AFFINITIES OF CERTAIN QUINONE REPELLENTS FOR DETERGENT-SOLUBILIZED PROTEINS FROM PERIPLANETA AMERICANA ANTENNAE

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Summary

Certain quinones bound with high affinity to a receptor isolated in a detergent-soluble protein fraction from P. americana antennae. The relative affinities of 4 of these quinones were the same as their relative repellent potencies and degrees of induced polarographic half-wave potential shifts in the same proteins. Two other quinones also had high affinities for the receptor, but did not cause shifts in the half-wave potential, nor did they repel the insects. Explanations for the high affinities of certain quinones for the receptor in the absence of behavioral repellency are given.

Two genetically distinct strains of P. americana, LAB and WARF, exhibited markedly different in vivo sensitivities to certain naphthoquinones as determined in bioassays for chemical repellency (1). Radiolabelled menadione as well as other SH reagents bound selectively to one protein band which was resolved from a crude detergent extract of antennal proteins and was electrophoretically indistinguishable between each strain (2). By direct competition experiments against (^{14}C)-menadione, we now have demonstrated that the binding constants between different naphthoquinones and this protein band are in the same order as their repellent potency, the most repellent having the highest affinity constant. However, two quinones which do not significantly repel but do bind with high association constants also were found. Finally, no differences were seen between the LAB and WARF proteins insofar as their affinity constants or binding capacities for quinones are concerned.

Methods

Proteins were extracted separately from the two strains of cockroaches. Two saline (33 ml of:0.9% NaCl; 0.01 M potassium phosphate buffer, pH 7),

followed by one detergent (7.5 ml of: 0.9% NaCl; 0.6% Triton X-100; 0.01 M potassium phosphate buffer, pH 7) extractions were made from 75 pairs of antennae (3).

Competitive binding demonstrations

The detergent extract from each 75 pairs of antennae was separated into ten 0.75 ml aliquots. To each aliquot was added enough 2-methyl (^{14}C)-1,4-naphthoquinone (menadione), mixed with unlabelled menadione in methanol, to yield a final concentration of 5 x 10^{-4}M, the saturation level for this ligand. Increasing concentrations of one of five naphthoquinones or p-benzoquinone were added simultaneously as competing ligands. In each experiment, aliquots were saturated with menadione in the absence of competitor, and these served as controls. Experiments were normalized using such controls as a standard. Each experimental sample was next incubated at room temperature for 2 hr and then subjected to electrophoresis as previously described (3).

Nonspecific binding demonstrations

Nonspecific binding was determined from the naphthoquinone binding capacity of aliquots of a detergent extract from similarly treated cockroach heads. Two sets of 5 heads were extracted twice each in 33 ml of saline. The pellets were then combined and extracted in 7.5 ml of the detergent solution. The final extract yielded a protein concentration similar to that obtained from the antennal detergent preparation (3). The previously described labelling and competition experiments were then replicated with aliquots from the detergent-soluble proteins from the heads. Ligand bound to a protein band from heads which is electrophoretically similar to the one which contains the naphthoquinone receptor in antennae was subtracted as nonspecific binding from the total amount bound to that band in antennal aliquots.

