Pulsatile signaling in intercellular communication

Periodic stimuli are more efficient than random or chaotic signals in a model based on receptor desensitization

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ABSTRACT The efficiency of various patterns of pulsatile stimulation is determined in a model in which a receptor becomes desensitized in the presence of its stimulatory ligand. The effect of stochastic or chaotic changes in the duration and/or interval between successive pulses in a series of square-wave stimuli is investigated. Before addressing the effect of random variations in the pulsatile signal, we first extend the results of a previous analysis (Li, Y. X., and A. Goldbeter. 1989. *Biophys. J.* 55:125–145) by demonstrating the existence of an optimal periodic signal that maximizes target cell responsiveness whatever the magnitude of stimulation. As to the effect of stochastic or chaotic variations in the pulsatile stimulus, three kinds of random distributions are used, namely, a Gaussian and a white-noise distribution, and a chaotic time series generated by the logistic map. All these random distributions are symmetrically centered around the reference value of the duration or interval that characterizes the optimal periodic stimulus yielding maximal responsiveness in target cells. Stochastically or chaotically varying pulses are less effective than the periodic signal that corresponds to the optimal pattern of pulsatile stimulation. The response of the receptor system is most sensitive to changes in the time interval that separates successive stimuli. Similar conclusions hold when stochastic or chaotic signals are compared to a reference periodic stimulus differing from the optimal one, although the effect of random variations is then reduced. The decreased efficiency of stochastic pulses accounts for the observed superiority of periodic versus stochastic pulses of cyclic AMP (cAMP) in *Dictyostelium* amoebae. The results are also discussed with respect to the efficiency of periodic versus stochastic or chaotic patterns of hormone secretion.

INTRODUCTION

Many hormones (Crawley and Hofler, 1987; Leng, 1988) as well as intracellular messengers such as cyclic AMP (cAMP) (Gerisch, 1987) and Ca^{2+} (Berridge et al. 1988; Cuthbertson, 1989) vary in a pulsatile manner. These observations raise the possibility that the physiological effects of these signals might be governed by the frequency of their temporal variation (Knobil, 1981). Such a frequency encoding would prove more advantageous than amplitude-modulated control (Rapp et al., 1981; Rapp, 1987). Frequency encoding of signals in intercellular communication is exemplified by the synthesis and release of cAMP in response to cAMP pulses in the cellular slime mold *Dictyostelium discoideum* (Martiel and Goldbeter, 1987; Goldbeter, 1990; Li and Goldbeter, 1990) and by the gonadotropin release from pituitary cells in response to pulses of gonadotropin-releasing hormone (GnRH) emitted by the hypothalamus (Knobil, 1980; Wildt et al., 1981; Pohl et al., 1983).

To understand the possible molecular bases of frequency encoding of pulsatile stimuli, we have studied in detail (Li and Goldbeter, 1989; Goldbeter and Li, 1989) a simple, general model of a receptor undergoing reversible desensitization in the presence of its stimulatory ligand; this model was originally proposed in the study of sensory adaptation to constant stimuli in microorganisms (Goldbeter and Koshland, 1982; Segel et al., 1986). Our previous analysis showed that owing to the kinetic characteristics of desensitization, the cellular response generated upon binding of the ligand to the receptor strongly depends on the frequency of the pulsatile stimulus. Moreover, there exists an optimal pattern of periodic stimulation that maximizes the responsiveness of target cells. This pattern, which corresponds to a particular pair of values of the duration and interval between successive stimuli, was shown to be strongly influenced by the kinetic constants governing receptor desensitization and resensitization.

These results were subsequently corroborated (Li and Goldbeter, 1990) by the analysis of a more realistic model for cAMP signaling in *D. discoideum* based on receptor desensitization through reversible phosphorylation (Martiel and Goldbeter, 1987), in which the nature of the cellular response was specified explicitly in terms of cAMP-induced cAMP synthesis. That an optimal pattern of pulsatile stimulation is recovered in that specific model justifies the recourse to the more general model for the desensitized receptor, which presents the advantage of wider applicability and increased amenability to analytical and numerical investigation.

Having established the existence of an optimal pattern of periodic stimulation, the natural question arises as to what happens when the duration of a pulse or the

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interval between successive pulses (or both) varies stochastically. Will the resulting responsiveness be greater than that obtained for the optimal, periodic pattern of pulsatile stimulation? In *D. discoideum,* an experimental study of the effects of stochastic cAMP pulses on cAMP-mediated cell differentiation showed that randomly spaced pulses of fixed duration were less effective than periodic pulses in inducing differentiation (Nanjundiah, 1988). The present analysis aims at providing an explanation for these observations. Another, closely related question pertains to the physiological significance of chaotic versus periodic signals in intercellular communication. The present model provides an opportunity of assessing the possible advantages of chaos in physiology (Pool, 1989); thus, how do periodic stimuli compare with aperiodic stimuli as to their effect on target cell responsiveness?

To address these questions, we investigate in this paper how stochastic variations in the characteristics of pulsatile stimuli influence cellular responsiveness. To this end, we consider the general receptor model previously used in our analysis of frequency encoding (Li and Goldbeter, 1989), and examine its response to square-wave stimuli. We first extend our previous results by establishing the existence of an optimal pattern of periodic stimulation at subsaturation levels of stimulatory ligand, and determine the dependence of this pattern on the magnitude of stimulation. We then turn to the effect of stochastic variations in the square-wave signal. Two different kinds of stochasticity, as well as deterministic chaos, are considered. We show that in this receptor system, signals with stochastic variations are always inferior to the optimal, periodic pattern of stimulation. The loss in responsiveness to stochastic signals is estimated quantitatively. It appears that cellular responsiveness is more sensitive to variations in intervals between successive pulses than to changes in the duration of each pulse. The efficiency of chaotic signals is also reduced as compared to that of periodic stimulation. These results are extended to the case where the reference, periodic signal differs from the optimal one.

Because the decrease in responsiveness due to stochastic or chaotic variations remains limited, it appears that the pulsatile nature of the stimulus might well be the characteristic feature of the signal that matters most for the response. That the signal is strictly periodic or subjected to stochastic or chaotic variations is indeed of lesser consequence than the fact that the stimulus is pulsatile rather than continuous. Such a conclusion will be discussed in relation to pulsatile patterns of hormone secretion.

