EGF and PDBu binding has been reported to correlate generally with their activity in promoting mouse skin carcinogenesis (*9, 12*). However, even though debromoaplysiatoxin did not increase MCA-initiated cell transformation, this polyacetate derivative markedly inhibited the binding of EGF and PDBu to cell receptors. There was an apparent dissociation between the activity of debromoaplysiatoxin in binding or modulating receptors and its activity in increasing MCA-induced cell transformation. We earlier reported a similar observation, namely that dihydroteleocidin B was 100 times more effective than TPA in increasing MCA-initiated transformation of BALB/3T3 cells, whereas dihydroteleocidin B and TPA were equally effective in inhibiting the binding of EGF and PDBu to cell receptors (*5*). However, TPA has strong activity in stimulating DNA synthesis in arrested cells, whereas debromoaplysiatoxin has no activity at all. This suggests that the cause of the inability of debromoaplysiatoxin to increase transformation is different from that of TPA. There may be qualitative differences in the binding of aplysiatoxin and debromoaplysiatoxin to receptors. The aplysiatoxin-debromoaplysiatoxin pair provides clues important for elucidating the factors (or steps) involved in tumor promotion and the functional role of the hydrophilic region of promoters.

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References and Notes

1. T. Sugimura, *Gann* **73**, 499 (1982).
2. H. Fujiki, M. Suganuma, M. Nakayasu, H. Hoshino, R. E. Moore, T. Sugimura, *ibid.*, p. 495.
3. L. Diamond, T. G. O'Brien, W. M. Baird, in *Advances in Cancer Research*, G. Klein and S. Weinhouse, Eds. (Academic Press, New York, 1980), p. 1.
4. T. Kakunaga and J. D. Crow, *Science* **209**, 505 (1980).
5. T. Hirakawa, T. Kakunaga, H. Fujiki, T. Sugimura, *ibid.* **216**, 527 (1982).
6. T. Kakunaga, *Int. J. Cancer* **12**, 463 (1973).
7. K. Umezawa, B. Weinstein, A. Horowitz, H. Fujiki, T. Matsushima, T. Sugimura, *Nature (London)* **290**, 411 (1981).
8. L.-S. Lee and I. B. Weinstein, *Science* **202**, 313 (1978).
9. M. Shoyab, J. E. DeLarco, G. J. Todaro, *Nature (London)* **279**, 387 (1979).
10. S. H. Yuspa *et al.*, *ibid.* **262**, 402 (1976).
11. A. D. Horowitz *et al.*, *Cancer Res.* **43**, 1529 (1983).
12. M. Shoyab and G. J. Todaro, *Nature (London)* **288**, 451 (1980).
13. T. Kakunaga, *Int. J. Cancer*, **14**, 736 (1974).

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Nitrous Oxide Production in Nearshore Marine Sediments

Abstract. *Coastal marine sediments are shown to be a net source of nitrous oxide. The rates of nitrous oxide flux from sediments in Narragansett Bay, Rhode Island, ranged from 20 to more than 900 nanomoles per square meter per hour. Sediments from a eutrophic area had higher rates of net nitrous oxide production than sediments from relatively unpolluted sites. The benthic nitrous oxide source exceeds the nitrous oxide source to the bay from sewage treatment plant effluent.*

Recognition of the role of N_2O in the destruction of stratospheric O_3 (*1*) and in the radiative heat budget of the troposphere (*2*) has led to increased interest in the sources, sinks, and biogeochemical cycles of this trace gas. Anthropogenic increases in the global production rate of N_2O have been predicted as a consequence of increasing use of nitrogen fertilizer (*3*) and the combustion of fossil fuels (*4, 5*). The marine environment plays a major part in the global cycling of N_2O, with sources identified in surface ocean waters (*6–9*) and sinks in low-oxygen environments (*7–10*). Although it has been suggested that marine sediments may be net sinks or sources of N_2O (*8, 11*), as far as we know, no direct measurements of the N_2O flux to or from marine sediments have been made. We summarize here the results of measurements of N_2O fluxes from nearshore marine sediments taken from Narragansett Bay, Rhode Island, over an annual cycle, demonstrate that N_2O production per unit area is higher from polluted sediments than from relatively unpolluted sediments, assess the importance of benthic N_2O production relative to other terms in the nitrogen budget for the bay, and compare benthic N_2O production to estimated N_2O inputs to the bay from sewage treatment plant (STP) effluent.

