A Class of Parametrically Excited Calcium Oscillation Detectors

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ABSTRACT Intracellular Ca^{2+} oscillations are often a response to external signals such as hormones. Changes in the external signal can alter the frequency, amplitude, or form of the oscillations suggesting that information is encoded in the pattern of Ca^{2+} oscillations. How might a cell decode this signal? We show that an excitable system whose kinetic parameters are modulated by the Ca^{2+} concentration can function as a Ca^{2+} oscillation detector. Such systems have the following properties: (1) They are more sensitive to an oscillatory than to a steady Ca^{2+} signal. (2) Their response is largely independent of the signal amplitude. (3) They can extract information from a noisy signal. (4) Unlike other frequency sensitive detectors, they have a flat frequency response. These properties make a Ca^{2+}-sensitive excitable system nearly ideal for detecting and decoding Ca^{2+} oscillations. We suggest that Ca^{2+} oscillations, in concert with these detectors, can act as cellular timekeepers to coordinate related biochemical reactions and enhance their overall efficiency.

INTRODUCTION

Intracellular Ca^{2+} oscillations are often a response to external signals in cells as diverse as fish eggs and human neurons. Changes in the external signal are often reflected in changes in the frequency, amplitude, or form of these oscillations, suggesting that the information carried by the external signal may be encoded in the pattern of Ca^{2+} oscillations. In hepatocytes, for example, changes in the angiotensin concentration modulate the Ca^{2+} oscillation frequency, while the amplitude, baseline value, and waveform remain constant. This observation by Woods et al. (1986) led to their proposition that hepatocytes use frequency modulation (FM) to encode information, as in FM radio broadcasting.

What a cell does with this information depends on the response of the *receiver* to the Ca^{2+} oscillations. In this paper we describe a class of biochemical systems that can act as receivers and decoders of the Ca^{2+} oscillation-encoded information. These biochemical systems, called parametric Ca^{2+} oscillation detectors, or PCODs, become entrained to the Ca^{2+} oscillations and produce large amplitude oscillations. These large amplitude oscillations, the output of the receiver, may serve as a starting point of other cellular chemical reactions. We use the term parametric because the modulation of some kinetic parameter of the biochemical system by Ca^{2+} is central to the activation of these systems.

The essential dynamic property of PCODs is excitability. Excitable is used in the same sense as in nerve axons; that is, the system is stable to small disturbances but "fires" when the disturbance exceeds some threshold. Excitable behavior is not uncommon in biological systems, and many oscillating systems can, by a change in parameter values, be made excitable. Because the known cellular oscillators number over a hundred (Rapp, 1979) the class of PCODs may be quite large. Thus PCODs are not part of a novel class of dynamical systems but rather part of a large and familiar one. What is novel is our *interpretation* of the response of excitable systems to periodic parameter modulation.

We will show that PCODs have the following properties: (1) PCODs are more sensitive to an oscillatory Ca^{2+} signal than to a steady elevated Ca^{2+} concentration. (2) The response of PCODs is largely independent of the amplitude of the Ca^{2+} oscillations. (3) PCODs can extract information from a very noisy signal. (4) Unlike other frequency-sensitive detectors, PCODs have an almost flat frequency response. We show how these properties allow a PCOD to operate as a receiver within the constraints imposed by using Ca^{2+} as a signaling molecule.

We suggest that Ca^{2+} oscillations, in concert with PCODs, can act as cellular timekeepers to temporally coordinate related biochemical reactions and, perhaps, enhance their efficiency and throughput.

A MODEL PARAMETRIC Ca^{2+} OSCILLATION DETECTOR

A mechanical energy analog

The operation of a PCOD and most of its important properties can be understood using a mechanical energy analog of an excitable system shown in Fig. 1. Fig. 1 *A* shows the ball at rest at the local energy minimum $x = x_0$. Distorting the energy profile (Fig. 1 *B*) causes the ball, starting from x_0, to roll down to the new energy minimum. In this case the distortion is small enough that the ball makes only a small local adjustment to the new energy minimum. If, however, the energy profile distortion is large enough (Fig. 1 *C*) the ball finds itself beyond the local energy maximum (the threshold value) and rolls down the big energy hill to land far from its starting position. This is analogous to the threshold phenomenon in nerve axons.

