tinental shelf southeast of Nantucket Island suggest that at least part of the texturally correlated ice limit (Fig. 1) is a result of the late Wisconsinan glacial advance. This conclusion also is in support of the hypothesis (*20*) of an extensive Laurentide ice sheet that may have extended to Georges Bank during the late Wisconsinan.

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War Gases as Olfactory Probes

Abstract. *The tear gas ethyl bromoacetate is a fruity-smelling alkylating agent that blocks the ability of the frog nose to respond to esters and a variety of other odorants, but leaves sensitivity to amines unimpaired. Lachrymators and chemical warfare agents of other functional types such as sulfides (mustard gas) and amines (nitrogen mustards) may have similarly specific actions that will enable their use as chemical probes of the sense of smell.*

The initial step in odor recognition by the nose is the momentary binding of odorant molecules to receptor sites embedded in the dendritic membranes of olfactory receptor cells. The interaction between odorant molecules and at least some receptor sites must be highly specific, because humans and animals can discriminate odorants with a high degree of chemical similarity, for example between optical isomers of the same compound (*1*). Even single olfactory receptor cells respond differently to molecules as similar as ethyl and methyl acetate (*2*), propanol and butanol (*3*), or stereoisomers of the same complex alcohol molecule (*4*).

The physiological function and biochemistry of receptor proteins is most effectively studied when specific and irreversible inhibitors are available for use as chemical probes, for example, when α-bungarotoxin is used to inhibit and tag acetylcholine receptors (*5*). Getchell and Gesteland (*6*) pioneered studies of olfactory receptors using the group-specific sulfhydryl reagent *N*-ethylmaleimide (NEM). The NEM blocked the electrical responses of the frog olfactory epithelium to a variety of odors, presumably by inactivating receptor proteins through attack on sulfhydryl groups near the odorant binding site. When the fruity-smelling ester ethyl-*n*-butyrate was applied before and during NEM treatment, responses to it and to similar esters were maintained and those to dissimilar odorants abolished (*7*).

We adopted Getchell and Gesteland's approach, but searched for reagents that would not require application in liquid solution and would not require the simultaneous application of other chemicals to protect specific receptor sites from the effects of the inhibitor. Vaporous inhibitors are preferable for olfactory studies since application of any liquid agent to the olfactory mucosa impairs responses to odors for a time and prevents monitoring the progressive effect of the inhibitor. We used volatile alkylating agents from a novel source—obsolete war gases of pre-World War II vintage. The agent most studied to date, ethyl bromoacetate, inhibits olfactory responses in a specific and unexpected way.

More than 50 different chemical agents were tested for antipersonnel use during World War I (*8*). Many are alkylating agents that attack proteins by forming covalent bonds with sulfhydryl and amino groups (*9*). We sought chemical agents with distinctive odor qualities associated with the functional groups they contain, expecting that such agents would more selectively attack receptors for those specific odor qualities (*10*). Ethyl bromoacetate (EBA) was chosen for our first tests because it is a fast-acting tear gas with a high vapor pressure and an identifiable fruity odor. We reasoned that a fruity-smelling alkylating agent should preferentially bind to receptors for fruity-smelling compounds and deactivate them, leaving responses to other classes of odorants unaffected. Haloacetates such as EBA have been widely used for modification of enzymes and other proteins. They are much more reactive to sulfhydryl groups than amino groups (*11*), and in this regard are similar to NEM.

Electroolfactogram (EOG) potentials of the frog (*Rana pipiens*) olfactory epithelium were recorded with an automatic, programmable stimulating and recording system (*12*). The system delivered 0.5-second test pulses of a reference odorant, isoamyl acetate, at 100-second intervals. Frogs were immobilized with curare, and EOG responses were detected with Ag-AgCl Ringer-agar-filled glass electrodes in contact with surface of the surgically exposed olfactory mucosa (*13*).

The EOG response recorded at the epithelial surface is a mass potential or summated d-c voltage change composed of many individual receptor potentials produced by tens to hundreds of olfactory neurons in the vicinity of the recording electrode (*14*). Single 0.5-second pulses of a variety of odorants—including alcohols, aldehydes, amines, esters, ketones, organic acids, and sulfides—generally produced EOG amplitudes of 2 to 6 mV. A typical experiment included a 40- to 60-minute period of stimulation with the reference odorant (0.5-second test pulses at 100-second intervals) until a stable baseline (amplitude variation of

