Energy Transduction: Inhibition of Cockroach Feeding by Naphthoquinone

Abstract. *1,4-Naphthoquinones inhibit feeding of* Periplaneta americana *by complexing with sulfhydryl groups of receptor protein in sensory neurons, by oxidizing the sulfhydryl groups, and by being reduced.*

We (*1–3*) have established the role of certain quinols and quinones as stimulants or inhibitors of gustation and olfaction by certain insects. Quinones behave chemically like alpha-beta unsaturated ketones, and may react with sulfhydryl groups by forming complexes with them (*4*), oxidizing them, or both (*5*); such reactions may change the conformation of macromolecules (*6*). Thus, quinol-quinone systems apparently have the requisites for an energy exchange with sulfhydryl groups of proteins in receptor macromolecules in living systems but in no case (*5*) has a natural biological action of a quinone been proven to operate by way of a specific mechanism. We now report that the inhibition of feeding by *Periplaneta americana* by 1,4-naphthoquinones involves an exchange of energy between the quinone and sulfhydryl groups of receptor macromolecules during formation of a complex with, and oxidation of, the receptor sulfhydryl groups and reduction of the quinone.

In studying possible relationships between chemical structure and relative inhibition of *P. americana* feeding by 1,4-naphthoquinones by means of the bioassay method detailed by Norris *et al.* (*3*), we found that the mean weight loss per assay tablet, relative to that in the positive control, was 20.4 percent with 5-hydroxy-1,4-naphthoquinone; 23.1 percent with 1,4-naphthoquinone; 31.3 percent with 2-hydroxy-1,4-naphthoquinone; and 50.7 percent with 2-methyl-1,4-naphthoquinone. The order of relative inhibition of cockroach feeding among these naphthoquinones was the same as that for *Scolytus multistriatus* feeding (*2*). Such an agreement between changes in chemical structure and changes in gustatory inhibition in two very different insect species strongly suggests a mechanism of energy transduction in the inhibition of feeding.

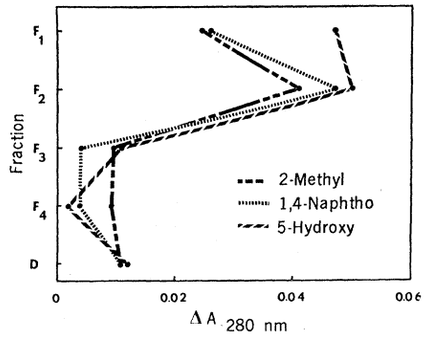

Fig. 1. Differential absorbance at zero time at 280 nm ($-\Delta A_{280}$) upon addition of a given 1,4-naphthoquinone (1.7×10^{-7} mole/liter) to sample of an antennal fraction or the decantate (*D*) standardized to produce an optical density of 0.2 units.

Studies (*3*) of the effects upon the chemical stimulation or inhibition of feeding of partially or wholly removing the antennae or maxillae (or both) of *P. americana* indicated that chemoreceptors on the antennae are especially important. Our (*7*) high-resolution autoradiographic studies of the penetration of radiolabeled quinol stimulants and quinone inhibitors into chemoreceptor sensilla on the antennae of both *P. americana* and *S. multistriatus* have shown that these chemicals penetrate into the lumen of such sensilla by way of pores through the cuticle, and that the labeled chemicals complex especially with membranes of dendritic branches of sensory neurons in the sensilla.

We added 5-hydroxy-1,4-naphthoquinone, 1,4-naphthoquinone, or 2-methyl-1,4-naphthoquinone to optically standardized samples of particulate fractions (F_1 to F_4) of a homogenate of adult male cockroach antennae separated by density gradient centrifugation to determine whether the quinones especially complexed with those fractions which were rich in fragments of nerve membranes. The interaction of each naphthoquinone (1.7×10^{-7} mole/liter) with each sample caused a change in ultraviolet absorbance at 280 nm, an indication of the formation of complexes. The greater changes in absorbance occurred with fractions F_1 and F_2, which are especially rich in fragments of nerve membranes (*3*). The order of relative binding (that is, complexing) among the three naphthoquinones with F_1 and F_2 (Fig. 1) was the same as the order of their relative inhibition of cockroach feeding.

