Facilitation in Arthropod Photoreceptors

Dear Sir,

A paradoxical effect of light has been observed in some invertebrate photoreceptors: an increased amplitude of electrophysiological responses to test flashes following illumination by a step or flash of light. This apparent increase in photoreceptor sensitivity is known as facilitation, and is in contrast to the better known case of reduced sensitivity following illumination (light adaptation). Facilitation was first reported in 1954 by Ruck and Jahn for isopod crustaceans. Subsequently, facilitation has been reported for: arthropods of two other classes (spiders—DeVoe, 1972; insects—Giulio and Lucaroni, 1967; Ventura and Puglia, 1977); the barnacle (Stratten and Ogden, 1971; Shaw, 1972); and cephalopod mollusks (Hartline and Lange, 1974). Facilitation has never been reported for vertebrate photoreceptors, although the effect of light in temporarily reducing sensitivity has been intensively investigated.

A paper by Hanani and Hillman (1976) reported verification of facilitation in barnacle lateral photoreceptors, and proposed that facilitation might be understood by processes which are opposite (see below), but related, to those believed to be involved in light adaptation. From the results of experiments in which the conditioning (i.e. facilitation-inducing) light intensities and extracellular calcium concentrations were varied, Hanani and Hillman explained that facilitation might be due to a decreased intracellular calcium concentration, or to a decreased activation affinity of ion channel-blocking sites.

I should like to call attention to the fact that the temporary changes found in photoreceptor cells after termination of illumination involve not only sensitivity but also the time course of the electrophysiological response. It is well known, at least for arthropod photoreceptors, that the time scale of responses recorded from light-shaped photoreceptors is compressed (and the latency of response is decreased) when compared to dark-adapted responses (Fuortes and Hodgkin, 1964; Fein and Charlton, 1975). If processes opposite to those involved in light adaptation were indeed the basis of facilitation, that is decreased intracellular calcium ion concentration or reduced affinities of ion-channel blocking sites, then an expansion of the time scale (i.e. increased response latencies) would be expected together with increased amplitude. Hanani and Hillman did not report latency data in their paper.

Ventura and Puglia (1977), however, performed a careful parametric study of facilitation and ant photoreceptors, but their records showed no systematic differences in time scale between facilitated and unfacilitated electrophysiological responses. This finding relates directly to Hanani and Hillman's membrane-ionic hypothesis. There is good evidence that illumination results in an increased intracellular calcium concentration (Brown and Blinks, 1974), and that this calcium may have a dual effect on the electrophysiological response: there are sensitivity losses (Lisman and Brown, 1975a, b; Fein and Lisman, 1975) and a decreased latency of the receptor potential (Brown and Lisman, 1975). Although facilitation meets one requirement to be a case opposite to light adaptation (response amplitude increments), from Ventura and Puglia's work it seems not to meet the other (an increase in time scale of response). Latency measurements which are correlated with response amplitude measurements are crucial to the test of a
facilitation hypothesis involving changes in calcium concentration and membrane binding sites.

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Local Anesthetics: Do Benzocaine and Lidocaine Act at the Same Single Site?

Dear Sir:

Hille (1977) in a recent examination of whether amine local anesthetics (such as lidocaine) and a neutral analog (benzocaine) act by common or by different mechanisms has assumed that, "as a first approximation, all inhibitory actions of the drugs are attributed to binding to a single receptor" in the membrane. The one support for this idea of a single receptor was his finding that benzocaine produces the same kind of large negative voltage shift of the sodium inactivation curve as does lidocaine. However, this support becomes somewhat weakened by the demonstration of Shrivastav et al. (1976) that the volatile general anesthetic trichloroethylene produces a similar shift in the steady-state inactivation curve, unless the volatile general anesthetics also act at this same single site. The present experiments show that the hypothesis of a single site of action for all local anesthetic drugs, charged and neutral, is inadequate.