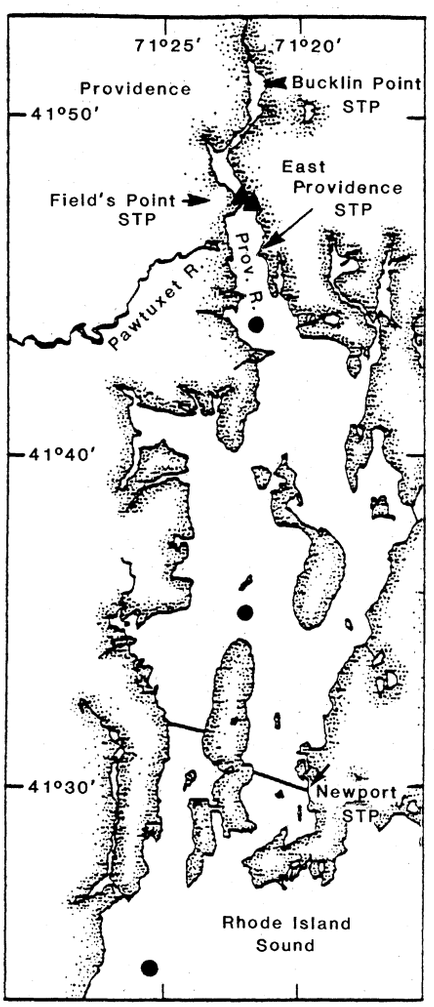

Fig. 1. Sediment (●) and water (▲) sampling stations in Narragansett Bay. The arrows indicate the points of discharge by four major sewage treatment plants (STP). Field's Point STP serves 200,000 people, Bucklin Point STP 95,700, East Providence STP 42,000, and Newport STP 39,000.

Sediment cores were collected between July 1978 and November 1979 by scuba divers from three stations in the Narragansett Bay area (Fig. 1). These stations lie along gradients in salinity, water depth, sediment organic content, silt-clay content, O_2 consumption, NH_4^+ production, pore-water H_2S concentration, and dominant macrofauna (Table 1). The cores were incubated in gastight chambers (*12*). The N_2O was measured in the water-equilibrated gas phase over the cores by electron-capture-detection gas chromatography (*12*), and N_2O production was calculated from the difference in the concentration of N_2O in sequential samples taken during the first 7 hours after the water was changed over a core. Sampling intervals were generally from 1 to 3 hours. Two to five flux measurements were made on each core.

There was a net flux of N_2O from the sediments in all cores from all three stations (Fig. 2), ranging from 19 nmole m^{-2} $hour^{-1}$ at the mid-Bay station to more than 900 nmole m^{-2} $hour^{-1}$ at the Providence River station, located close to numerous sources of sewage pollution. The N_2O production for these two stations showed an approximately exponential increase with increasing temperature. This temperature relation was more pronounced in the Providence River sediments (Q_{10} = 9.4) than in the mid-Bay sediments (Q_{10} = 2.3) (Q_{10} is the increase in the rate of chemical reaction for each 10°C increase in temperature). For the two experiments in which Rhode

Table 1. Some characteristics of the three stations at which sediment cores were collected for N_2O flux measurements; N.D., not detectable.

Station	Salinity* (per mil) (*26*)	Water depth (m)	Silt-clay (%) (*26*)	Organic carbon (%) (*26*)	Pore-water H_2S, 0 to 5 cm (μM) (*27*)	Benthic NH_4^+ flux† (μmole nitrogen m^{-2} $hour^{-1}$) (*18, 26, 28*)	Benthic O_2 consumption (mg m^{-2} $hour^{-1}$) (*26*)	Dominant microfauna (*26*)
Providence River	27.5 to 30.5	7	84	4.5	~ 500	300 to 700	70	*Mulina lateralis*
Mid-Bay	30 to 32	8	82	2	N.D.	30 to 350	38	*Nucula annulata*
Rhode Island Sound	32	30	49	1		40 to 225	28	*Nucula annulata*

*Range of bottom-water values. †Summer values.