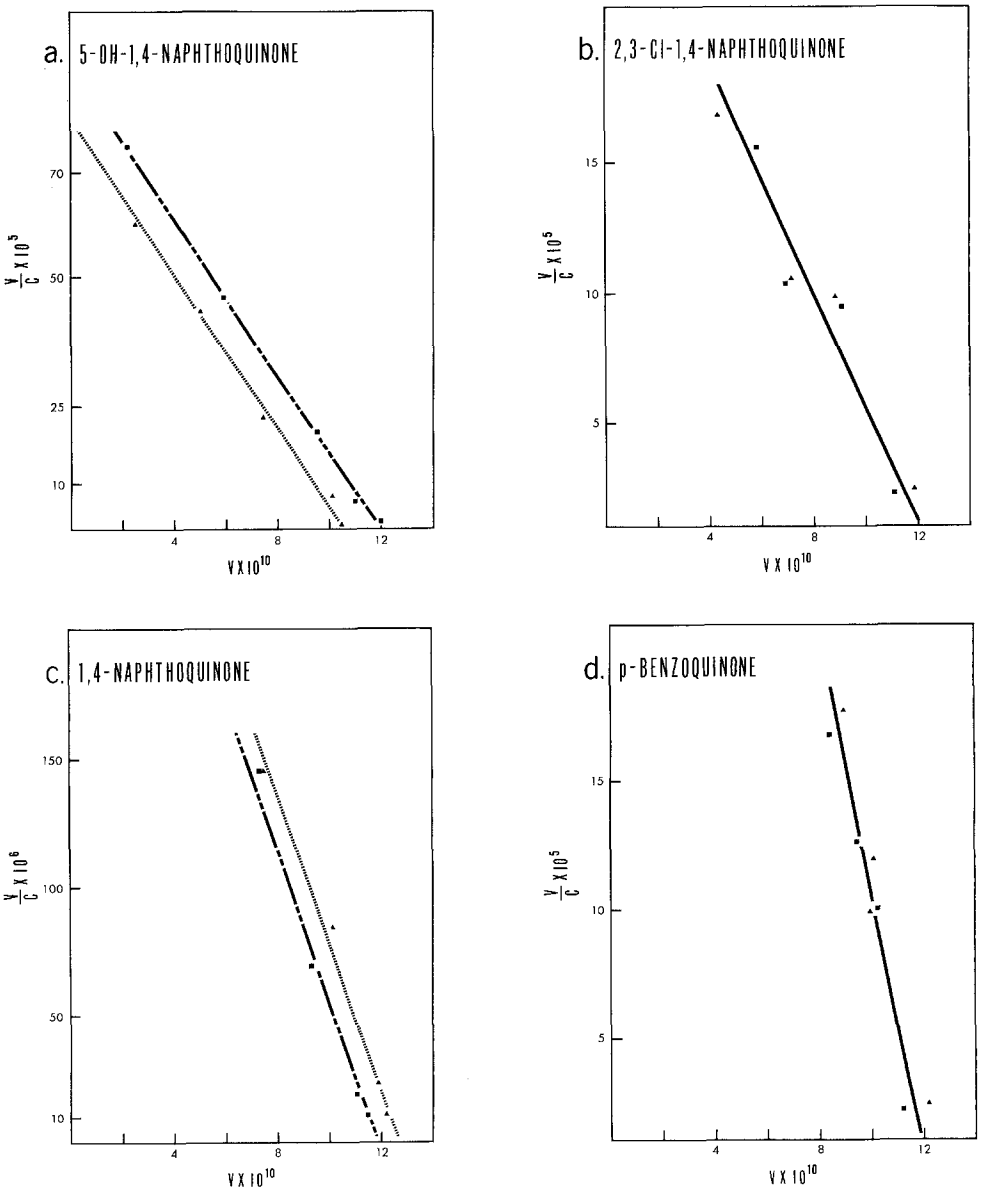
Equilibrium dialysis assays

Equilibrium dialysis assays for (^{14}C)-menadione binding to receptor and the competition against such binding brought by increasing concentrations of 5-hydroxy-1,4-naphthoquinone (juglone) were run. One ml of protein extract was placed inside a dialysis sac. The sac was put in a flask containing 24 ml of the detergent solution to which had been added enough naphthoquinone to yield a desired final concentration in 25 ml. Buffer exchanges were accomplished by previous dialysis for 45 min against each of 3 changes of a 100-fold volume excess of the new buffer. These buffers were either the saline without Triton, or the Tris/glycine buffer used in the electrophoresis reservoirs. Upon insertion of the dialysis sac, the flask was flooded with nitrogen, sealed, and allowed to equilibrate at 4°C for 24 hr. Aliquots of 0.25 ml were then taken from each sac and from each external solution, put into counting vials to which scintillation fluid was added, and counted for radioactivity. All data were plotted according to Scatchard (4).

Results

Incubating the receptor-containing fraction from either the LAB or WARF strain of cockroaches with increasing concentrations of radiolabelled menadione yielded a saturation value of $1.3 \pm 0.3 \times 10^{-3}$ umol/0.25 ml of protein extract. Considering that the protein band which contains the receptor represents approximately 13.7 ug of protein (5), this yielded a

value of 9.5 $\pm$ 0.3 X 10^{-5} umol/ug of receptor-containing band. This value compared favorably with the value of 5.8 X 10^{-5} umol/ug of receptor-containing band obtained from the Scatchard plots of data from the equilibrium dialysis assay (Fig 2), and 6.3 X 10^{-5} umol/ug from similar plots of data from the electrophoresis experiments (Fig 1).