To investigate further the binding affinity of naphthoquinone for particulate material in the antennal homogenate, we incubated 0.1 μc of 2-[^{14}C]methyl-1,4-naphthoquinone with the crude homogenate or antennae for 15 minutes at room temperature. This mixture was then centrifuged at 20,000*g*, and the pellet obtained was resuspended in 0.25*M* sucrose and again centrifuged three times to remove radioactivity not bound to particulate matter. Samples of each wash were analyzed for radioactivity. The final pellet obtained was fractionated on the sucrose-density gradient as described previously. Samples of each fraction were placed in 10 ml of methyl cellusolve–based counting solution and counted for radioactivity in a Packard 3380 liquid scintillation counter. Quenching was monitored by an external standard. Counts per minute per milligram of protein in each fraction were as follows: F_1, 7742; F_2, 8089; F_3, 5716; and F_4, 4489. Thus, the fractions rich in nerve membranes, F_1 and F_2, contained much greater amounts of bound radiolabeled naphthoquinone than F_3 or F_4 did.

To intensify any reaction between $10^{-3}M$ 1,4-naphthoquinone and a sample of fraction F_2 so that reaction products might be better detected by spectrophotometry, we froze a mixture of the two. A visible change in color indicative (*6*) of energy transfer occurred upon freezing the mixture. The preparation was then thawed and immediately analyzed spectrophotometrically.

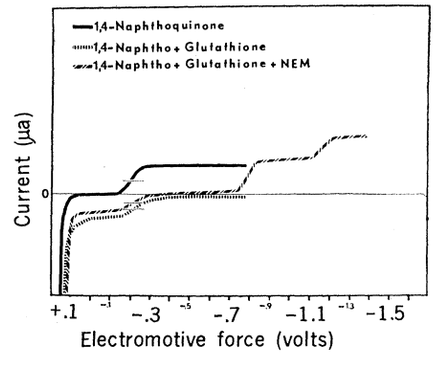

Fig. 2. Polarograms of unsubstituted 1,4-naphthoquinone; 1,4-naphthoquinone and reduced glutathione; and 1,4-naphthoquinone, reduced glutathione, and NEM, at 20 μa, full-scale sensitivity, and a damping setting of 4. All concentrations of tested chemicals were 5×10^{-4} mole/liter; the buffer used was 75 ml of phosphate (*p*H 7.0) and 25 ml of 95 percent ethanol. Note the small shift in half-wave potential of the wave for the 1,4-naphthoquinone when mixed with glutathione or glutathione and NEM.

The interaction of $10^{-3}M$ 1,4-naphthoquinone and F_2 produced new absorbance caused by the corresponding quinol formed upon reduction of 1,4-naphthoquinone by the receptor chemical.

When a sulfhydryl group inhibitor [$10^{-3}M$ *N*-ethyl maleimide (NEM)] was added to either the above mixture or a mixture of the quinone and $10^{-3}M$ reduced glutathione, a change in color occurred upon freezing; however, it was different from that observed in the mixture of quinone and F_2 or glutathione. The known specificity of NEM for sulfhydryl groups would indicate that the naphthoquinones react at sulfhydryl groups on the receptor chemical in F_2 and on reduced glutathione.