Island Sound sediments were used, the N_2O production was similar to that from the mid-Bay sediments (Fig. 2). The N_2O production from the Providence River sediments, for a given temperature, was from 1.5 to over 10 times that of the relatively unpolluted sediments from the other two stations.

Nitrous oxide can be produced during bacterial nitrification (*13, 14*) and can be both produced (*15*) and consumed (*16*) during denitrification. Since both nitrification and denitrification are active processes in the sediments at all three stations (*12, 17, 18*), the production of N_2O is likely to be the result of all three of these pathways.

Several factors may be responsible for the higher N_2O production in the Providence River sediments relative to the other two stations. (i) The rate of metabolism in these sediments is greater, as evidenced by their higher O_2 consumption and NH_4^+ fluxes (Table 1). (ii) High H_2S concentrations in these sediments (Table 1) have been shown to inhibit N_2O reduction during denitrification (*19*). With rates of denitrification (N_2 production) at the Providence River station being more than an order of magnitude greater than N_2O production (*12*), even a 10 percent inhibition of N_2O reduction by denitrifying bacteria due to high H_2S concentrations could produce the observed N_2O fluxes at this station. (iii) Higher N_2O fluxes could also be related to nitrification. Goreau *et al.* (*14*), using cultures of the marine nitrifying bacteria *Nitrosomonas*, showed that the yield of N_2O per mole of NH_4^+ oxidized increased from 0.25 percent at high O_2 (~ 20 percent) concentrations to nearly 10 percent at low O_2 (0.5 percent) concentrations. Such an increased yield of N_2O would be favored in the organic-rich, low-O_2 Providence River sediments.

The benthic production of N_2O is small relative to fluxes of other forms of nitrogen across the sediment-water interface. For example, summer NH_4^+ production rates are high for sediments from the Providence River station but low for sediments from the other two stations (Table 1). The net N_2O production rates (in micromoles per square meter per hour) in July at these stations are only a fraction of these values, 0.56 to 0.92 at Providence River, 0.08 to 0.18 at mid-Bay, and 0.03 to 0.04 at Rhode Island Sound. Benthic N_2O production is a negligible sink for fixed nitrogen in Narragansett Bay as a whole, since the average input of fixed nitrogen from rivers and sewage is approximately 200 μmole m^{-2} $hour^{-1}$ (*20*).

To our knowledge, this is the first direct demonstration that coastal marine sediments are a source of N_2O. McElroy *et al.* (*9*) identified chlorinated STP effluent as well as nitrification in the water column as sources of N_2O in the Potomac River estuary. In order to estimate N_2O sources to the Narragansett Bay area associated with chlorinated STP effluent, we measured the N_2O concentration of water at the outfall pipe in Field's Point, the major STP discharging into the bay, in September 1978 (*21*). The N_2O concentration of the effluent was ~ 925 nM (*22*) or about 109 times the air-saturation value. This value is similar to the N_2O concentration reported for chlorinated effluent from a Michigan STP, which was 108 times the saturation value (*23*).

We estimate that the magnitude of the STP N_2O source to the Narragansett Bay area is ~ 17 mole $hour^{-1}$, assuming that the N_2O concentration of all chlorinated STP effluent entering the bay is similar to that of Field's Point (*24*). In the Narragansett Bay system as a whole, benthic N_2O production (37 mole $hour^{-1}$) is about twice the estimated STP N_2O input (17 mole $hour^{-1}$). Even in areas of high sewage input, such as the Providence River, benthic N_2O production (17 mole $hour^{-1}$) is approximately equal to the STP N_2O input (15 mole $hour^{-1}$) (*25*).