MODEL AND MEASURES OF CELLULAR RESPONSIVENESS

The model considered, schematized in Fig. 1, is that of a simple receptor-mediated response system in which the receptor undergoes a reversible transition between two conformational states, R and D; the former state is supposed to be more active than the latter (Segel et al., 1986; Knox et al., 1986). Upon binding the ligand L, the two states form, respectively, the complexes RL and DL which are also interconvertible. All the four states contribute to the cellular response but with different efficacies (or weights) measured by the "activity coefficients" $a_i (i = 1, \ldots, 4)$. As a measure of ligand-elicited activity at a given moment, we take the following linear, weighted combination of the concentrations of the four states, called "activity" (Segel et al., 1986; Knox et al., 1986):

$$A(t) = a_1[\mathrm{R}] + a_2[\mathrm{RL}] + a_3[\mathrm{DL}] + a_4[\mathrm{D}]. \tag{1}$$

The simplest form of pulsatile signal to be considered here is that of a square-wave (see Fig. 1, as well as Li and Goldbeter, 1989). Such a square-wave stimulus is characterized by two parameters, namely, the duration of each pulse, τ_1, and the interval between pulses, τ_0. Results

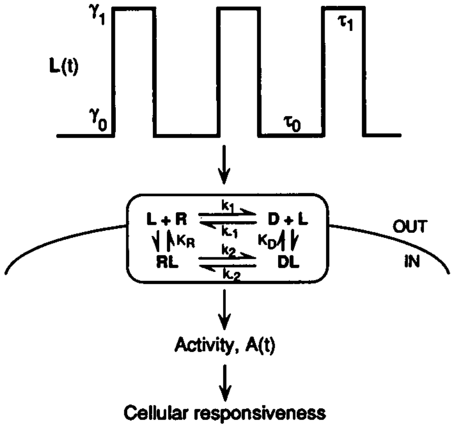

FIGURE 1 Schematic diagram of a pulsatile, square-wave stimulus applied to a receptor undergoing reversible desensitization. Binding of the ligand L results in a change in activity $A(t)$ (see Eq. 1) which is coupled to the cellular response. The interconversions between the free receptor forms R and D, and the liganded forms RL and DL are shown; the forms R (RL) and D (DL) represent active and desensitized states of the receptor, respectively. The rate constants for receptor desensitization (k_1, k_2) and resensitization (k_{-1}, k_{-2}) are indicated as well as the dissociation constants of L for the two receptor states (K_R, K_D). The square-wave stimulus is characterized by the duration τ_1 of each pulse, and the interval τ_0 between successive pulses; γ_0 and γ_1 denote the values of the dimensionless ligand concentration $\gamma = [L]/K_R$ during the off- and on-phases of stimulation, respectively.

similar to those obtained with the square-wave stimulus are recovered when considering more realistic situations such as that where the stimulus undergoes an instantaneous increase followed by an exponential decrease as a function of time (Li and Goldbeter, 1989). After determining the influence of the amplitude of the periodic square-wave signal in the next section, we shall fix this amplitude to a close-to-saturation value in the subsequent sections devoted to the analysis of stochastic or chaotic pulsatile stimulation.

The first quantity of interest previously defined in the analysis of periodic, square-wave stimuli is the integrated activity α_T, normalized by division through the total receptor concentration R_T, over a period $T = \tau_0 + \tau_1$:

$$\alpha_T = \frac{1}{R_T}\int_0^{\tau_1}[A(t) - A_0]\mathrm{d}t. \tag{2}$$

In Eq. 2, A_0 represents the basal activity generated in the absence of ligand. The value of A_0 could be regarded as a threshold that needs to be exceeded for triggering a response. If we assume that the rate of synthesis or release of a hormone elicited by ligand binding is proportional to the activity, then α_T would yield a measure of the total amount of hormone synthesized or secreted during one period of the pulsatile stimulus. The integration is carried out only over the on-phase because the above-basal activity for square-wave signals can only be generated during that phase (Li and Goldbeter, 1989).

To assess the capacity of target cells to generate a large number of significant responses in a given amount of time, we shall use the *cellular responsiveness* α_R previously defined (Li and Goldbeter, 1989) as the product of two terms related, respectively, to the magnitude of a single response scaled by the response to a step increase in ligand ($\alpha_{T\mathrm{step}}$), and to the average level of response during a period:

$$\alpha_R = \left(\frac{\alpha_T}{\alpha_{T\mathrm{step}}}\right)\left(\frac{\alpha_T}{T}\right). \tag{3}$$

Let us now introduce quantities corresponding to α_T and α_R in the presence of stochastic variations in τ_0 and/or τ_1, when the magnitude of these parameters changes for each of the successive periods n ($n = 1, 2, \ldots$). In such a case, the response over a certain period $T_n = \tau_0^{(n)} + \tau_1^{(n)}$ is determined by integrating during that period the activity generated above the basal level A_0. We denote this integrated activity by $\alpha_T^{(n)}$ and define it in Eq. 4.

$$\alpha_T^{(n)} = \int_{t_{n-1}}^{t_{n-1}+\tau_1^{(n)}}[A(t) - A_0]\mathrm{d}t \quad \text{with } t_{n-1} = \sum_{j=0}^{n-1} T_j, \tag{4}$$

where the index n indicates the number of the pulse within the series, with $T_0 = 0$.

As a result of the stochastic variation in τ_1 or/and τ_0, $\alpha_T^{(n)}$ changes from period to period (i.e., for different values of n). Thus, a reasonable measure of the level of response over a single period is the average of $\alpha_T^{(n)}$ over a large number (N) of periods. We define this quantity as the mean integrated activity, $\langle\alpha_T\rangle$:

$$\langle\alpha_T\rangle = \frac{1}{N}\sum_{n=1}^{N}\frac{\alpha_T^{(n)}}{\alpha_{T\mathrm{step}}}. \tag{5}$$

In Eq. 5, $\alpha_T^{(n)}$ is scaled by $\alpha_{T\mathrm{step}}$ so as to make $\langle\alpha_T\rangle$ dimensionless.