Discussion

These values, though in good agreement, are approximations because the receptor protein which specifically binds naphthoquinones is yet impure. Similar constraints are appropriate in considering the binding capacities for all tested quinones. Numerical differences in the binding capacity of the receptor-containing band from the two strains for a given quinone are probably artifacts introduced in the extracting or aliquoting steps. Nonspecific binding amounted to a maximum of 7%.

The association constants obtained from slopes of the plots show that there is no significant difference in this parameter per quinone between the LAB and WARF strains (Table 1). Among the tested repellent quinones,

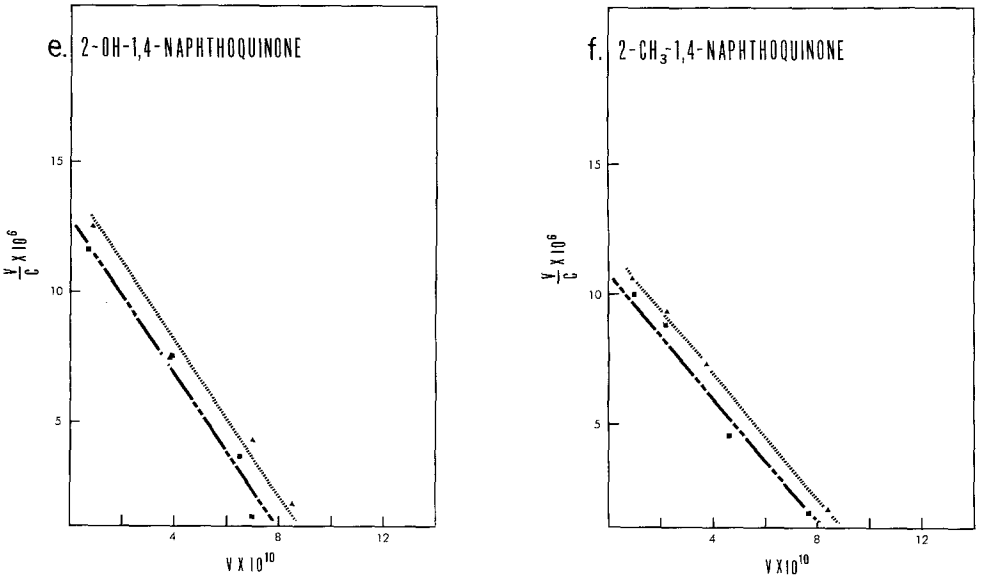


Figure 1. Scatchard plots of the competition of (^{14}C)-menadione binding to antennal proteins brought by increasing concentrations of: a) 5-hydroxy-1,4-naphthoquinone; b) 2,3-dichloro-1,4-naphthoquinone; c) 1,4-naphthoquinone; d) p-benzoquinone; e) 2-hydroxy-1,4-naphthoquinone; and f) 2-methyl-1,4-naphthoquinone. ■ LAB; ▲ WARF.