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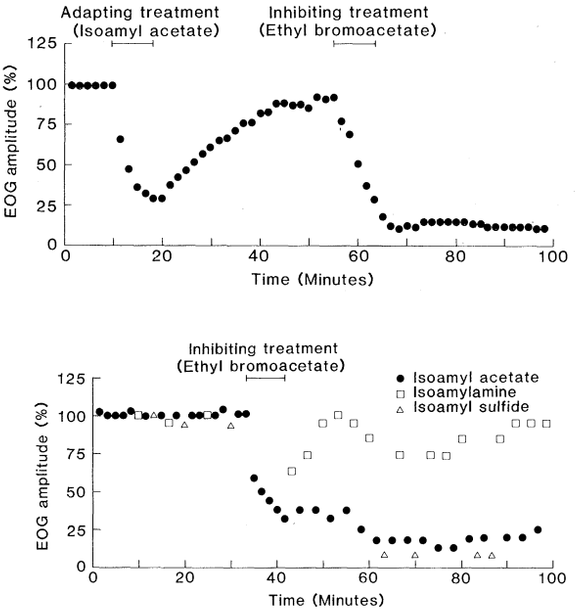

Fig. 1 (top). Adaptation to isoamyl acetate and inhibition by ethyl bromoacetate. Circles represent the magnitude of EOG responses to 0.5-second pulses of the reference odorant, isoamyl acetate, given at 100-second intervals. Adaptation: Six 18-second pulses of the reference odorant depressed the EOG response. Recovery followed when the adapting treatment with isoamyl acetate ended. Inhibition: Six 18-second pulses of ethyl bromoacetate, an alkylating agent, irreversibly inhibited the EOG response to the reference odorant. Fig. 2 (bottom). Retention of the amine response. Treatment with ethyl bromoacetate inhibited responses to isoamyl acetate and to isoamyl sulfide. Responses to isoamylamine were only slightly inhibited.

± 5 percent) was achieved. Test pulses with other odors before EBA treatment were delivered at 200- to 500-second intervals in place of the reference odorant. The inhibiting treatment was applied as a series of 18-second pulses of EBA delivered at 100-second intervals at 1200 ml/min from a reservoir containing air saturated with the reagent.

Six 18-second pulses of EBA reduced EOG responses to the reference odorant, isoamyl acetate, to approximately 10 percent of the pretreatment magnitude (Fig. 1). This reduction in EOG magnitude was true inhibition, not simple sensory adaptation, because responses to the reference odorant did not return even when the recovery period was extended to 10 hours. Each pulse of EBA during the inhibitory treatment was preceded by a test pulse of the reference odorant given 2 seconds before the EBA pulse. In this way, the progressive increase in inhibition produced by successive pulses of EBA was monitored (Fig. 1).

Responses to other odorants such as isoamyl alcohol and isoamyl sulfide were similarly inhibited. However, responses to certain amines were an exception. Ethyl bromoacetate did not inhibit responses to isoamylamine, diethylamine, and similar aliphatic amines (Fig. 2). Receptor sites for these amines are thus resistant to the alkylating agent, EBA. Further treatment with EBA will reduce the amine response, but resistance to inhibition is striking. A level of EBA treatment which completely abolishes responses to all other odorant classes also reduces isoamylamine responses to approximately 50 percent of pretreatment levels, but this amine response is sustained for hours without diminution and without the slightest recovery of responses to non-amines.

We conclude that olfactory neurons contain specific amine receptors that account for the ability of the nose to distinguish the fishy-smelling quality of these amines from other odorants such as the fruity-smelling reference odorant, isoamyl acetate. Amine receptors resist inhibition either because the interaction of EBA with the active binding site is limited by a poor steric fit or by competing reactions, or because this site contains no sulfhydryl or amino groups for EBA to attack.

Protection from inhibition was obtained when isoamyl acetate was simultaneously applied during EBA treatment. In fact, isoamyl acetate is a universal protector because it prevented inhibition of responses to all other odorant classes as well. However, simultaneous application of other, non-ester odorants during EBA treatment produced neither specific nor general protection. Isoamyl sulfide, for example, did not protect responses to itself or to any other odorant. This is a surprise and a disappointment, because specific protection could be used as a powerful tool to isolate the responses to other classes of odorants. Nonprotection by isoamyl sulfide and other non-esters may be explained by the fact that the reaction of haloacetates with sulfhydryl groups can be either suppressed or enhanced by the presence of substrates, allosteric effectors, or other specifically interacting substances (*11*).

Other war gases may have unique inhibitory effects related to their odors. For example, mustard gas [bis(2-chloroethyl)sulfide] has a characteristic sulfide odor reminiscent of garlic, whereas nitrogen mustard (mechloroethamine) has a distinctly fishy odor. The arsines are a particularly interesting group, judging by comments on their odors from the literature: "like geranium" (vinylchloroarsine), "fruity" (ethylarsinedichloride), "mixture of garlic and cyanic acid" (diphenylcyanarsine), "onion-like" (phenylcarbylamine chloride), and "characteristic odor" (chlorpicrin) (*8*). Although the term war gas is something of a misnomer—most were dispersed on the battlefield as aerosols—virtually all these compounds have sufficient vapor pressures to be applied to the nose as vapors. They represent a powerful new tool for the study of olfaction.

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