Besides the magnitude of the response over a single period, we should again take into account the number of significant responses that can be generated in a given amount of time. Such a measure is provided by the mean cellular responsiveness $\langle\alpha_R\rangle$ defined by Eq. 6.

$$\langle\alpha_R\rangle = \langle\alpha_T\rangle\frac{\sum_{n=1}^{N}\alpha_T^{(n)}}{\sum_{n=1}^{N}T_n}. \tag{6}$$

For strictly periodic square-wave pulses, the mean integrated activity $\langle\alpha_T\rangle$ and the mean responsiveness $\langle\alpha_R\rangle$ reduce to the integrated activity α_T (scaled by $\alpha_{T\mathrm{step}}$) and to the responsiveness α_R analyzed in our previous study of periodic stimulation (Li and Goldbeter, 1989).

OPTIMAL PATTERN OF PERIODIC STIMULATION AS A FUNCTION OF STIMULUS MAGNITUDE

Our previous analysis demonstrated the existence of a particular pair of values (τ_1^*, τ_0^*) that maximizes cellular responsiveness α_R. This optimal pattern of periodic stimulation was obtained at a saturating ligand level, and its dependence on the various parameters of the model was determined. The main result of that analysis was that the optimal pattern (τ_1^*, τ_0^*) closely depends on the kinetic constants governing reversible receptor desensitization: the optimal stimulus duration τ_1^* is primarily related to the rate constant k_2 measuring ligand-induced desensitization (see Fig. 1), whereas the optimal interval τ_0^* is mainly influenced by the resensitization rate constant k_{-1} (Li and Goldbeter, 1989). Qualitatively similar results were also obtained in the more specific model proposed for cAMP signaling in *Dictyostelium* cells (Li and Goldbeter, 1990).

Before addressing the effect of stochastic or chaotic variations in τ_1 and τ_0, it is important to extend these results by determining whether and how the optimal pattern (τ_1^*, τ_0^*) varies in the general receptor model with the magnitude of the periodic stimulus. Shown in Fig. 2 is the dependence of α_R on τ_1 and τ_0 at three different magnitudes of stimulation, namely, $\Delta\gamma = \gamma_1 - \gamma_0 = 0.1$, 1, and 10, where γ_1 and γ_0 denote the level of stimulus during stimulation and between stimuli, respectively (γ is the ligand concentration L normalized by division through its dissociation constant K_R with the R state of the receptor).

On each of the three surfaces in Fig. 2, an optimum pattern of stimulation maximizing cellular responsiveness is apparent. The particular set of parameter values considered in Fig. 2 was taken so as to yield an optimal pattern (τ_1^*, τ_0^*) close to that observed for GnRH (Knobil, 1980), namely, $\tau_1^* = 6.1$ min and $\tau_0^* = 53.9$ min. The data of Fig. 2 demonstrate that the existence of an optimal pattern (τ_1^*, τ_0^*), previously established at a saturating level, is recovered at all levels of stimulation.

Whereas the optimal cellular responsiveness α_R^* rises up to a plateau value as $\Delta\gamma$ increases, the optimal pattern of stimulation also varies with the magnitude of the stimulus. A comparison of the three panels of Fig. 2 as well as a more detailed analysis of the dependence of the optimal pattern on $\Delta\gamma$ (Fig. 3) indicate that the optimal duration τ_1^* is more affected than the interval τ_0^* by a change in the magnitude of the stimulus: the value of τ_1^* significantly decreases as the magnitude of the stimulus increases, whereas the value of τ_0^* only slightly diminishes. Thus, in Fig. 2, the optimal pattern (τ_1^*, τ_0^*) changes (in minutes) from (24.1, 64.4) to (9.6, 56.4) and (6.3, 54.1) when $\Delta\gamma$ goes from 0.1 to 1 and 10.

STOCHASTIC DISTRIBUTIONS AND CHAOTIC DYNAMICS CONSIDERED FOR THE PULSATILE SIGNAL

Stochastic variations in τ_1 (τ_0) are introduced through random deviations from the optimal value τ_1^* (τ_0^*) of the reference, periodic signal. Therefore, the random distribution is always centered at the optimal value of the relevant quantity, which will be denoted by τ^* for simplicity. When only one of the parameters, τ_1 or τ_0, changes stochastically, the other is fixed at its optimal value. The results remain valid when considering a reference periodic signal differing from the optimal one (see the next section).

Three kinds of randomness are considered, namely, the truncated Gaussian distribution (Fig. 4 *a*), the white-noise, uniform distribution (Fig. 4 *b*), and the distribution corresponding to deterministic chaos generated by the logistic iterate map (see, e.g., Hao, 1989): $r_{n+1} = \mu r_n(1 - r_n)$ with $\mu = 4$ (Fig. 4 *c*). The Gaussian distribution will be truncated symmetrically at $\tau^* - \sigma$ and $\tau^* + \sigma$