What underlies the distortion of the energy profile? The key assumption in our model is that changes in the Ca^{2+} concentration modulate the velocity field (described below),

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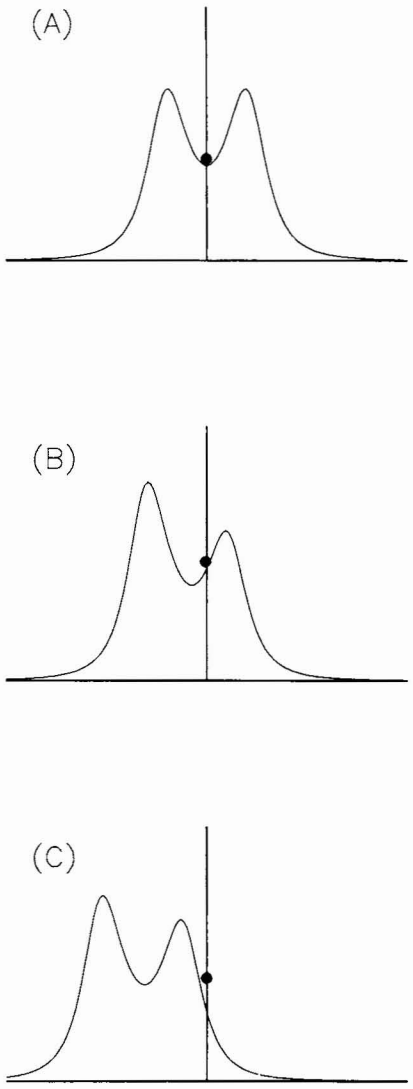

FIGURE 1 Energy analog for the parametric Ca^{2+} oscillation detector (PCOD). Ca^{2+} oscillations modulate the velocity field of the PCOD, which is analogous to the energy profile in a mechanical system. (*A*) The ball is at the local energy minimum at $x = x_0$. (*B*) A small change in k distorts the energy profile, moving the energy minimum to a new location, and the ball moves only a little to adjust to the new energy minimum. (*C*) A sufficiently large change in k distorts the energy profile enough so that the ball no longer is attracted to the new local energy minimum; instead it rolls down the large energy well to land far from its starting point.

which is the analog of the energy profile. So suppose that the Ca^{2+} oscillations cause the energy profile to alternate between that in (Fig. 1 *A*) and (Fig. 1 *C*). If after falling the ball is repeatedly returned to the little energy well, then the ball will periodically drop from a great height. Thus small periodic changes in the energy profile can produce large periodic changes in the height of the ball.

A model PCOD

Many oscillatory systems can become excitable by a change in parameter values. One such system is Goldbeter and Segel's (1977) model for cyclic adenosine 3′,5′-monophosphate (cAMP) oscillations in the slime mold *Dictyostelium discoideum*. Segel's (1989) simplification of the original model provides a convenient system to demonstrate the properties of PCODs. The simplified model has two differential equations, so phase plane methods can be used to analyze and explain how PCODs operate; numerical simulations show that the simplified model yields the same conclusions as the original. Segel's equations are

$$\dot{\alpha} = f(\alpha, \beta) = 1 - \sigma\phi(\alpha, \beta), \tag{1}$$

$$\dot{\beta} = g(\alpha, \beta) = q\sigma\phi(\alpha, \beta) - k_t\beta, \tag{2}$$

where α and β are the intracellular concentrations of adenosine triphosphate (ATP) and cAMP, respectively. Segel's simplification comes from eliminating the differential equation for the extracellular cAMP (γ), approximating it as an algebraic function of β, $\gamma(\beta) = k_t\beta/(hk)$.

σ is the ratio of the maximum cAMP production rate to the substrate input rate, k_t is the decay rate of intracellular cAMP, and k is the ratio of the decay rate of extracellular cAMP to the maximal input rate of ATP. h is the ratio of extracellular volume to total intracellular volume, and $q = K_s/K_p$, where K_s is the Michaelis constant for adenylyl cyclase and K_p is the dissociation constant for extracellular cAMP. ϕ describes the conversion of ATP to cAMP and is given by

$$\phi(\alpha, \beta) = \frac{\alpha(1 + \alpha)(1 + \gamma(\beta))^2}{L + (1 + \alpha)^2(1 + \gamma(\beta))^2}, \tag{3}$$

where L is the allosteric constant.

