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Comparison of Time Constants of Single Channel Patches, Quantum Bumps, and Noise Analysis in *Limulus* Ventral Photoreceptors

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Summary. The characteristic time constants derived from three different experimental procedures for measuring light-evoked currents in photoreceptors are compared; these procedures include single-channel patch-clamp measurements, noise analysis, and current relaxation studies. Recent patch-clamp measurements of the mean open times of single light-activated channels in the ventral photoreceptor of *Limulus* (Bacigalupo, J., Lisman, J.E. (1983), *Nature* (*London*) **304**:268–270) yield a disagreement of the measured mean open time with the relaxation time of the falling phase of quantum bumps and with the inverse characteristic frequency of the noise power spectrum, measured by Wong (Wong, F. (1978), *Nature* (*London*) **276**:76–79). We present new experimental results which show that the relaxation time of the falling phase of bumps is markedly shortened by light-adaptation. Hence the state of light-adaptation has to be taken into account when comparing different experiments. Secondly, we investigate three simple models for the mechanism of channel opening and closing, and conclude that an agreement of the mean open time of single channels, the relaxation time of the falling phase of bumps, and the inverse characteristic frequency of the noise power spectrum cannot be expected.

Key Words ventral photoreceptor of *Limulus* · quantum bumps · ion channel open times · (photoreceptor) noise power spectrum · light-adaption · time constants of membrane signals

Introduction

The experimental analysis of the properties of a single ionic membrane channel measured by the patch-clamp method yields the mean open and the mean close time for this channel. These time constants can also be calculated from the characteristic frequency of a noise power spectrum measured from an extended membrane region containing a large number of channels, provided that certain preconditions are met, e.g., if the channels open and close spontaneously and independently from each other. The relationship between the results of these two types of experiments, however, depends upon the mechanism that controls the opening and closing of the channels. Agreement cannot be expected for the correlated opening and/or closing of channels, as is found in light-activated channels in photoreceptor cells. We shall present arguments that results from patch-clamp and noise analysis experiments measure different properties of the transduction system. For example, single-channel observations do not take account of the correlations between channels. Hence, results from different experimental procedures cannot appropriately be compared without further information concerning the mechanism of transduction and channel opening and closing.

With some photoreceptors, for example the ventral photoreceptor of *Limulus,* there exists a third experimental method of obtaining information on the opening and closing of the light-activated channels and the transduction: the measurement of "quantum bumps," i.e., the response of the photoreceptor to the absorption of single photons. A bump is generally considered as a superposition of the correlated, transient opening of several hundred (or thousand) membrane channels. As we shall report, the falling phase of a bump can be fitted by an exponential $\propto \exp.(-\lambda t)$, where t is time and λ is a decay rate constant. The question arises whether the rate constant λ can be expected to coincide with the characteristic frequency of a noise power spectrum, or if $1/\lambda$ can be expected to coincide with the mean open time τ obtained from a patch-clamp experiment. We shall argue that in general the answer is "no": the bump decay reflects a property of the system which need not be identical with those properties reflected by single channel observations or by the noise behavior of the system. An appropriate comparison of λ with the results of patch-clamp and noise experiments requires information of the underlying mechanisms.

We report new experiments which show that the above-mentioned rate constant λ of the falling

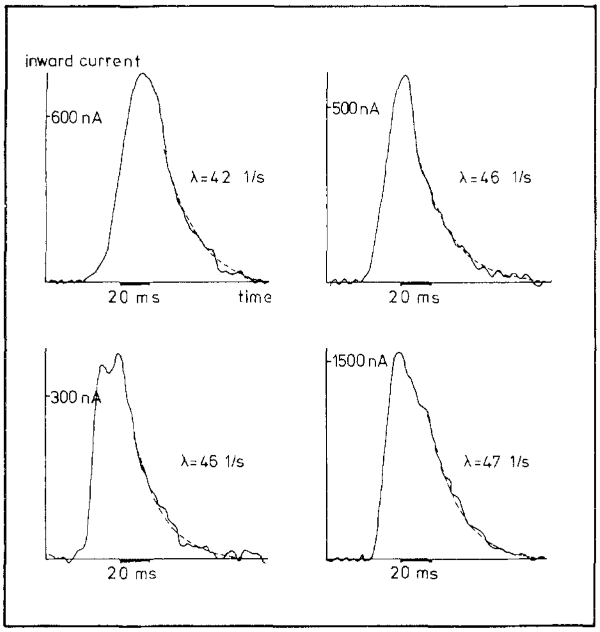

Fig. 1. Four experimentally observed quantum bumps of the ventral photoreceptor of *Limulus* (inward current *vs.* time). Exponential $e^{-\lambda t}$ were fitted to the falling phase by a computer program

phase of bumps increases markedly with increasing light-adaptation of the photoreceptor; the falling phase of the bumps becomes shorter. If experimental results depend upon light adaptation, then this has to be taken into account when comparing results from different experiments.