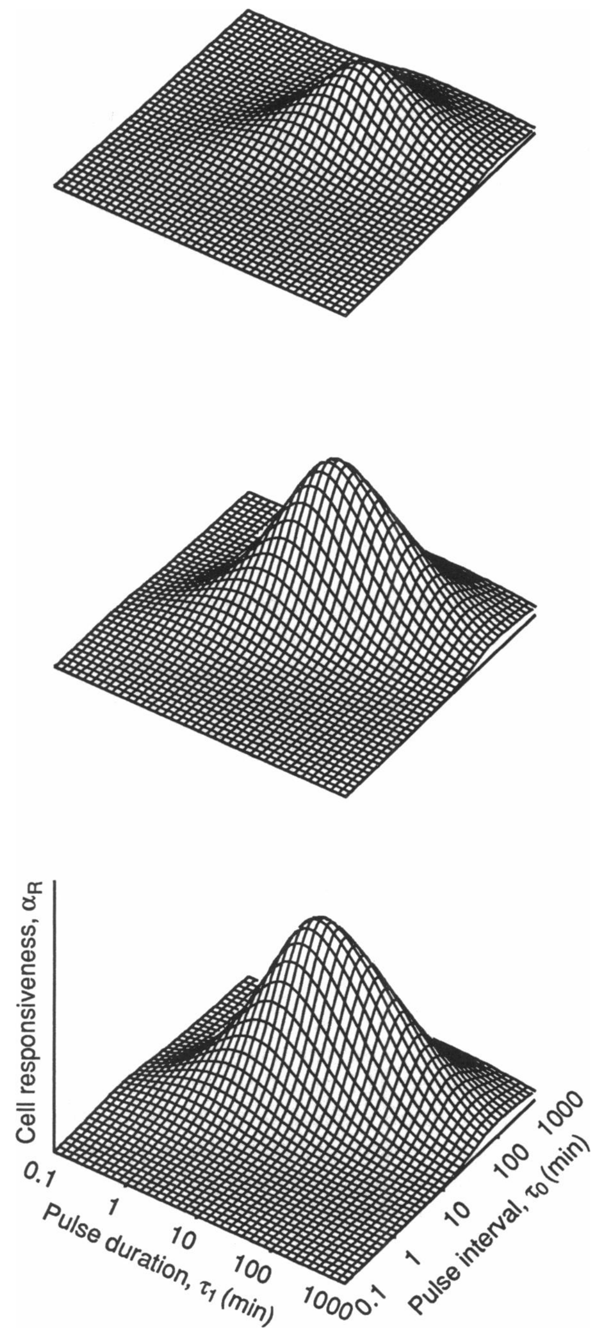

FIGURE 2 Cellular responsiveness as a function of pulse duration (τ_1) and pulse interval (τ_0) at three different magnitudes of stimulation. From top to bottom, the amplitude of the stimulus, measured by $\Delta\gamma = \gamma_1 - \gamma_0$ (see Fig. 1), increases from 0.1 to 1 and 10; the basal ligand level (γ_0) is fixed at the value 10^{-3}. The vertical bar in the lower panel corresponds to $\alpha_R = 1$. Cellular responsiveness α_R is determined according to Eq. 3 for the following kinetic parameter values: $k_1 = 0.00195$ min^{-1}, $k_{-1} = 0.0195$ min^{-1}, $k_2 = 0.645$ min^{-1}, $k_{-2} = 0.0645$ min^{-1}. To ensure exact adaptation (Segel et al., 1986; Li and Goldbeter, 1989), the activity coefficients of the four receptor states (see Fig. 1) are taken equal to $a_1 = 20$, $a_2 = 101$, $a_3 = 10$, $a_4 = 1$, whereas $c = K_R/K_D = 100$.

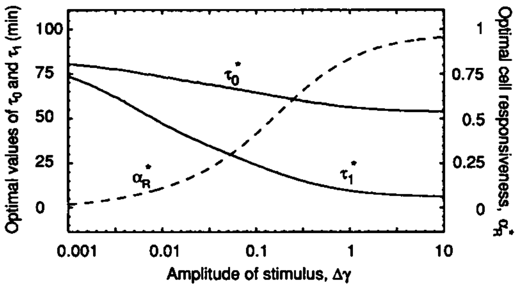

FIGURE 3 Optimal pattern of square-wave stimulation as a function of the magnitude of the pulsatile signal. Shown as a function of $\Delta\gamma = \gamma_1 - \gamma_0$ are the optimal values τ_0^* and τ_1^* maximizing cellular responsiveness (see Fig. 2), as well as the corresponding value of α_R. The curves are obtained as previously described (Li and Goldbeter, 1989), for the parameter values of Fig. 2, with $\gamma_0 = 10^{-3}$.

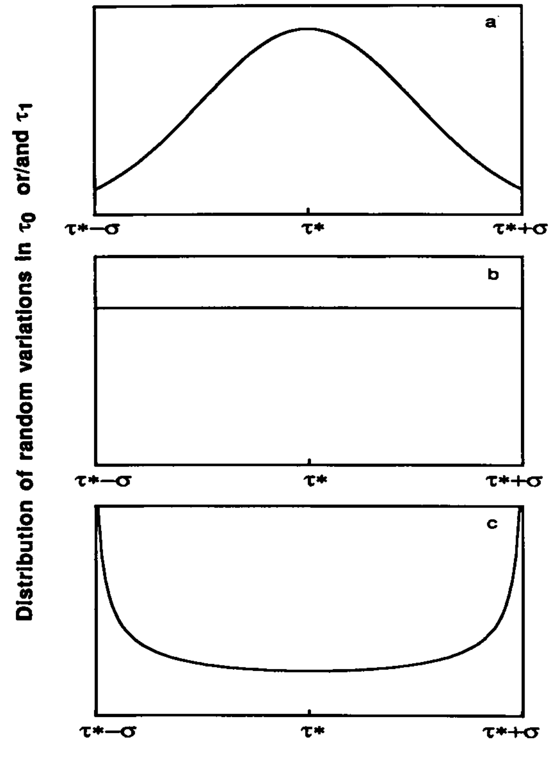

FIGURE 4 Probability distributions characterizing the three types of randomness considered for the stochastic or chaotic variation of the pulsatile signal. (*a*) Truncated Gaussian distribution, obeying the equation $p(\tau) = \exp[-(\tau - \tau^*)^2/2\sigma^2]/[\sigma\sqrt{2\pi}]$, $(0 < \tau < 2\sigma)$. (*b*) Uniform, white noise distribution $p(\tau) = 2\sigma$, $(0 < \tau < 2\sigma)$. (*c*) Distribution generated by the logistic map $r_{n+1} = \mu r_n(1 - r_n)$; for the value $\mu = 4$ considered, the dynamics is chaotic and the distribution reduces to the invariant (Chebychev) distribution $p(\tau) = \sigma/[\pi\sqrt{1 - (\tau - \tau^*)^2}]$, $(0 < \tau < 2\sigma)$ (see for example, Hao, 1989). In the cases *b* and *c*, the variance σ simply represents the width of the distribution considered.

(Fig. 4 *a*) where σ represents the variance of the distribution. Thus, as σ goes to infinity, the truncated Gaussian distribution reduces to a uniform distribution. In the white-noise and chaotic distributions (Fig. 4 *b* and *c*), the variance σ simply specifies the domain of variation in τ.

As the optimal values of τ_1^* and τ_0^* generally differ, we shall use the relative variance $\sigma_r = \sigma/\tau^*$ to facilitate the representation of the results in the next section. Thus, changing the value of σ from 0 to τ^* corresponds to a change of the value of σ_r from 0 to 1. Values of σ larger than τ^* are not considered in our study because they might render the values of τ_1 and/or τ_0 negative. Our choice for the range of σ matches that considered in the experimental study of Nanjundiah (1988), where the value $\sigma = \tau^* = 5$ min was used in determining the effect of stochastic pulses of cAMP on *Dictyostelium* development.

The white-noise distribution is obtained by means of a computer random number generator that produces numbers uniformly distributed within the interval between 0 and 1. These uniformly distributed numbers can be used to generate by the direct method (Abramowitz and Segun, 1968) random numbers obeying Gaussian distribution. As for the logistic iterate map, initial values were chosen randomly in different numerical experiments.

