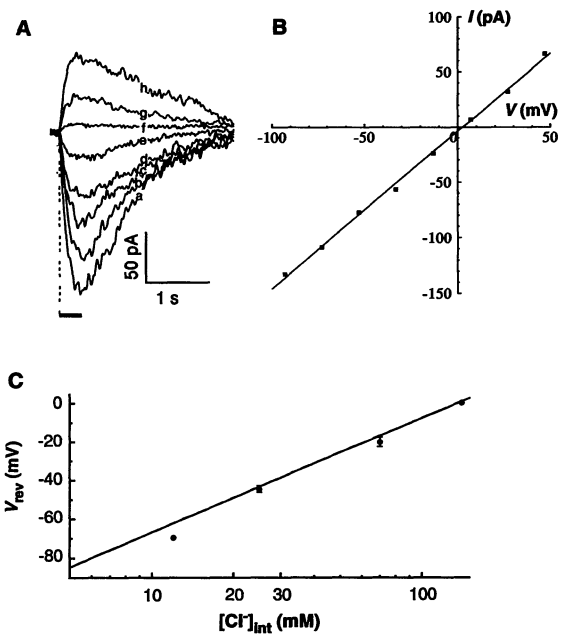

Fig. 1. (**A**) Whole cell recordings of GABA responses in a nerve terminal of the posterior pituitary. The holding potential was varied from −90 to 50 mV in 20-mV intervals (a to h) and 50 μM GABA was applied in pulses, indicated by solid bar, from a pressure pipette held near the nerve ending. The channel noise during the responses is consistent with the number of channels activated. (**B**) GABA-activated currents plotted as a function of membrane potential. The response reversed near 0 mV, which, for a recording pipette containing 140 mM CsCl, is consistent with a Cl^--selective channel. (**C**) Reversal potential of the GABA response plotted as a function of the natural logarithm of internal Cl^- concentration. The line drawn is the Nernst equation for Cl^-. Number of measurements for each concentration: 11 for 140 mM, 3 for 70 mM, 4 for 25 mM; and 5 for 12 mM. Error bars indicate SEM. For whole cell recordings, patch pipettes were filled with CsCl, KCl, or potassium gluconate (mixed in various proportions so that Cl^- and gluconate added up to 140 mM), 10 mM EGTA, 4 mM MgATP, and 10 mM Hepes (pH 7.3). Potassium gluconate and CsCl were mixed to give 12, 70, and 140 mM Cl^-. In the solution with 25 mM Cl^-, the other monovalent ion concentrations were 115 mM gluconate, 130 mM K^+, and 10 mM Na^+. Potentials were corrected for the liquid junction potential between the pipette and bathing solutions.

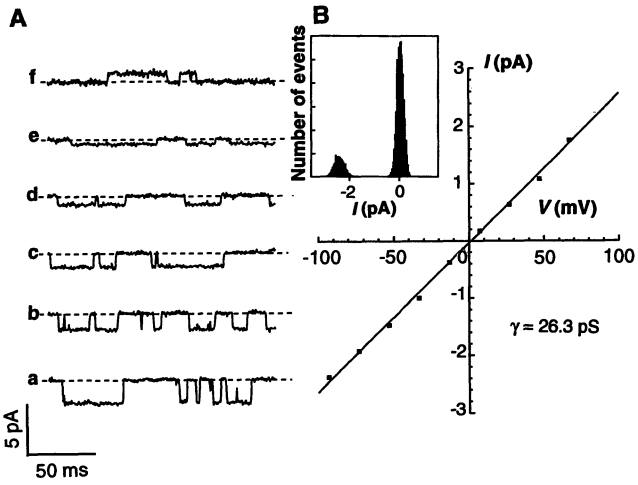

Fig. 2. (**A**) Currents through single GABA receptor channels in an excised outside-out patch. Holding potential varied from −90 to 30 mV at 20-mV intervals, +10 mV not included (a to f). Dashed lines indicate closed-channel level. Solutions were the same as in Fig. 1, A and B, with nearly symmetrical Cl^-. (**B**) Single-channel current varied linearly with membrane potential and reversed at 0 mV. (Inset) Amplitude distribution of 556 individual openings recorded at a membrane potential of −90 mV. Mean current at −90 mV was −2.4 pA in this particular experiment. Data were digitized at 2 kHz and filtered at 1 kHz.