The actual reason for our considerations and model calculations comes from recent patch-clamp experiments of single light-activated channels in *Limulus* ventral photoreceptors by Bacigalupo and Lisman [1]. After sonification of the cells and treatment with proteolytic enzymes, about 30% of the attempts yielded successful seals, among which 4 out of 77 contained light-activated channels. One of their results showed that the distribution of the open times of the light-activated channels could be fitted by an exponential with a mean open time τ between 1.2 and 4.2 msec. These values differ appreciably from those of 18.7 msec for the inverse characteristic shoulder frequency of the noise power spectrum and 14.4 msec for $1/\lambda$ of dark-adapted bumps obtained by Wong [7]. This disagreement led Bacigalupo and Lisman to conclude that the falling phase of a bump is not rate limited by the closing of channels, but follows rather the concentration of the transmitter molecule that causes the channels to open.

This conclusion seems not unequivocal to us, since their patch-clamp experiments were performed at a much higher level of light adaptation than the bump experiments used for comparison. In the following, we present our measurements and describe our arguments in more detail in order to confirm our point of view.

Materials and Methods

SHORTENING OF THE FALLING PHASE OF BUMPS BY LIGHT-ADAPTATION

We have evaluated the rate constants for the falling phase of a number of current bumps of the *Limulus* ventral photoreceptor evoked by a 50 μsec test flash of constant energy (1.3×10^{12} photons/m^2). Two seconds prior to the test flash, the cells were exposed to light-adapting flashes of 10 msec duration and variable numbers of photons up to 8×10^{13}/m^2. For details of the experimental procedure *see* [6]. The membrane currents of the cells, which were superfused with saline at 15°C, were recorded under voltage clamp, and an exponential $e^{-\lambda t}$ was fitted to each bump's falling phase by a best-fit computer program (*see* Fig. 1). Figure 2 shows two distributions of λ-values obtained from the same cell under dark-adaptation and under light-adaptation by 8×10^{13} photons/m^2, respectively, as described above. Figure 3 shows the variation of the mean value of the individual λ's as a function of the number of photons contained in the light-adapting flash (2 different cells).

Results

Our results show that the rate constants of the falling phase of bumps significantly increase with increasing numbers of light-adapting photons. Within the adaptation range of our experiments, some cells show almost a doubling of the rate constants corresponding to a reduction of the relaxation times $1/\lambda$ from a mean value of 20 msec for the dark-adapted state to 12 msec for our maximum light-adapted state.

Since Bacigalupo and Lisman [1] applied a constant light stimulus of about 3.5×10^{16} photons/m$^2 \cdot$ s to the cell in their experiments, their results cannot be directly compared with ours. If one accepts, however, that stationary light-adaptation by a constant stimulus is reached within a time period of at least 200 msec [5], the effective number of light-adapting photons in Bacigalupo's and Lisman's [1] patch-clamp experiments is at least 7×10^{15}/m^2. This value is far beyond the maximum number of light-adapting photons in our bump experiments. An extension of our measurements of λ to Bacigalupo's and Lisman's [1] adaptation level is not possible, since soon beyond our maximum number of light-adapting photons we find that the bumps following the test flashes become extremely small in amplitude and very short in duration, thus cease to be observable at all (*compare* the "Adapting Bump