Examples of the different sorts of pulsatile stimuli considered are shown in Figs. 5 and 6. Represented in Fig. 5 are (*a*) the optimal periodic square-wave stimulus, (*b*) the stimulus subjected to Gaussian noise in τ_1 and τ_0, and (*c*) the stimulus with white noise in τ_1 and τ_0. Shown in Fig. 6 is the pulsatile signal with chaotic variation in τ_1 (*a*), τ_0 (*b*), and in both τ_1 and τ_0 (*c*).

EFFECT OF RANDOM AND CHAOTIC PULSATILE STIMULATION

The effect of the above mentioned kinds of random variations in τ_1 and/or τ_0 on the mean responsiveness $\langle\alpha_R\rangle$ was studied for increasing values of the variance σ ranging from 0 to τ^*, i.e., for a relative variance σ_r varying from 0 to 1 (Fig. 4). Similar results were obtained for $\langle\alpha_T\rangle$ (data not shown). For each value of σ_r, $\langle\alpha_R\rangle$ is obtained by averaging over 600 randomly distributed, successive pulsatile stimuli. Effects of random variations obeying the truncated Gaussian distribution, the uniform distribution and the chaotic distribution of the logistic map are given, respectively, in Fig. 7, *a–c*. In each of these panels, the effect of a stochastic variation only in τ_1, only in τ_0, or in both quantities is represented by a dotted, dashed, or solid curve, respectively (from top to bottom, on each panel). The dashed horizontal line in each panel represents the reference value α_R^*

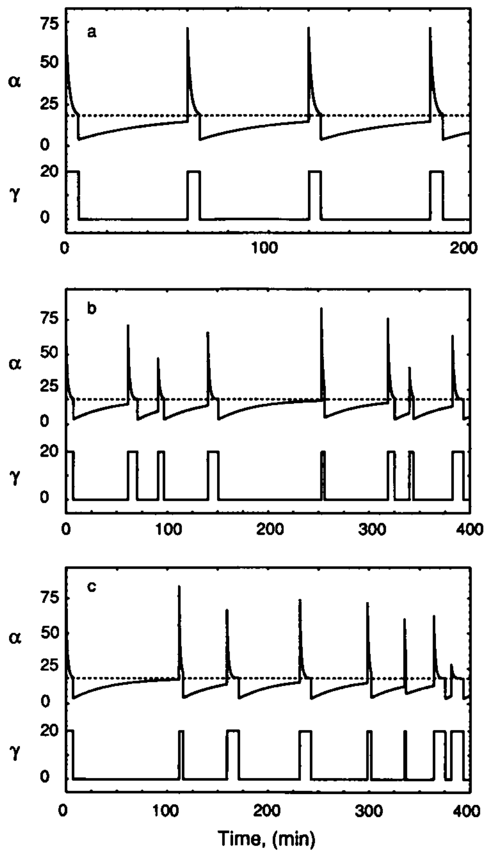

FIGURE 5 Periodic and stochastic pulsatile signal considered for receptor stimulation. In each panel, the activity (*upper curve*) generated by the pulsatile signal (*lower curve*) is indicated as a function of time. (*a*) Optimal periodic signal maximizing cellular responsiveness. (*b* and *c*) Stochastic variation in τ_1 and τ_0, around the optimal values τ_0^* and τ_1^*, generated by the Gaussian and white noise distributions, respectively (see Fig. 4, *a* and *b*). Parameter values are those of Fig. 2, with $\Delta\gamma = 20$. The relative variance σ_r equal to unity.

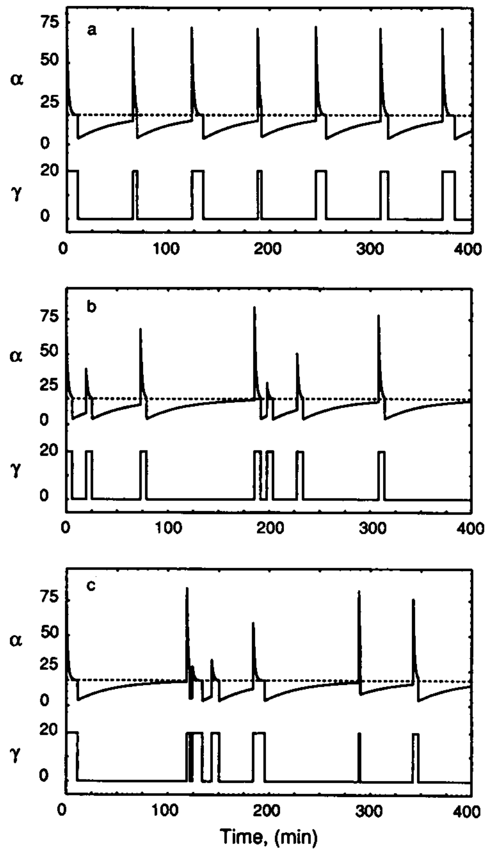

FIGURE 6 Chaotic variations in pulsatile stimulation. Aperiodic variations generated by the logistic map (see Fig. 4 *c*) affect the duration τ_1 of each pulse (*a*), the interval τ_0 between successive pulses (*b*), or both τ_1 and τ_0 (*c*). As in Fig. 5, the upper curve in each panel represents the variation of the activity A(t) (Eq. 1) associated with the pulsatile stimulus. Parameter values are as in Fig. 5.

obtained when the receptor system is stimulated by the optimal periodic signal (τ_1^*, τ_0^*).

The following conclusions emerge from the comparative analysis of periodic versus stochastic or chaotic pulsatile stimuli, as illustrated by the typical results of Fig. 7. (*a*) Stochastic or chaotic variation in τ_1, τ_0 or both always results in a reduced responsiveness as compared to the value obtained for the optimal, strictly periodic stimulus (Figs. 2 and 5 *a*). The magnitude of the decrease in responsiveness augments as the variance of the random distribution increases.

(*b*) The reduction of responsiveness is more significant when both τ_1 and τ_0 vary stochastically than when only one of the two quantities varies while fixing the other at its optimal value (compare the solid curve with the other ones in Fig. 7). However, the effects of simultaneous random variations in τ_1 and τ_0 are not additive.