Ca^{2+} activates some classes of adenylyl cyclase (type I and III; Choi et al., 1992; Wu et al., 1993) and inhibit others such as those found in cardiac myocytes (Yu et al., 1993; Colvin et al., 1991) and in *D. discoideum* (Monk and Othmer, 1989). We therefore assume that changes in the Ca^{2+} concentration alter the value of q while leaving the other parameters unchanged. The properties of PCODs presented below are not unique to the assumption that q is Ca^{2+} dependent. We got similar results, not shown here, by assuming that any one of the other parameters k, σ, h, k_t, or L, is modulated by Ca^{2+}.

The basic ideas illustrated in the mechanical energy analog translate naturally to the dynamical system given by Eqs. 1 and 2. The appropriate setting for this translation is the α-β plane, or *phase plane* (Segel, 1989). Analogous to the ball's coordinate x is the *state* or *phase* of the PCOD ($\alpha(t)$, $\beta(t)$). In the mechanical energy analog the energy profile or potential energy gradient determines the motion of the ball. Here, the velocity field $(\alpha, \beta) = (f(\alpha, \beta; q), g(\alpha, \beta; q))$ determines the motion of the phase point in the phase plane. The movement of the phase point is like that of a ball moving on a complex landscape of hills, valleys, cols, and depressions. The key assumption in our model is that changes in Ca^{2+} alter the velocity landscape through changes in the parameter q. In effect, changes in Ca^{2+} move the location of the hills and valleys, flattening some, raising others.

For a system to function as a PCOD it suffices that it possess three dynamic properties: (P1) existence of a unique

stable steady state; (P2) global boundedness; and (P3) threshold behavior, that is, large changes in (α, β) result from arbitrarily small deviations beyond a certain region of attraction. Properties (P1) and (P2) ensure that the solution returns to the steady state (SS). Collectively, we call these three properties excitability.

Response of the PCOD to disturbances

To understand how the cAMP system responds to periodic q modulation we begin by studying its response to a special kind of perturbation. In the phase plane, shown in Fig. 2, the vertical ($\dot{\alpha}$) and horizontal ($\dot{\beta}$) velocity components of the phase point anywhere in the plane are given by Eqs. 1 and 2, respectively. Of special importance are the loci of points where $\dot{\alpha} = 0$ and $\dot{\beta} = 0$. These are the α- and β-nullclines, shown as the solid curves in Fig. 2. $\dot{\beta}$ is positive for points above the β-nullcline, so the trajectory moves rightward; conversely, below the β-nullcline, $\dot{\beta}$ is negative, and the trajectory moves leftward. Similarly, for points above (below) the α-nullcline, $\dot{\alpha}$ is negative (positive) so the trajectory moves downward (upward). Thus trajectories begin to reverse their direction on the nullclines, and any solution trajectory ($\alpha(t)$, $\beta(t)$), for example the one shown by the dashed loop, crosses the α-nullcline (β-nullcline) horizontally (vertically) because at their intersection $\dot{\alpha} = 0$ ($\dot{\beta} = 0$). Thus the nullclines mark the horizontal and vertical limits of the solution. This system has a *unique* SS found at the intersection of the nullclines.

Property (P1), stability of the unique SS, is satisfied when the α-nullcline intersects the ascending part of the β-nullcline near the local maximum. Linear stability analysis (Segel, 1989) shows that the SS is a stable focus. Thus after a *small* perturbation the system returns to the SS through a series of damped oscillations as shown in Fig. 3 *A*, and the trajectory is shown as the small dashed-dotted loop in Fig. 2. A sufficiently large perturbation, however, causes the system to "fire," generating a large spike seen in Fig. 3 *B*; the corresponding trajectory is shown as the dashed curve in Fig. 2.

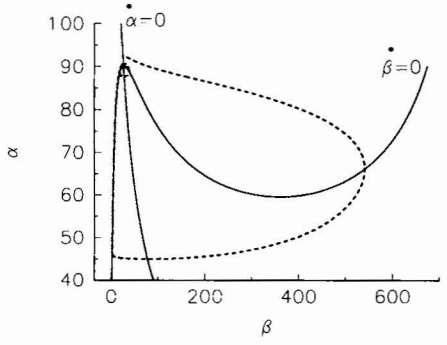

FIGURE 2 The phase plane for Eqs. 1 and 2. The nullclines are shown by the solid curves. The β-nullcline has a characteristic n-shape common to many excitable systems. The small loop (*dotted-dashed*) is the solution trajectory for a subthreshold perturbation; the large loop (*dashed*) is the trajectory for a suprathreshold perturbation. The parameters used were $q = 105$, $\sigma = 30$, $k_t = 4$, $h = 10$, $L = 10^6$, and $k = 10$. These parameter values, except for q, are used throughout the paper.