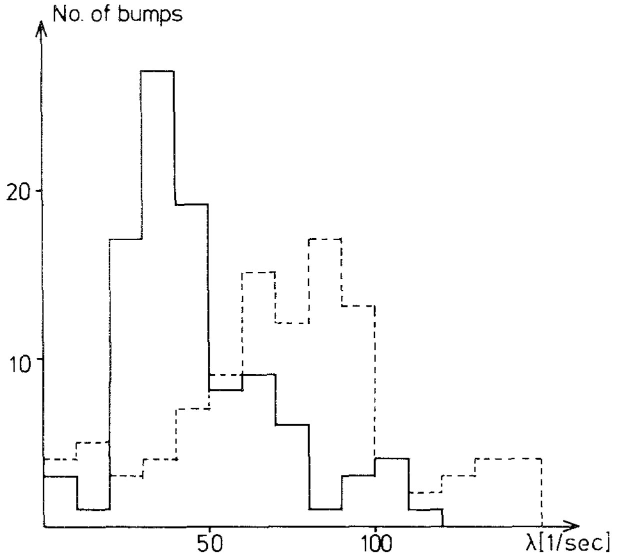

Fig. 2. Distribution of the rate constant λ of the falling phase of *Limulus* photoreceptor bumps. Continuous line: Rate constant λ of 99 bumps recorded from a dark-adapted cell; Dashed line: λ-distribution of 106 bumps from the same cell in a weakly light-adapted condition

Model,'' [3, 8]). In order to illustrate the effect of different orders of magnitude of numbers of light-adapting photons on the rate constants, we have marked Bacigalupo's and Lisman's $1/\tau$ – values for $\tau = 4$ msec at the estimated adaptation level (Fig. 3). This shows that the comparison of the mean open times of Bacigalupos' and Lisman's [1] patch-clamp experiments with the relaxation times $1/\lambda$ of the falling phase of dark-adapted bumps seems to be inadequate, since light adaptation markedly shortens $1/\lambda$. The possibility that the values of the mean open times in their experiments might be significantly reduced by light adaptation and that the relaxation times of the falling phase of dark-adapted bumps yet reflect the mean open times of the channels in the dark-adapted state cannot be excluded.

A conceivable explanation of the shortening of the channel open times by light adaptation could be a dependence of the channel-closure rate on the local intracellular calcium level. As will be shown below, however, an agreement of $1/\lambda$ with the mean open time even under equal light-adaptation conditions is to be expected only for very particular and simple channel opening and closing mechanisms.

A further detail that points to an inadequacy in a comparison of the two types of experiments is the fact that in Bacigalupo's and Lisman's [1] patch-clamp experiments latency times in the order of up to several seconds are observed, whereas those for bumps of dark-adapted cells are in the order of 200 msec, and less than 100 msec in light-adapted cells.

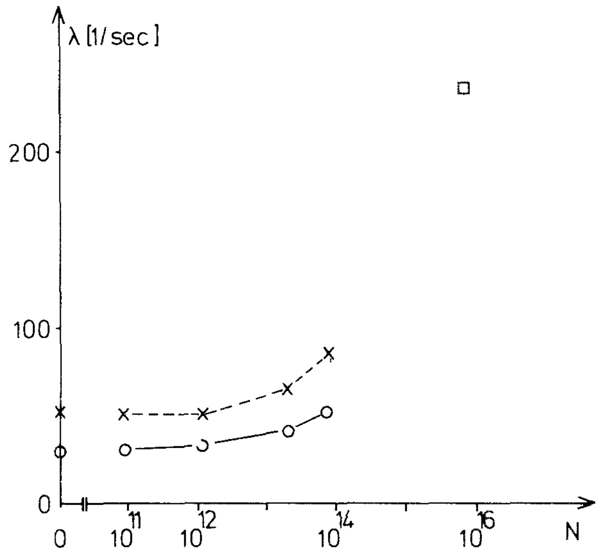

Fig. 3. Dependence of the average rate constant λ on light adaptation. Abscissa: Number N of photons per m^2 contained in the light-adapting flash. (×, ○): Average λ of ~100 bumps each from two different cells. (□): Channel closure rate constant $(1/\tau)$, or inverse of the mean open time τ, of single light-evoked channels, as reported by Bacigalupo and Lisman [1]. The corresponding number of light-adapting photons per m^2 was estimated by us to be at least 7×10^{15} per m^2 (*see* text)

This disagreement could be due to the rather incisive treatment of preparing the cell in order to obtain patches. It seems possible that this treatment could produce degenerate channels with open times that deviate from those in other experiments.

Discussion

Can the Mean Open Times τ and the Bump Relaxation Times $1/\lambda$ be Expected to Coincide?

We have calculated the rate constants of the falling phase of bumps on the basis of three kinds of simple models for the opening and closing mechanism of a channel. For each of the models we have adopted the hypothesis of a "transmitter" molecule that causes the channels to open [1, 2]. Bumps are assumed to be caused by short pulses of transmitter production, activation, or release (subsequently referred to as "transmitter production"), following the absorption of a photon. In all three models, the transmitter is given a spontaneous inactivation rate constant μ. Whether this transmitter inactivation is the rate-limiting step of the whole process or not, remains open at this stage. The three models differ as follows:

A) The transmitter open channels "enzymatically"; the time the transmitter is bound is assumed to be much shorter than the mean channel open time. Subsequently the transmitter may open further channels. The opened channels close at random with a time constant k, thereby giving a mean open time expression $\tau = 1/k$.