(*c*) Cellular responsiveness is more sensitive to variations in the time interval between pulses, τ_0, than to variations in the duration of each pulse, τ_1 (compare the dashed curve with the dotted one in Fig. 7). Thus, a recovery time long enough for the system to resensitize is of crucial importance to maintain high responsiveness.

(*d*) The drop in responsiveness depends on the type of random variation considered. Thus, the truncated Gaussian distribution results in the smallest decrease in cellular responsiveness because it is more tightly centered at the optimal value (Fig. 4 *a*). A slightly larger reduction of the response is obtained when uniformly distributed randomness is used (Fig. 4 *b*). Chaotic randomness generated by the logistic map results in the most significant decrease in response. The reason is that the corresponding distribution is tilted toward the two

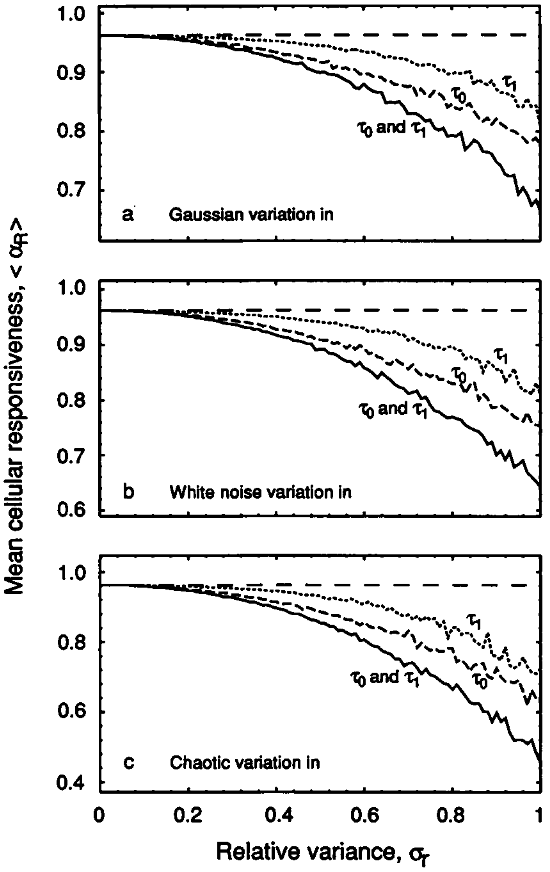

FIGURE 7 Mean responsiveness $\langle\alpha_R\rangle$ as a function of the relative variance $\sigma_r(=\sigma/\tau^*)$ characterizing the stochastic or chaotic variations in the pulsatile signal. Considered are random variations obeying the truncated Gaussian distribution (*a*), the uniform, white noise distribution (*b*), and the chaotic distribution generated by the logistic map (*c*). The value of $\langle\alpha_R\rangle$ associated with stochastic variations only in τ_1 (*dotted curve*), only in τ_0 (*dashed curve*) or in both τ_1 and τ_0 (*solid curve*) is plotted in each panel. The upper, dashed horizontal line indicates the optimal value α_R^* of cell responsiveness corresponding to the optimal periodic signal. For each value of σ_r, the value of $\langle\alpha_R\rangle$ is obtained by averaging over a series of 600 successive pulses. Parameter values are as in Fig. 2, with $\Delta\gamma = 20$. For the parameter values considered, $\tau_1^* =$ 6.1 min and $\tau_0^* =$ 53.9 min, $\langle\alpha_R\rangle^* = 0.962$, whereas the scaling factor $\alpha_{T\text{step}}$ is equal to 103.47.

ends (Fig. 4 *c*) which are most distant from the optimal value located at the center.

So far, we have compared the efficiency of stochastic or chaotic stimuli with that of the optimal periodic signal which served as reference pattern of pulsatile stimulation. The question arises as to whether the conclusions reached above extend to situations where the reference periodic signal differs from the optimal one. In other words, if one considers an arbitrary periodic signal corresponding to a particular pair of values (τ_1, τ_0) differing from (τ_1^*, τ_0^*), will stochastic or chaotic variation in τ_0 and/or τ_1 result in an enhancement of the responsiveness α_R $(<\alpha_R^*)$? This question has been addressed by numerical calculations. Although an exhaustive study is necessarily out of reach, a number of different reference patterns (τ_1, τ_0) have been tested; typical results are shown in Fig. 8. This study confirms the results established in the case where the optimal periodic pattern (τ_1^*, τ_0^*) is taken as reference signal: a