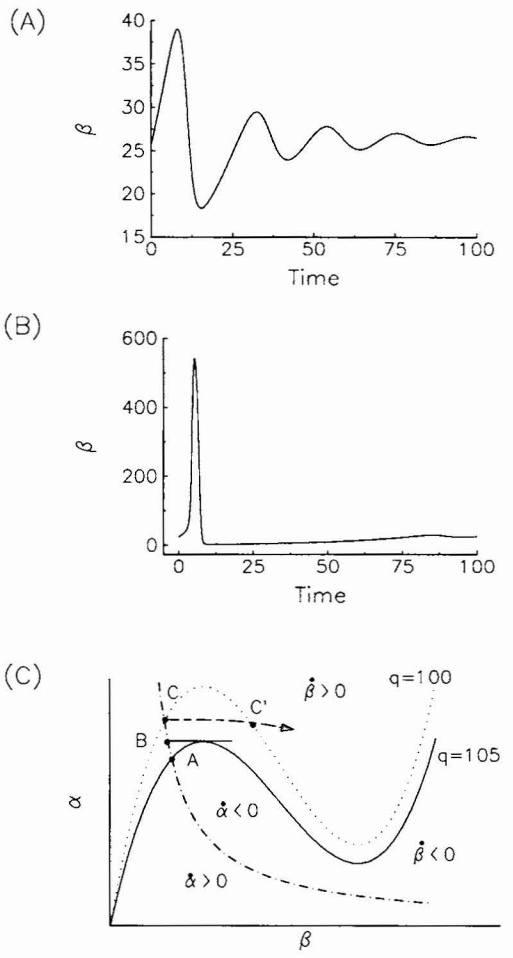

FIGURE 3 Response to subthreshold (*A*) and suprathreshold (*B*) perturbations. *C* is a caricature of the nullclines for two values of q. The β-nullclines have an n shape, shown dotted for $q = 100$ and solid for $q = 105$. Only a single α-nullcline (*dotted-dashed*) is needed because this nullcline is independent of q. The steady states (points A and C) lie at the intersection of the α- and β-nullclines. Point B on the α-nullcline is level with the local maximum of the β-nullcline for $q = 105$; any perturbation from A along the α-nullcline that is below B will not generate a large spike. α is negative above the α-nullcline and positive above it. β is positive above the β-nullcline and negative below it.

The origin of these qualitatively distinct responses to perturbations can be understood by using the phase plane schematic in Fig. 3 *C*. These curves are topologically accurate representations of the nullclines for $q = 100$ (dotted curve) and $q = 105$ (solid curve). We use these schematic nullclines because the curvatures of the actual β-nullclines are too subtle near the SS to clearly illustrate the dynamics in this region. Only a single α-nullcline is needed because this nullcline is independent of q.

The β-nullcline has a characteristic n shape that is common to many excitable systems such as FitzHugh's BVP model for axons (FitzHugh, 1961). The importance of the n-shaped nullcline becomes evident when we make subthreshold or suprathreshold perturbations to the SS. Let q be

equal to 105 and displace the state from point A (the SS) along the α-nullcline toward C to a location below point B that is level with the local maximum of the β-nullcline. The reason for displacing the state along the α-nullcline instead of along an arbitrary direction is to prepare for the next section where q will be modulated. In that case the initial point will lie along the α-nullcline at a position determined by the instantaneous value of $q(t)$. Starting from the initial displacement the state will follow a trajectory that moves downward and toward the right (because $\dot{\beta}$ is positive and $\dot{\alpha}$ negative) until, very shortly, it intersects the β-nullcline to the left of the local maximum. At the β-nullcline the rightward movement is arrested and the trajectory begins to turn back to the left. The net result is a small damped oscillation shown as the dashed-dotted curve in Fig. 2.

Now consider a suprathreshold displacement from A to C, which is the SS for $q = 100$. The state follows a trajectory (labeled CC′) leading downward and to the right as before. But because the state starts sufficiently high, it clears the local maximum of the β-nullcline and shoots off to the right. The rightward movement is eventually bounded by the ascending part of the β-nullcline. This perturbation generates the large spike in Fig. 3 *B* whose trajectory is shown as the dashed curve in Fig. 2.