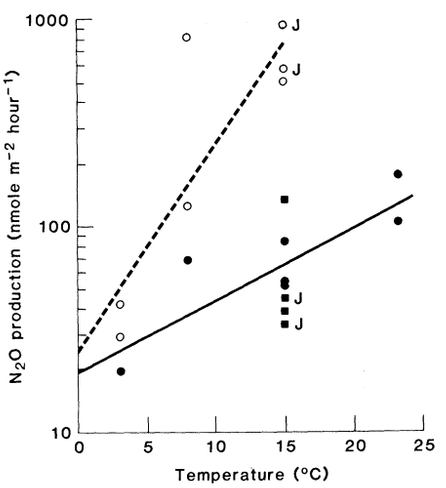

Fig. 2. The N_2O production from Narragansett Bay sediment cores collected between July 1978 and November 1979 from three stations: Providence River (○), mid-Bay (●), and Rhode Island Sound (■). Incubation temperatures were as follows: March 1978, 3°C; July 1978, 23°C; July 1979, 15°C; October 1979, 15°C; and November 1979, 8°C. Each point represents the mean rate from one core. The standard deviations of replicate measurements from a single core ranged from 1 to ~ 50 percent of the mean flux. For the least-squares regression lines (the logarithm of N_2O flux versus temperature), r (correlation coefficient) = 0.85 for Providence River data (dashed line) and r = 0.85 for mid-Bay data (solid line); J refers to cores collected in July 1969. No production of N_2O was detected in water incubated without sediments.

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References and Notes

1. P. J. Crutzen, *Q. J. R. Meteorol. Soc.* **96**, 320 (1970); J. Hahn and P. J. Crutzen, *Philos. Trans. R. Soc. London Ser. B* **296**, 521 (1982).
2. W. C. Wang, Y. L. Yung, A. A. Lacis, T. Mo, J. E. Hansen, *Science* **194**, 685 (1976).
3. P. J. Crutzen, *Ambio* **3**, 201 (1974); *Geophys. Res. Lett.* **3**, 169 (1976); N. D. Sze and J. Rice, *ibid.*, p. 343; S. C. Liu, R. J. Cicerone, T. M. Donahue, W. L. Chameides, *Tellus* **29**, 251 (1977).
4. D. Pierotti and R. A. Rasmussen, *Geophys. Res. Lett.* **3**, 265 (1976).
5. R. F. Weiss and H. Craig, *ibid.*, p. 751; R. F. Weiss, *J. Geophys. Res.* **86**, 7185 (1981).
6. J. Hahn and C. Junge, *Z. Naturforsch.* **32**, 190