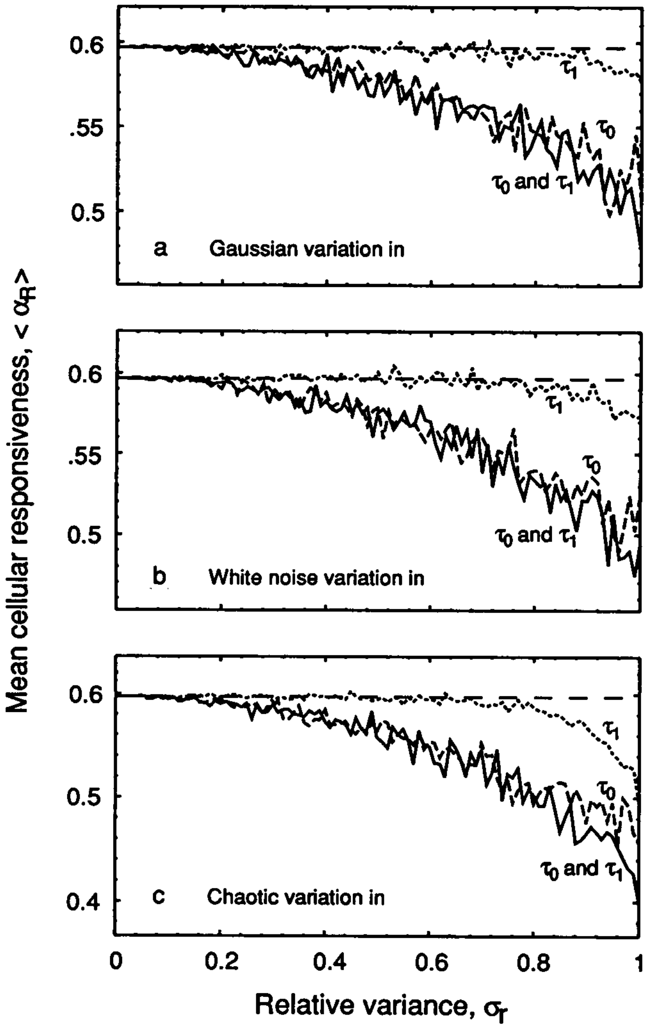

FIGURE 8 Mean cell responsiveness $\langle\alpha_R\rangle$ as a function of the relative variance σ_r when the stochastic or chaotic variations in the pulsatile signal occur around values of τ_0 and τ_1 differing from the optimal values τ_0^* and τ_1^*. The stochastic variations obey the Gaussian distribution (*a*), the white noise distribution (*b*), or the chaotic distribution generated by the logistic map (*c*). As in Fig. 7, the dotted, dashed and solid curves in each panel refer to the effect of random variations in τ_1, τ_0, or both τ_1 and τ_0. The upper, dashed horizontal line relates to the cell responsiveness corresponding to the reference signal considered: $\tau_1 = 15$ min, $\tau_0 = 25$ min. This reference pulsatile signal differs from the optimal one for which $\tau_1^* = 6.1$ min, $\tau_0^* = 53.9$ min (see Fig. 7). Other parameter values are as in Fig. 7.

periodic signal is generally more efficient than the corresponding pulsatile signal with stochastic or chaotic variations in τ_1 (Fig. 8, dotted curves), which is itself more efficient than the pulsatile signal with random variations in τ_0 (Fig. 8, dashed curves). Finally, the less efficient signal is the one subjected to random variations in τ_0 and τ_1.

A comparison of Figs. 7 and 8 indicates that the difference between periodic and stochastic or chaotic stimuli is relatively smaller when the reference periodic signal differs from the optimal one. In several instances where $\tau_1 > \tau_1^*$ and $\tau_0 < \tau_0^*$, the reference values of τ_0 and τ_1 tested were such that at low variance the signal subjected to stochastic or chaotic variation in τ_1 proved as efficient or even slightly more efficient than the strictly periodic, reference signal; an example of such a situation is shown in Fig. 8. At larger variances, however, the periodic signal again yields higher responsiveness in target cells.

DISCUSSION

The present study is devoted to the effect of periodic or randomly varying pulsatile stimuli on target cells whose response is mediated by a receptor undergoing reversible desensitization upon prolonged stimulation by its ligand. With respect to strictly periodic stimuli, the analysis extends previous results obtained at saturating levels of the ligand (Li and Goldbeter, 1989), by demonstrating the existence of an optimal pattern of periodic stimulation maximizing target cell responsiveness at all magnitudes of stimulation. The optimal pattern of pulsatile stimulation corresponds to a pair of values (τ_1^*, τ_0^*) characterizing the duration (τ_1) of each pulse and the interval (τ_0) between successive pulses in the square-wave stimulus. The present results indicate how the magnitude of the stimulus influences the characteristics of the optimal periodic signal.

The effect of stochastic variations in the pulsatile signal was tested by considering Gaussian or white noise fluctuations in τ_1 and/or τ_0 around the optimal, reference values τ_1^* or τ_0^*. Moreover, the case of an aperiodic signal was dealt with by assuming that τ_1 and/or τ_0 fluctuate around these optimal reference values according to a simple chaotic dynamics generated by the logistic map. The results of such a comparative analysis indicate that periodic pulses are more efficient than stochastic or chaotic ones in physiological systems where continuous stimulation induces a decrease in the responsiveness of target cells owing to receptor desensitization.

Refining this conclusion, we note that cellular responsiveness to the pulsatile stimulus progressively decreases from the level observed for the reference periodic signal when only the duration τ_1 fluctuates, when only the interval τ_0 varies, or when both τ_1 and τ_0 are subject to stochastic or chaotic changes. Moreover, Gaussian noise is less detrimental to the response of target cells than white noise, which is itself associated with higher responsiveness when compared to the particular type of chaotic dynamics considered (see Fig. 7). The decrease in responsiveness observed for stochastic or chaotic signals, however, is not very important as long as the extent of the fluctuations remains reduced; thus, this decrease is $<20\%$ for random variations whose variance is less than half the optimal reference value ($0 < \sigma_r < 0.5$) (see Fig. 7).

That periodic signaling represents an optimal mode of intercellular communication (Goldbeter, 1988) can be explained by the fact that cell responsiveness results from a balance between two antagonistic demands: on the one hand, a reasonable time should elapse for the receptors to resensitize up to a sufficient level, whereas on the other hand the system should not wait too long for the arrival of the next stimulus, as this would reduce the number of significant responses that the receptor system can produce in a given amount of time. Any deviation from this balance should be detrimental to cell responsiveness.

The above conclusions on the comparative efficiency of stochastic, chaotic, and periodic signals should hold regardless of the molecular mechanism underlying target cell desensitization. That mechanism may involve receptor phosphorylation as in *Dictyostelium* cells (Meier and Klein, 1988; Vaughan and Devreotes, 1988), allosteric conformational changes, or inactivation of a Ca^{2+} channel as in pituitary gonadotrophs stimulated by GnRH (Stojilkovic et al., 1989).

As desensitization happens primarily at the level of the cAMP receptor in *D. discoideum,* our present study provides insights into the experimentally observed superiority of periodic signals over stochastic ones in these amoebae (Nanjundiah, 1988). In these experiments, the interval between successive cAMP pulses was varied in a white noise manner from 0 to 10 min, with a mean of 5 min. Such conditions correspond to the case considered for the dashed curve in Fig. 7*b* when the relative variance σ_r is equal to unity, i.e., when the value of τ_0 varies around τ_0^* from 0 to 2 τ_0^*. The data of that figure indicate that cell responsiveness is then decreased by $\sim 20\%$. Although such a decrease is relatively limited, it nevertheless supports the view that the reduction in the synthesis of cAMP in target cells underlies the lack of physiological effect of stochastically varying pulses.

The magnitude of the decrease in responsiveness due to random variations in the pulsatile stimulus can be expected to be more significant when the optimum on the surface yielding α_R as a function of τ_0 and τ_1 (Fig. 2) becomes sharper. Such is the case when a pattern of stimulus more realistic than the square-wave is considered, e.g., a series of instantaneous increases in ligand followed by exponential decay (see Fig. 14 in Li and Goldbeter, 1989). A sharper optimum in α_R was also obtained in the more specific model for cAMP signaling in *Dictyostelium* based on receptor phosphorylation (Li and Goldbeter, 1990).