*B*1) The transmitter opens channels by being bound, the bound complex representing an open channel and decaying with a time constant k as in *A*. The transmitter is assumed to be inactive afterwards.

*B*2) As *B*1, but the transmitter is assumed to be active after the decay of the complex.

It is obvious for each of the models, which represent three particularly simple examples from a much larger variety of conceivable mechanisms, that $\tau = 1/k$ represents the mean open time to be observed in a patch-clamp experiment. The calculation of the rate constants for the falling phase of bumps yields a pair of constants for each of the models. These constants are independent of the time course of transmitter production since we consider the "late" falling phase of bumps (*see* Fig. 1), where it is assumed that the transmitter production has come to an end, or is extremely small.

Within these pairs of constants, the constant with the lower value is rate limiting at large times in the sense that it will control the falling phase. For models *A* and *B*1, one of the calculated constants coincides with $k = 1/\tau$; on the basis of these models one might then expect an agreement of the mean open time with the relaxation time of the falling phase if k is rate limiting in the above mentioned sense and if, as we would like to emphasize again, the same light-adapted state is realized for the two experiments. For model *B*2, none of the calculated rate constants concides with $k = 1/\tau$. In this model, both constants are combinations of k, μ and further model parameters; this also applies to the second constant of model *B*1. In model *A*, however, the second constant coincides with the time constant μ of the transmitter decay. For more complex models, the calculation will yield not only pairs, but three or more rate constants. We expect the possibility of one of them coinciding with the mean open time of a single channel to be rather unlikely.

Can the Characteristic Frequencies of Noise Power Spectra be Expected to Coincide with Either the Inverse Mean Open Time $k = 1/\tau$ or with the Bump Decay Rate Constant λ?

We have calculated the noise power spectra for a constant light stimulus on the basis of our three simple models. The transmitter production is now assumed to occur at some rate that shows a constant and finite mean value and Poisson-like fluctuations around it (*compare* Fain & Lisman [4]). For each of our models, we obtain a power spectrum that contains two corner frequencies. For none of our models, however, does any of the calculated characteristic frequencies coincide with the time constant $k = 1/\tau$ of channel closing. The time constant k enters into the calculated expressions for the characteristic frequencies only in combination with the mean steady-state transmitter concentration level, which in turn will depend on the stimulating light intensity. We expect that this finding will also apply to more complicated mechanisms for channel opening and closing. Experimental power spectra of cells under constant illumination therefore cannot be expected to yield the rate constant k directly. Bumps, on the other hand, are single-photon events; hence the individual bump's falling phase does not depend on the intensity of the evoking light. Coincidence of the rate constant λ of the falling phase of bumps in dark-adapted cells with a characteristic frequency of the power spectra would be accidental.

Wong's experimental results [7] for the noise power spectrum show only one corner frequency instead of two. This corner frequency depends slightly on the stimulating light intensity, as would be expected on the basis of our three models. That the experimental power spectrum shows only one shoulder may be due to the fact that the graphic picture of a power spectrum dissolves two frequencies only if their values differ by at least two orders of magnitude. It is also possible that one of the measurable frequencies is very high and beyond the top end of the frequency spectrum reported. In any case, neither of the two corner frequencies need coincide with the rate constant λ of the bump falling phase with the time constant $k = 1/\tau$ of channel closing.

Conclusion

Summarizing our results, we would like to point out that an agreement between the mean open time of single channels and the relaxation time of the falling phase of bumps can be expected only for certain types of channel opening and closing and only if the closing of channels is the slowest, i.e., rate-limiting process during the falling phase of bumps. Moreover, the relaxation time of the falling phase of bumps depends strongly on light adaptation such that its agreement with the mean open time of single channels presumes equal light adaptation conditions in both measurements. While Bacigalupo and Lisman's [1] assumption that the falling phase of bumps is controlled by the time course of the transmitter and not by the closing of channels is not ruled out, it is inconclusive. The interpretation of the characteristic frequencies of the noise power spectrum again depends on the type of underlying control mechanism for the opening and closing of the channels. An agreement of the inverse characteristic frequency with either the mean open time of single channels or with the relaxation time of the falling phase of bumps seems to be unlikely and at most accidental.

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