brane potential to its resting value, action potentials were easily generated, and their width at half-height decreased only slightly for both isolated action potentials (2.6 ± 0.9% reduction in width at half-height; n = 4) and action potentials broadened by 20-Hz stimulation (7 ± 4%; n = 4) (these action potential duration experiments were done with 12 mM intracellular Cl^- to prevent depolarization and allow us to focus on the shunting action). The duration of a presynaptic action potential is a well-established determinant of transmitter secretion (*8*, *20*). Because the GABA-induced increase in membrane conductance does not make action potentials repolarize much more rapidly, it is not likely to alter the efficacy of an action potential in triggering secretion. On the other hand, when steady current was injected in the absence of GABA to produce depolarizations similar to those induced by GABA, action potential generation was prevented (Fig. 4E) in a manner similar to the accommodation of axons during slow depolarization (*21*). Depolarization suffices to block action potentials, and the most likely mechanism for this is Na^+-channel inactivation. Thus, by depolarizing the membrane, GABA can retard the propagation of action potentials into the thousands of swellings and nerve endings that emanate from a single hypothalamic peptidergic neuron (*22*).

GABA inhibits peptide release from the posterior pituitary (*23*); GABA-containing fibers densely innervate this structure (*24*). The pharmacological profile of inhibition suggests the involvement of a $GABA_A$ receptor (*23*). Although the $GABA_B$ receptor is generally regarded as the more prevalent GABA receptor type in nerve endings (*25*), many systems possess presynaptic $GABA_A$ receptors (*1*, *3*, *4*). The present characterization of the ion channels and pharmacological specificity of the $GABA_A$ receptor in posterior pituitary nerve terminals has narrowed the gap between our understanding of the mechanism of action of GABA at nerve terminals and nerve cell bodies. In nerve cell bodies, GABA activates a Cl^- channel to inhibit action potential generation. In the nerve terminals of the posterior pituitary, GABA activates a Cl^- channel to retard action potential propagation.

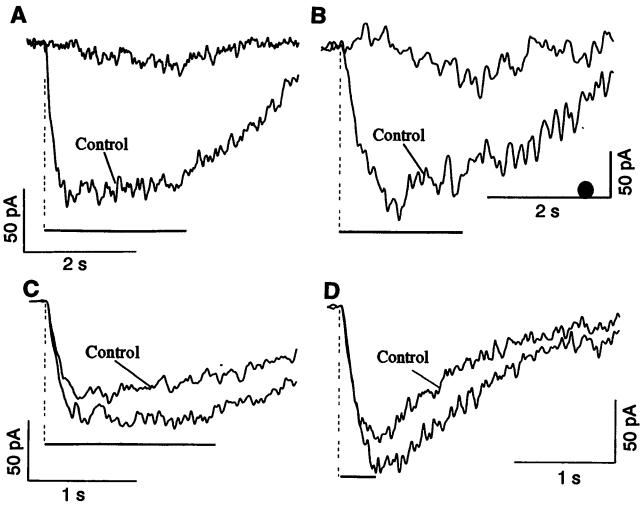

Fig. 3. Drug effects on GABA responses. Whole cell recordings were made as in Fig. 1. (**A**) Bicuculline (100 μM) and (**B**) picrotoxin (100 μM) blocked GABA responses (four nerve terminals each). (**C**) Muscimol (50 μM) produced a response similar to the GABA response but 28 ± 8% larger (n = 4). In these measurements, the concentration of GABA was 50 μM. (**D**) Chlordiazepoxide (20 μM), applied along with 40 μM GABA, produced a response 27 ± 8% larger than the response of the same nerve ending to the same concentration of GABA (n = 8). Bars indicate time of drug application from pressure pipettes.