It is neither easy nor necessary to determine analytically the precise perturbation magnitude needed to reach the threshold for firing. Nevertheless, it is useful to note that point B marks the lower bound (but not the greatest lower bound since the trajectory has a downward component) for suprathreshold perturbations along the α-nullcline. As the parameters change and bring the intersection of the α- and β-nullclines (that is, the SS) closer to the the local maximum, the threshold for firing becomes smaller. When the SS coincides with the β-nullcline's local maximum the firing threshold is zero: the SS is no longer stable and the system oscillates spontaneously.

A study of the vector field throughout the phase plane shows that the trajectories of Eqs. 1 and 2 are globally bounded. Thus the cAMP system has all the properties of excitability: unique SS, global boundedness, and threshold behavior.

Response of the PCOD to periodic parameter modulation

To see one of the most important advantages of using oscillatory instead of steady Ca^{2+} signaling, we first need to establish the system's response to different values of *constant* q. Fig. 4 shows the relationship between q and the amplitude of the spontaneous cAMP oscillations. For $q < q_c \approx 109$, the system is stable, and no spontaneous oscillations occur. Large amplitude oscillations arise for $q \geq q^* \approx 111$. Note in particular that no sustained oscillations occur when q is held fixed between 100 and 105.

Now suppose q increases monotonically with increasing Ca^{2+} concentrations and that Ca^{2+} oscillations modulate q between 100 and 105. In other words, we assume that a Ca^{2+} rise inhibits adenylyl cyclase. Ca^{2+} oscillations can vary, in the same cell, from spiky to sinusoidal depending on experimental conditions, such as a neurotransmitter concentration (Lakatta et al., 1985; Wakui et al., 1989). We start by using rectangular modulation of q not only to simulate spiky Ca^{2+} oscillations but also because in this case the origin of parametric oscillations can be readily understood in terms of the super- and subthreshold perturbations. Fig. 5 shows the surprising result that periodically modulating q generates large amplitude cAMP oscillations that continue as long as the modulation occurs. Why does the modulation of q between two values produce large amplitude oscillations while no oscillations occur with q fixed at a value in that range?

As q jumps between 100 and 105, the β-nullcline in Fig. 3 *C* alternates between the dotted and solid curves. The system starts with $q = 100$ at the stable SS, point C in Fig. 3 *C*. The system remains in this state until q suddenly jumps

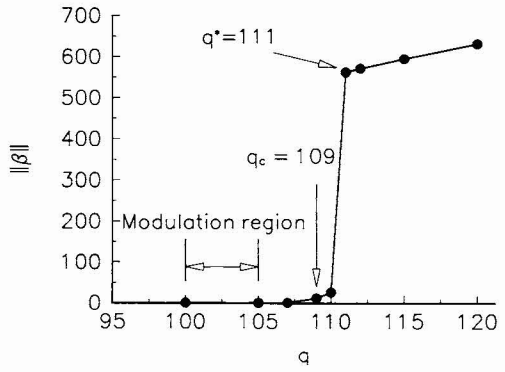

FIGURE 4 Amplitude of spontaneous oscillations for constant q. The amplitude $\|\beta\|$ is defined as the difference between the maximum and minimum values of β in one period. Spontaneous oscillations (indicated by a nonzero amplitude) occur when the steady state loses stability, which happens when $q > q_c \approx 109$. Large amplitude oscillations occur for $q \geq q^* \approx 111$. The double-headed arrow marks the extreme values of $q(t)$ that generated the large cAMP oscillation in Fig. 5.

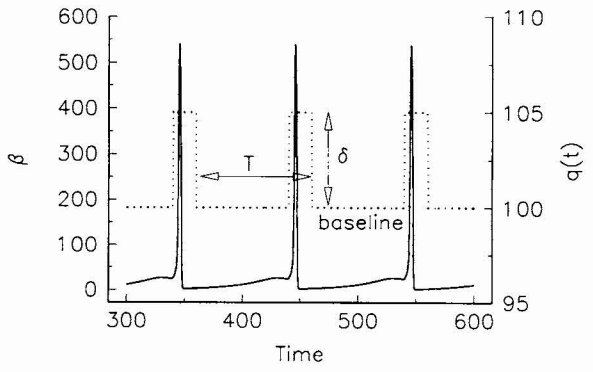

FIGURE 5 Large amplitude cAMP oscillations (*solid curve*) resulting from periodic modulation of q (*dotted curve*). The rectangular modulation function has baseline value $q_0 = 100$, period $T = 100$, amplitude $\delta = 5$, and duty cycle DC = 0.2. These large oscillations occur despite the maximum $q(t)$, 105, being less than q_c, the minimum value of *constant* q needed to generate spontaneous oscillations. The modulation period is large enough that each $q(t)$ pulse triggers a cAMP spike.