(1977); C. Junge, B. Bockholt, K. Schutz, R. Beck, *Meteor Forschungsergeb.* **6**, 1 (1971); T. Yoshinari, *Mar. Chem.* **4**, 189 (1976).
7. Y. Cohen and L. I. Gordon, *Deep-Sea Res.* **25**, 509 (1978).
8. J. W. Elkins, S. C. Wofsy, M. B. McElroy, C. E. Kolb, W. A. Kaplan, *Nature (London)* **275**, 602 (1978).
9. M. B. McElroy *et al.*, *Limnol. Oceanogr.* **23**, 1168 (1978).
10. Y. Cohen, *Nature (London)* **272**, 235 (1978).
11. J. Sorensen, *Appl. Environ. Microbiol.* **36**, 809 (1978).
12. S. P. Seitzinger, S. W. Nixon, M. E. Q. Pilson, S. Burke, *Geochim. Cosmochim. Acta* **44**, 1853 (1980); S. P. Seitzinger, S. W. Nixon, M. E. Q. Pilson, *Limnol. Oceanogr.*, in press.
13. A. M. Blackmer, J. M. Bremner, E. L. Schmidt, *Appl. Environ. Microbiol.* **40**, 1060 (1980); G. A. F. Ritchie and D. J. D. Nicholas, *Biochem. J.* **126**, 1181 (1972); T. Yoshida and M. Alexander, *Soil Sci. Soc. Am. Proc.* **34**, 880 (1970).
14. T. J. Goreau *et al.*, *Appl. Environ. Microbiol.* **40**, 526 (1980).
15. E. P. Greenberg and G. E. Becker, *Can. J. Microbiol.* **23**, 903 (1977).
16. J. M. Barbaree and W. J. Payne, *J. Mar. Biol.* **1**, 136 (1967).
17. S. P. Seitzinger, thesis, University of Rhode Island (1982).
18. S. W. Nixon, C. A. Oviatt, S. Hale, in *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*, J. M. Anderson and A. Macfadyen, Eds. (Blackwell, London, 1975), pp. 269–283; H. Elderfield, N. Luedtke, R. J. McCaffrey, M. Bender, *Am. J. Sci.* **281**, 768 (1981).
19. J. Sorensen, J. M. Tiedje, R. B. Firestone, *Appl. Environ. Microbiol.* **39**, 105 (1980); T. Y. Tam and R. Knowles, *Can. J. Microbiol.* **25**, 1133 (1979).
20. S. W. Nixon, in *Estuaries and Nutrients*, B. Nielson and L. E. Cronin, Eds. (Humana, Williamsburg, Va., 1981), pp. 111–138.
21. Water samples were collected on 28 September 1978 with a Niskin bottle; the temperature and salinity were measured, and 84 ml of water was drained through a Tygon tube into a 125-ml serum bottle containing 1.0 ml of 160 m*M* $HgCl_2$. No differences were seen between $HgCl_2$-treated samples and untreated samples. The bottles were then capped with serum-bottle stoppers. The samples were brought to room temperature in the laboratory and then equilibrated by shaking by hand for 2 minutes. Duplicate 1.5-ml samples of the water-equilibrated gas phase were analyzed for N_2O as described by Seitzinger *et al.* (*12*). The water temperature was measured within 5 minutes after sampling. We calculated the total N_2O in the bottles and the N_2O concentrations of the water samples, using the N_2O solubility equation of R. Weiss and B. A. Price for the appropriate temperature and salinity [*Mar. Chem.* **8**, 347 (1980)]. We calculated the original percent saturation of the samples by assuming a dry-air mixing ratio for N_2O of 301 parts per billion (ppb). We based this on data (*5*) showing that the tropospheric N_2O concentration is increasing at a rate of ~ 0.2 percent per year and that the dry-air mole fraction as of 1 January 1978 in the Northern Hemisphere was 300.2 ppb. An N_2O mixing ratio of 301.25 ppb was calculated for 1 October 1979.
22. The Field's Point sample was taken directly above the outfall pipe, which was approximately 2 m below the water surface. The salinity of that sample was 18 per mil and the N_2O concentration was 295 n*M*. It is known that some seawater enters the Field's Point STP; although the salinity of the effluent was not known precisely, we estimated it to be 4 per mil on the basis of information supplied by the plant engineer. The effluent was assumed to mix with river water of 24 per mil and 25 n*M* N_2O, as measured in a surface-water sample taken about 200 m from the outfall pipe. Using this information, we calculated that the effluent concentration was ~ 925 n*M*.
23. R. J. Cicerone, J. D. Shetter, S. C. Liu, *Geophys. Res. Lett.* **5**, 173 (1978).
24. The STP discharge to the Providence River area includes Field's Point (11×10^3 m^3 $hour^{-1}$), Bucklin Point (4×10^3 m^3 $hour^{-1}$), and East Providence (1.2×10^3 m^3 $hour^{-1}$). The only STP discharge of any consequence to Narragansett Bay is at Newport with an input of 1.7×10^3 m^3 $hour^{-1}$ (Rhode Island Department of Environmental Management 1979 files). The calculated Field's Point N_2O concentration (925 n*M*) (*22*) was used as an estimate to calculate N_2O input from all treatment plants.
25. Area of Providence River is 2.4×10^7 m^2 (U.S. Department of Commerce, National Ocean Survey Chart 13224); the N_2O production for September, assuming that the Providence River station is typical of all of river is ~ 700 nmole m^{-2} $hour^{-1}$ (see Fig. 2); area of Narragansett Bay is 24.37×10^7 m^2 [J. N. Kremer and S. W. Nixon, *A Coastal Marine Ecosystem* (Springer, Berlin, 1978)]; the N_2O production for September is ~ 80 nmole m^{-2} $hour^{-1}$, assuming that the mid-Bay station is representative of the rest of the bay area.
26. "Marine Ecosystems Research Laboratory Annual Report, 1980" (University of Rhode Island, Kingston, 1981).
27. H. Elderfield, R. J. McCaffrey, N. Leudtke, M. Bender, V. W. Truesdale, *Am. J. Sci.* **281**, 1021 (1981).
28. J. Garber, thesis, University of Rhode Island (1982); J. R. Kelly, thesis, University of Rhode Island (1982).
29. This research was supported by NSF grants OCE 76-22149 and OCE 78-27124. We thank S. Burke, who assisted in the field and with laboratory analyses.
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Ethanol Modulation of Opiate Receptors in Cultured Neural Cells