The pharmacological principle involved is simple: if solutions of two drugs known to act at the time receptor site are chosen in concentrations that each separately produce the same equilibrium response (binding to site, block of sodium current, block of action potential, etc.), then any mixture of the two solutions must also produce that same equilibrium response. If they do not produce the same response (at equilibrium), they cannot be acting at the identical site. The virtue of this approach is that it is essentially a null-point method that does not depend on the linearity of the system. The proof is relatively simple. Consider the reaction, in unit volume, of either of two drugs (in concentrations A and B) with the sodium channel. The concentration of the latter in the drug-free, "open", conducting state is O (in Hille's notation). Because the channel can exist at equilibrium in three drug-free states (O, I, R), the total amount of drug-free receptor (conducting + inactivated + resting) is γ·O (where γ is constant under the conditions of the experiment, being greater than one and determined by voltage-dependent rate constants). Similarly, if the concentrations of the drug-receptor complex in the open but non-conducting, drug-modified, state (O· in Hille's notation) are (OA) and (OB), respectively, the total amounts in the drug-complexed states are α·(OA) and β·(OB), respectively (α and β each being greater than one and being determined by voltage-dependent, rate constants). If the concentrations A and B are equiactive when applied separately, it is easy to show that the fraction of the receptor pool in the drug-free conducting state O, ρ₀, which will be the same in both cases, is given by

\[ \rho_0 = \left(\gamma + \alpha \cdot A / K_a\right)^{-1} = \left(\gamma + \beta \cdot B / K_b\right)^{-1}, \]

where \( K_a \) and \( K_b \) are the equilibrium dissociation constants of the reactions \( O + A \rightleftharpoons (OA) \) and \( O + B \rightleftharpoons (OB) \), respectively. What happens when the solutions are mixed in the proportion \( a:1-a \), i.e., the two drugs are present together in respective concentrations \( aA \) and \( (1-a)B \)? Again it is easy to show that the fraction in state O, i.e., \( \rho'_0 \), is given by

\[ \rho'_0 = \left(\gamma + a\alpha \cdot A / K_a + (1-a) \beta \cdot B / K_b\right)^{-1}. \]

But from Eq. 1, \( \alpha \cdot A / K_a = \beta \cdot B / K_b \), so that Eq. 2 becomes \( \rho'_0 = \rho_0 \); i.e., the number of free conducting sodium channels when the preparation is bathed in the mixture is the same as when in either of the two separate solutions. A more general treatment of the case of \( m \) drugs acting at a site with \( n \) different states \( (n = 3 \) in Hille's model) by Dr. David Colquhoun (personal communication) leads to the same conclusion.
Desheathed frog sciatic nerves were mounted in a chamber at room temperature (20°C) with three pools separated by silicone grease seals. The two pools on either side were filled with paraffin oil and contained a pair of stimulating and a pair of recording electrodes, respectively. Drug solutions were added to the center pool (150 μl) and left there until the compound action potential elicited at 2 s⁻¹ throughout the experiment had reached a steady value (10-50 min in the different tests), and the various drug interactions presumably reached equilibrium. The first (e.g., lidocaine, 1.2 mM) was added and left until the action potential reached a new equilibrium value. The drug solution was then removed, and the nerve was allowed to recover in drug-free solution, after which the response to the second drug (e.g., benzocaine) was similarly determined. The concentration of the second drug was chosen on the basis of preliminary tests, so that the response to it matched closely that to the first. After allowing recovery to occur, we then applied an equal mixture of the two solutions (a solution with half the concentration of each drug, i.e., a = 0.5) and noted the equilibrium response.

The results are summarized in Table I. In 14 tests, lidocaine, 1.2 mM, produced a TABLE I
RESPONSES TO EACH OF TWO SOLUTIONS OF LOCAL ANESTHETIC AGENT AND TO AN EQUAL MIXTURE OF BOTH

| Solutions                        | Response* to  | Number of tests | Solution 1          | Solution 2          | Mixture         |
|----------------------------------|--------------|-----------------|---------------------|---------------------|-----------------|
| First                            | Second       |                 |                     |                     |                 |
| Lidocaine                        | Benzocaine   | 14              | 1.00                | 1.03±0.08           | 1.69±0.09§      |
| Lidocaine                        | Benzy alcohol| 6               | 1.00                | 1.22±0.23           | 1.83±0.14§      |
| Benzocaine                       | Benzy alcohol| 7               | 1.00                | 0.99±0.04           | 1.17±0.02§      |
| Lidocaine                        | Marcaine     | 8               | 1.00                | 1.31±0.16           | 1.25±0.25       |
| Drug X†                          | Drug X†      | 12              | 1.00                | 1.09±0.16           | 1.11±0.07       |