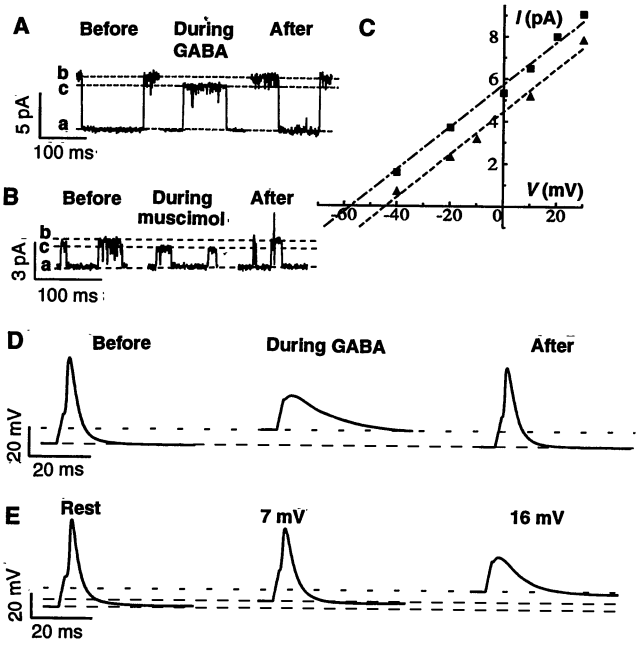

Fig. 4. (**A**) GABA application to a nerve terminal reduced the current through single K^+ channels in cell-attached patches by 1.09 ± 0.26 pA (n = 7). (**B**) Muscimol reduced single K^+ channel currents by 1.15 ± .06 pA (n = 5). The different amplitudes in (A) versus (B) reflect different K^+ channel types. (A) was from Ca^{2+}-activated K^+ channel [γ = 134 (*9*)]; the recordings in (B) were from delayed rectifier channel [γ = 27; for detailed characterization of K^+ channels of this preparation, see (*9*)]. Dashed lines indicate current through closed channel (a) and through open channel in absence (b) and presence (c) of 50 μM GABA or muscimol. (**C**) Amplitudes of single K^+ channels from (A) were plotted to give control (squares) and GABA (triangles) current-voltage curves. We saw similar depolarizations when we applied GABA either for several seconds continuously or in 500-ms pressure pulses (as in the recordings shown here). For these cell-attached experiments the patch electrodes were filled with 140 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes (pH 7.3). (**D**) We performed whole cell current-clamp experiments with patch electrodes filled with 25 mM KCl, 105 mM potassium gluconate, 10 mM sodium gluconate, 10 mM Hepes, 10 mM EGTA, and 4 mM MgATP (pH 7.3). With this filling solution and no injected current, GABA induced a depolarization of 15.9 ± 1.3 mV (n = 5). A 2-ms, 250-pA current pulse generated an action potential before GABA application but not while GABA was present. After GABA removal, the membrane potential recovered, and current injections once again generated action potentials. (**E**) Depolarization alone prevented action potential generation. The membrane was depolarized under current clamp by injection of steady positive current. Action potentials were generated with current pulses as in (D). A depolarization of 7 mV did not block action potentials, but a depolarization of 16 mV did. Similar results were obtained in five terminals for the experiments of both (D) and (E).

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10. The posterior pituitary was taken from male rats 3 to 5 weeks old. Slices 80 μm thick were cut in chilled artificial cerebrospinal fluid (125 mM NaCl, 4 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2

mM $CaCl_2$, 1 mM $MgCl_2$) bubbled with a mixture of 95% O_2 and 5% CO_2. For experiments, slices were bathed in the same solution. Patch-clamp recordings were made [O. P. Hamill *et al.*, *Pfluegers Arch.* **391**, 85 (1981)] from swellings ranging from 5 to 15 μm, which were clearly visible under Nomarski optics at ×600. Pipette-filling solutions for whole cell recordings are given in the figure legends. Additional methodological details can be found in (*8*) and (*9*).
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TECHNICAL COMMENTS