In the absence of adequate quantities of purified receptor chemical from F_1 and F_2, complexing between reduced glutathione and 1,4-naphthoquinone, with or without NEM, was further investigated by means of dropping-mercury-electrode polarography. The test solutions were polarographed in 75 ml of deoxygenated 0.1*M* phosphate buffer (*p*H 7.0) and 25 ml of 95 percent ethanol. Changes in the diffusion current of the wave for 1,4-naphthoquinone (Fig. 2) when mixed with reduced glutathione, compared to when analyzed alone, indicated that complexing of these chemicals occurred. In the presence of glutathione, an anodic wave for 1,4-naphthoquinone appeared (Fig. 2), whereas the quinone alone yielded only a cathodic wave. This indicated that 1,4-naphthoquinone had been reduced during its interaction with the glutathione. Upon addition of NEM to the mixture of 1,4-naphthoquinone and glutathione, the diffusion current for the 1,4-naphthoquinone wave fell between that obtained when the quinone was alone and that when it was combined with just glutathione. This would indicate that the addition of NEM reduced the amount of 1,4-naphthoquinone which was complexed with the glutathione. Thus, there was competition between 1,4-naphthoquinone and NEM for sulfhydryl groups on the reduced glutathione.

The findings from our various evaluations of the several naphthoquinones would support the hypothesis that their type of reaction with sulfhydryl groups of F_1 and F_2 is involved in the transduction of energy between the quinone and the sensory neuron. Our findings reveal a naturally operative mechanism for changing the conformation of a receptor macromolecule in nerve membrane. Farah *et al.* (*8*) presented evidence that such a change in conformation of macromolecules in membranes results in an altered flow of inorganic ions through the membrane; and Cole (*9*) discussed the evidence that such altered ion flow may lead to the generation of the electrical potential necessary to fire the neuron.

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Slow Synaptic Excitation in Sympathetic Ganglion Cells: Evidence for Synaptic Inactivation of Potassium Conductance

Abstract. *The slow excitatory postsynaptic potential (EPSP) was investigated in frog sympathetic ganglion cells. In contrast to the increased conductance associated with other known EPSP's, during the slow EPSP resting membrane conductance was decreased. Electrical depolarization of the membrane potentiated the slow EPSP, whereas progressive hyperpolarization decreased its size and then reversed it to a hyperpolarizing potential (the opposite of the effect of membrane polarization on other EPSP's). The reversal potential of the slow EPSP was close to the potassium equilibrium potential. We propose that the slow EPSP, in contrast to classical EPSP's, is generated by an inactivation of resting potassium conductance.*

Synaptic excitation results from an increased ion conductance of postsynaptic membrane at virtually all chemically transmitting junctions that have been investigated (*1*). During such typical excitatory postsynaptic potentials (EPSP's), membrane resistance is decreased. The equilibrium potential for the increased ion conductances involved is in the depolarizing direction so that electrical hyperpolarization of the membrane increases the EPSP, whereas progressive depolarization decreases and then reverses the EPSP to a hyperpolarizing potential. The slow EPSP of frog sympathetic ganglion cells has recently been reported to differ from typical EPSP's in that the membrane resistance is increased during the slow EPSP and hyperpolarizing current reverses the slow EPSP to a hyperpolarizing potential (*2*). We have investigated the mechanism of this slow synaptic excitation; we now report evidence consistent with the hypothesis that the slow EPSP is generated by a decrease of resting potassium conductance.

The tenth lumbar sympathetic ganglion of the bullfrog (*Rana catesbeiana*) was studied in vitro with continuous perfusion of oxygenated Ringer solution (*3*). Intracellular records were taken from sympathetic ganglion cells with 15- to 25-megohm microelectrodes filled with 3*M* KCl. Standard techniques of electrophysiological recording were used, current being injected through the recording microelectrode by means of a Wheatstone bridge (*4*). Preganglionic B fibers were stimulated in the sympathetic chain between the sixth and seventh ganglia (*5*). Type B ganglion cells were identified on the basis of antidromic conduction velocity (*6*).

The fast EPSP in frog sympathetic ganglion cells, like most EPSP's, is due to increased ion conductance (*7–9*). Hyperpolarization of the membrane increases the fast EPSP, and depolarization decreases its size (Fig. 1A); fur-