Abstract. *The mouse neuroblastoma–rat glioma hybrid cell line NG108-15 was used to study the acute and chronic interaction of ethanol with intact neural cells. In the short term, ethanol inhibited opiate receptor binding, but after long-term exposure the cells exhibited an apparent adaptive increase in the number of opiate binding sites; this was reversible when ethanol was withdrawn. High concentrations of ethanol (200 m*M*) increased opiate binding after 18 to 24 hours, whereas lower concentrations (25 to 50 m*M*) produced similar changes after 2 weeks. This model system has potential for exploring the cellular and molecular mechanisms underlying ethanol intoxication, tolerance, and withdrawal.*

Ethanol intoxication produces well-known behavioral and neurological effects. Tolerance develops to many actions of ethanol, and physical dependence follows chronic ethanol abuse. These phenomena may derive from the physical interaction of ethanol with neural membranes and the adaptation of these membranes to the continued presence of ethanol (*1–3*). Short-term ethanol exposure can disorder membrane lipids (*2*). After long-term ethanol administration, the membranes may exhibit several possibly adaptive alterations: (i) resistance to further disordering by ethanol; (ii) occasionally increased membrane order; and (iii) modifications in cholesterol, phospholipid, or fatty acid composition (*3*). Since these changes in membrane properties can alter the function of critical membrane proteins (*4*), many investigators have begun to characterize changes in neuroreceptors and related enzymes resulting from ethanol exposure (*5–9*). Biochemical studies in animals have been limited because of the heterogeneity of brain and the need to use disruptive tissue preparations. We have begun to use cells in culture as a rich source of intact and homogeneous neural tissue (*10*). We now report that in the mouse neuroblastoma–rat glioma hybrid cell line NG108-15, short- and long-term exposure to ethanol causes striking modifications in opiate binding in ways that may reflect the membrane events associated with intoxication, tolerance, and withdrawal.

Table 1. Response of opiate receptors to 4 days of ethanol treatment. The experiment was repeated four or five times, and means and standard errors (S.E.M.) were calculated. Long-term ethanol treatment did not significantly affect K_d ($F = 0.32$). The increase in B_{max} was tested by paired t-tests.

Ethanol concentration (m*M*)	B_{max} (Ethanol: control)	P	K_d (n*M*)
0			1.60 ± 0.09
100	1.40 ± 0.04	< 0.002	1.77 ± 0.09
200	1.85 ± 0.21	< 0.02	1.70 ± 0.21

The NG108-15 cells of passage 18 to 25 were cultured as previously described (*11*). When medium containing ethanol was added to cells, all flasks were kept tightly capped and the medium was changed daily. The concentration of ethanol in the medium, measured by gas-liquid chromatography, remained unchanged after 24 hours of incubation with confluent cells. Opiate receptor binding assays (*12*) were performed by incubating at 37°C a suspension of washed whole cells with tritiated methionine enkephalinamide ([^{3}H]MEA) (39.5 to 50.5 Ci/mm) (*11*). Specific binding, the difference between counts per minute in the presence or absence of 10^{-5} *M* naloxone, was normalized according to cell number (femtomoles per 10^6 cells). The standard deviation of triplicate samples ranged from 5 to 10 percent. The maximum number of binding sites (B_{max}) and receptor affinity (K_d) were calculated by linear regression analysis according to the method of Scatchard.

When ethanol was added to suspended whole cells immediately before the addi-