Polyethylene Bags and Solar Ultraviolet Radiation

In their study of the effects of solar ultraviolet (UV) radiation on natural phytoplankton assemblages in Antarctic waters, R. C. Smith *et al.* (*1*) used Whirlpak polyethylene bags as used by Z. Z. El-Sayed *et al.* (*2*) as sample containers during their in situ incubations. On a cruise in the Pacific from Chile to California, in March and April 1992, we placed replicate aliquots of water samples, taken from a depth of 5 to 10 m, in Whirlpak bags (18 ounces) and in round quartz glassware vessels (250 ml). Samples were then placed in deck incubators with the temperature controlled by flowing surface seawater and were exposed to direct solar radiation. Some samples were covered with plastic filters for 6 to 8 hours, centered at local noon. Rates of photosynthesis were determined by standard radiocarbon techniques (*3*); chlorophyll a concentrations were determined by fluorometry after extraction in methanol (*4*).

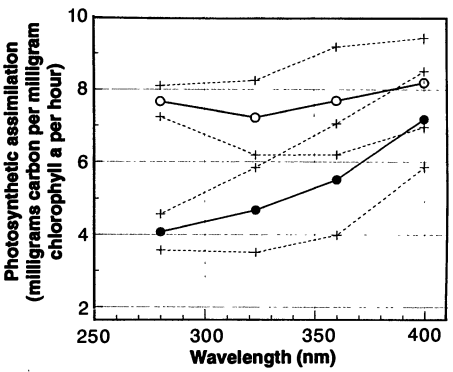

Fig. 1. Rates of phytoplankton photosynthetic assimilation in samples in glassware (open circles) compared with those in polyethylene bags (closed circles), as determined by analysis of variance tests. Some of the samples were covered with mylar (50% transmission at 323 nm), some with Plexiglas (50% transmission at 360 nm), and others with Plexiglas (UF3) (50% transmission at 400 nm). Circles represent the mean value for each treatment (n = 46 paired samples with no prefilter, 15 paired samples with UV radiation cut off at 323 nm, 15 paired samples with UV radiation cut off at 360 nm, and 38 paired samples with UV radiation cut off at 400 nm). Dashed lines indicate one standard deviation around the mean.

We found a significant difference ($P < 0.001$) in photosynthetic assimilation rates for samples in glassware as opposed to bags when the samples were exposed to solar radiation without any filter and when they were covered by mylar, which absorbs ultraviolet B (UVB) radiation (280 to 320 nm) (Fig. 1). This inhibitory effect was not decreased by leaching the bags in 1 N HCl for 12 hours. The results of our transmission tests agree with those in (*2*), which showed only 68% transmission of UVB at 300 nm. It is apparent that polyethylene bags absorb UVB, which results in a toxicity that significantly lowers the rate of CO_2 assimilation.

In spite of artifacts associated with the use of polyethylene bags, the general conclusions reached by Z. Z. El-Sayed *et al.* (*2*) and by Smith *et al.* (*1*) are similar to our results (*3*, *5*), which were obtained with glassware. There does not appear to be a temperature dependence associated with the toxicity produced by polyethylene bags, as similar results were obtained in Antarctic and in tropical waters. We do not know, however, whether different taxonomic groups of organisms would react in the same way as that noted in our experiments. It would therefore seem advisable for those studying the effects of solar UV radiation on microbial populations to first ascertain the validity of measurements made with the use of polyethylene bags.

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Response: Clean techniques and control studies (*1–3*) were performed before, during, and after the Icecolors '90 cruise. We checked and found no evidence for polyethylene bag contamination or toxicity.

Before the cruise, laboratory cultures and field samples of mixed phytoplankton communities were incubated in 250- or 500-ml untreated Whirlpak bags for different periods (up to 8 hours) while being exposed to darkness or to light-saturating fluorescence that had passed through a glass plate [photosynthetically available radiation (PAR) only]. When compared with replicate samples in wide-bottom glass Erlemeyer flasks, we found no decrease in volumetric production rates (mg C/m^3/hour) and no toxic effect.

At sea, we observed that extended (up to 14 hours) exposure to low amounts of UVB or UVA radiation, or both, had no effect on the in situ primary production