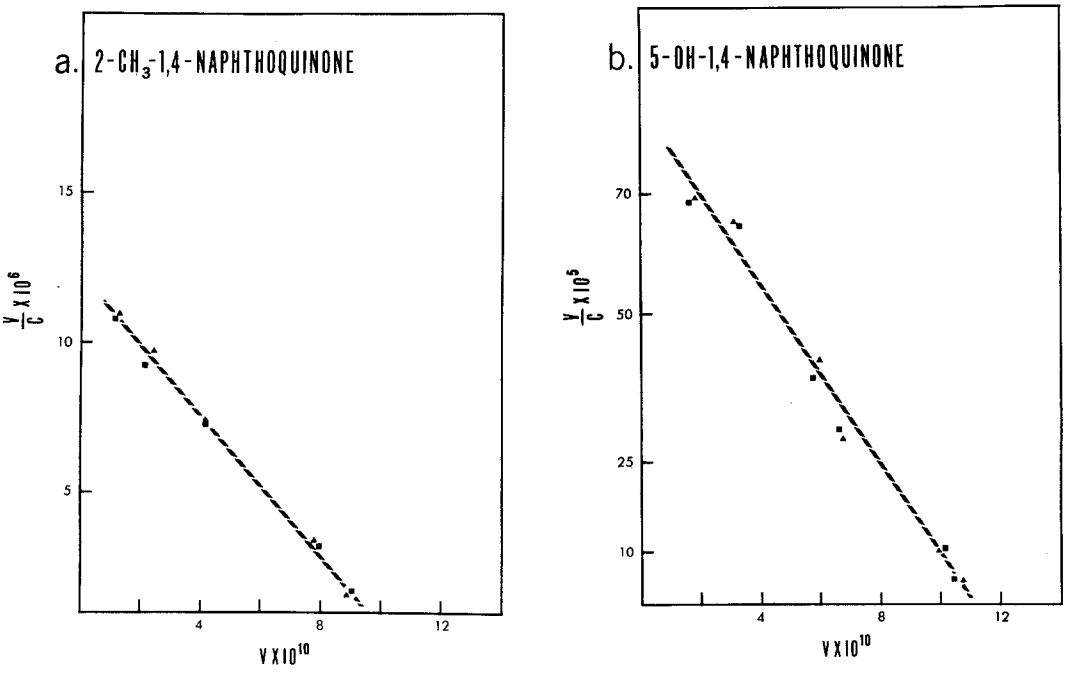


Figure 2. Scatchard plots of equilibrium dialysis assay data for: a) (^{14}C)-menadione binding to antennal proteins; and b) the competition of (^{14}C)-menadione binding to antennal proteins brought by increasing concentrations of 5-hydroxy-1,4-naphthoquinone. ■LAB; ▲WARF.

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the order of the association constants was: 5-hydroxy-1,4-naphthoquinone > 1,4-naphthoquinone > 2-hydroxy-1,4-naphthoquinone > 2-methyl-1,4-naphthoquinone (Table 1). This is the same order as for their relative behavioral effects as measured in bioassays for chemical repellency (1), and for the relative magnitudes of their induced shifts in the polarographic half-wave potential (E 1/2) of the receptor protein (5). The considerable affinities of 2,3-dichloro-1,4-naphthoquinone and p-benzoquinone for the binding site in the receptor (Table 1; Fig 1), and yet their lack of significant behavioral effects (repellency) or shifts in receptor E 1/2 are significant findings in terms of elucidation of the more complete chemoreception mechanism.

TABLE 1

Affinity constants of various quinones for the receptor-containing detergent-solubilized extract from adult male P. americana antennae.

Quinone	LAB	WARF
5-OH	7.1×10^5	7.3×10^5
2,3-Cl	6.25×10^5	6.25×10^5
1,4	3.13×10^5	3.04×10^5
p-BQ	2.5×10^5	2.5×10^5
2-OH	1.49×10^4	1.5×10^4
$2\text{-}CH_3$	1.27×10^4	1.3×10^4

The lack of repellent activity and receptor E 1/2 shift by dichloro napthoquinone and benzoquinone, even though these quinones bind comparably to repellent quinones with the protein, indicates that the receptor conformational transition, as measured by the E 1/2 shift, is essential to the chemoreception process. A reasonable explanation of why benzoquinone does not cause a conformational change is its relatively high redox potential. Thus, benzoquinone would tend to remain in the oxidized state after addition to the protein, and so lack important hydrogen bonding capability afforded by hydroxyl groups. In dichloro naphthoquinone, one chlorine is displaced from the quinoid ring after protein binding. The remaining chlorine would act as an electron withdrawing group and likewise tend to stabilize the complexed quinone in the oxidized state. Norris (6) stated that quinone repellent activity was highly correlated with redox potential and secondarily influenced by hydrogen bonding capabilities. A criterion for naphthoquinone behavioral activity thus may be further defined. In order to elicit a behavioral effect, the quinone must induce a conformational

change in the protein. The extent of the conformational change that occurs is related to the relative repellency of the quinones. The repellent quinone must ultimately be reduced after binding so it can interact via hydrogen bonds with some other part of the protein. It is thus possible that the actual binding of a naphthoquinone to a protein may be incidental to its elicited behavioral activity.

Exchanging the buffer to either a saline without Triton or to Tris/glycine had no effect on the binding of menadione. Some energy supplying (e.g., ATPase) or a reducing (e.g., NADH) system probably functions in the membrane to reverse the complex. In fact, a Na^{+}-K^{+}-Mg^{++}-ATPase was found to co-electrophorese in the same band as the receptor (7) and may exist in association with it in the antenna. Naphthoquinones have been found to exert at least two types of effects on the chemosensory system of P. americana, one consisting of a rather irreversible component and another being composed of reversible effects (8). Those naphthoquinones which can cause significant irreversible effects cause them in the same order as their relative inhibitions of ouabain-sensitive ATPase (9).

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