Besides the two examples of frequency encoding mentioned above, i.e., cAMP signaling in *Dictyostelium* and GnRH stimulation of pituitary cells, numerous examples of pulsatile signaling have been uncovered in intercellular communication. Thus, the pulsatile administration of platelet derived growth factor (PDGF) is indispendable to growth and transparency of the lens (Brewitt and Clark, 1988), whereas the physiological effect of insulin was shown, as for GnRH, to depend on the frequency of its pulsatile administration (Lefèbvre et al., 1987; Paolisso et al., 1991). It is likely that in other hormonal systems, pulsatile stimuli will also be found to be more efficient than constant ones. The conclusion that optimal periodic stimuli are more efficient than random pulsatile signals might thus bear on a large class of intercellular communication processes.

Another result of general significance relates to the compared efficiency of periodic and aperiodic stimuli. The present study provided an opportunity to test the possible benefits of chaos in cellular physiology (Pool, 1989). Much as for stochastically varying pulses, the responsiveness associated with chaotic signals is inferior to that related to the optimal periodic signal. The results on chaos were obtained by means of a particular aperiodic dynamics, namely, that of the logistic map; in view of the foregoing discussion, however, the conclusions as to the superiority of the optimal periodic signal versus chaotic stimuli should hold, at least qualitatively, for chaotic time series generated by other types of aperiodic dynamics.

Although most of the results were obtained by taking the optimal pattern of periodic stimulation as reference signal for comparison with stochastic or chaotic stimuli, other periodic signals differing from the optimal one were also considered, yielding essentially similar results. For most of these reference periodic stimuli, the same hierarchy in efficiency was found. In a few cases, however, the signal subject to stochastic or chaotic variations in τ_1 only was at least as efficient as the (nonoptimal) reference periodic signal at nonnegligible values of the variance (see Fig. 8).

When the reference periodic signals differs from the optimal one, cell responsiveness is smaller but the decrease in responsiveness due to stochastic or chaotic variations remains rather limited (compare Figs. 7 and 8) owing to the fact that the surface yielding α_R as a function of τ_0 and τ_1 is sharp only near the optimum (Fig. 2). This leads to the conclusion that the pulsatile (rather than continuous) nature of the stimulus is probably the most important characteristic of the signal. The fact that the latter is strictly periodic rather than subject to stochastic or aperiodic variations is of lesser consequence. This conclusion might be of physiological significance in view of the many examples of "noisy" episodic hormone secretion observed in endocrinology (see, e.g., Crowley and Hofler, 1987; Leng, 1988).

The question arises as to how the present results depend on the model considered and on the definition of cell responsiveness. As to the first aspect, we note that the desensitized receptor model analyzed here is of a very general form that should apply to a variety of experimental situations. From a kinetic point of view, the essential characteristics underlying the occurrence of an optimal response as a function of the pulsatile stimulus are as follows: (*a*) prolonged stimulation by the ligand elicits an initial response followed by a slower decay due to desensitization; (*b*) progressive recovery of cell responsiveness occurs upon removal of the ligand. Any physiological system possessing such properties should exhibit maximum responsiveness to an optimal pattern of pulsatile stimulation and the present results on the comparative efficiency of periodic versus stochastic or chaotic stimuli should hold qualitatively for such systems regardless of the molecular mechanism of desensitization. The latter process need not be directed only at the receptor. Thus, desensitization may involve the agonist-induced inactivation of a calcium channel as in pituitary cells (Stojilkovic et al., 1989), or the inhibitory action of G proteins as in cAMP signaling in *Dictyostelium* (Snaar-Jagalska and Van Haastert, 1990).

As to cellular responsiveness, definitions other than that given by Eq. 6 are possible. Thus, one could consider the amplitude of the response, or the mean response per pulse. None of these definitions, however, would provide a measure of the cellular response over a meaningful amount of time. For a sufficiently large pulse interval τ_0, the amplitude would simply approach a maximum value. Similarly, the mean response per pulse, equivalent to the integrated activity during one on-phase τ_1, saturates to a maximum value; as τ_0 increases (see Fig. 4 in Goldbeter and Li, 1989; and Fig. 3 in Li and Goldbeter, 1990). On the other hand, the mean response per unit pulsatile stimulation, i.e., the integrated activity α_T divided by the interval $(\tau_0 + \tau_1)$, should behave in a manner similar to cellular responsiveness α_R. That such is the case has been shown in the more specific model considered for cAMP signaling in *Dictyostelium* (Li and Goldbeter, 1990), where the maximum mean level of cAMP generated by the stimulus occurred for an optimal pattern of cAMP pulses very close to that yielding a maximum in cell responsiveness defined in a manner similar to that of Eq. 6. Therefore, we expect that measures of the cellular response will either saturate to a maximum value or exhibit an optimum as the interval τ_0 increases, according to whether or not they involve a division through the time period considered. The latter definitions are more meaningful from a physiological point of view since they provide an average, long-term measure of cellular behavior in response to stimulation.

In the present study, as well as in our previous analysis (Li and Goldbeter, 1989), the efficiency of the pulsatile stimulus was defined in terms of an activity generated above a basal level. Such a situation can be justified by assuming that it is the response that exceeds a certain threshold, taken equal to the basal activity level, that has to be maximized. Whereas the only changes in activity to be considered then take place during the on-phase of stimulation, the changes elicited upon ending each pulse could also be of physiological relevance. Thus, during the off-phase of the square-wave stimulus, the activity drops below the basal level (see Figs. 5 and 6). This drop is due to the sudden removal of ligand, characteristic of the square-wave stimulation. The drop below the basal level still occurs when considering a more realistic signal with exponential decay of the ligand, but it is generally less marked than for the square-wave stimulus (see Fig. 11 in Li and Goldbeter, 1989); the magnitude of the drop then depends on the rate of exponential decay.

The excursions below the basal level could be of physiological significance and might be associated with the withdrawal syndrome observed for a number of drugs in pharmacology (Gero, 1985). Minimizing the magnitude of these subbasal activity excursions so as to reduce withdrawal symptoms could represent an alternative or complementary mode of optimizing the pulsatile stimulus. The link with pharmacology can be extended by noticing that besides its applicability to physiological ligands, the present study bears on the search for optimal patterns of drug delivery.

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