* Mean (+SEM) fall in size of compound action potential. Response to first drug taken as unity.
† Drug X was either lidocaine (n = 8), marcaine (n = 1), lidocaine and marcaine (n = 1), benzy alcohol (n = 1), or benzy alcohol (n = 1).
§ Significantly different (t test) from unity (P < 0.01).
‖ Significantly different from drug X control (P < 0.01).

33.9 ± 2.3% fall in the size of the action potential. The appropriate concentration of benzocaine (usually 1.25 mM) produced a similar fall (1.03 ± 0.08 that produced by lidocaine). When mixtures of equal volumes of the two solutions (i.e., 0.6 mM lidocaine, 0.625 mM benzy alcohol) were added, the fall was much greater, being 1.69 ± 0.09 that produced by either solution alone. A similar enhanced effect was seen when lidocaine (1.2 mM) and benzy alcohol (15 mM) solutions were the two used. However, when mixtures of equiactive solutions of the neutral benzy alcohol (12–15 mM) and benzocaine (1.25 mM), or of the amines, lidocaine (1.2 mM) and marcaine (0.3–0.5 mM) were used, no significant enhancement of the response was seen. Table I also shows, as would be expected, that there was no significant enhancement when the two drug solutions being tested were identical (drug X).

Each test in Table I took several hours to complete, which might account for the observed drift in the sensitivity of the preparation with time. Thus, when the same drug (X in Table I) was tested three times in succession, the third response was 1.11 ± 0.07 that of the mean of the first two responses. Taking this as control, we found that only the responses to mixtures of lidocaine and benzocaine, and of lidocaine and benzy alcohol, were significantly different from the responses to each solution alone. The drug pairs benzocaine and benzy alcohol, and lidocaine and marcaine, showed little or
no significant enhancement of the response to their mixture. These experiments are thus consistent with the idea that lidocaine and marcaine act at the same site, and that benzyl alcohol and benzocaine act at the same site, but not with the idea that lidocaine and benzocaine act at only one site to block propagation of the action potential.

It should be noted that Hille (1977) studied the block of sodium channels directly, by measuring sodium conductance rather than the size of the compound action potential, but this difference is not necessarily important because the null-point method used does not depend on the linearity of the system. The method, however, does rely on the system being at equilibrium, but on the basis of his studies, Hille (1977) has concluded that the local anesthetic does not remain in equilibrium with the sodium channels during a transient perturbation of the membrane potential. The key postulate in his model is that inactivation of the sodium channel increases the affinity of the receptor for the local anesthetic, thus accounting for the frequency-dependent inhibition observed with local anesthetics. The present authors, however, assume that the channels already inactivated or already occupied by drugs at the time of initiation of a particular action potential do not contribute current to that action potential (almost by definition): only the open, drug-free, fraction existing at equilibrium at this time contributes. Rather, they assume (implicitly) that the action potential inactivates the existing drug-complexed (noncontributing) channels, so increasing their affinity for the drug, disturbing the equilibrium, and hence affecting a succeeding action potential (because of the enhanced binding of the drug to some channels at this later time) but not the one under study. These experiments thus in no way invalidate the general model for local anesthetics action proposed by Hille (1977) for the amine local anesthetics. Nor do they exclude the possibility that benzocaine can act at the same site as lidocaine. They do, however, indicate that whether or not benzocaine acts at the lidocaine site, at least one other site of action is involved. A plausible explanation is that, in addition to acting at the lidocaine site, benzocaine may be acting in the same way as do the alcohols and volatile general anesthetics.

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