to 105. At this instant the velocity landscape changes and the β-nullcline shifts to the solid curve. Note that at the instant q jumps to 105 the system's state is still at point C, so the conditions are identical to that which produced the large pulse in Fig. 3 *B*. The time that q remains equal to 105 is long enough for the state to move past C′ so that, when q drops back to 100, the trajectory will not be corralled by the dotted β-nullcline, and a large pulse is generated. Since the modulation period (T) is very long ($T = 100$) the system has ample time to return almost to the SS (point C) before q jumps back again to 105 and the cycle is repeated.

This analysis lies at the core of understanding the response of an excitable system to periodic parameter modulation. Thus it is worthwhile to expand and summarize the results of previous paragraph. Whether periodic q modulation will generate large cAMP oscillation depends on both the modulation amplitude $\delta(t) = q(t) - q_0$ (here the baseline q_0 equals 100) and the time q during which remains displaced from the baseline. This time, τ, is the product of the modulation period and the duty cycle (DC), which is the fraction of the modulation period where $\delta(t) \neq 0$. δ must be large enough that the β-nullclines for $q = q_0$ and $q = q_0 + \delta$ have the configuration shown in Fig. 3 *C*. In particular, δ must be large enough that the SS for $q = q_0$ lies above the local maximum of the β-nullcline for $q = q_0 + \delta$. Referring again to Fig. 3 *C*, τ must be long enough for the state to move for C at least to C′ before q returns back to q_0. For δ and τ smaller than these minimum values, large amplitude oscillations cannot be parametrically excited.

Small modulations can excite large amplitude oscillations

The cytotoxicity of high Ca^{2+} concentrations requires the cell to keep the Ca^{2+} concentration low. This requirement imposes a number of constraints on using Ca^{2+} as a signaling molecule (see Discussion). Fig. 6 shows two important properties of PCODs that allow them to compensate for these constraints. This figure shows how the amplitude of the cAMP oscillation depends on the amplitude of the rectangular modulation function. The abscissa is the percent change from the baseline value ($q_0 = 100$). The modulation period and the duty cycle were fixed to $T = 100$ and DC = 0.2.

The first important property to be gleaned from this graph is that the fractional change in q needed to excite large amplitude cAMP oscillations is small; ~3% modulation of q suffices. By contrast, if q were not periodically modulated but held at a fixed value, q would need to increase by ~11% (indicated by the arrow) from the baseline value before large cAMP oscillations would be seen at $q = q^*$. Even at this value the oscillation amplitude (indicated by the square) would be smaller than when q is modulated.

This property of PCODs shows an advantage Ca^{2+} oscillations have over a steady Ca^{2+} signal. Assuming again that q increases monotonically with increasing Ca^{2+} concentration, Fig. 6 shows that the PCOD responds more strongly to an oscillatory Ca^{2+} signal than to a high steady Ca^{2+} signal

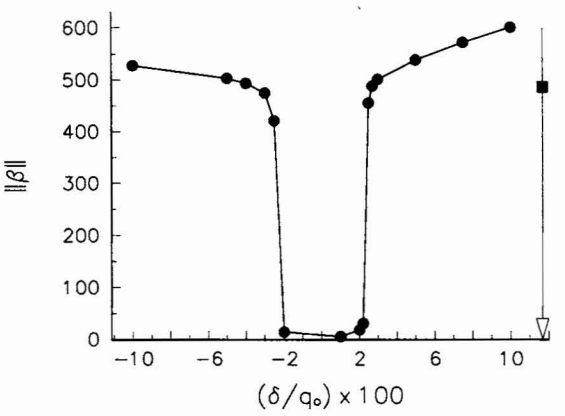

FIGURE 6 cAMP oscillation amplitude (circles) as a function of $q(t)$ modulation amplitude. The $q(t)$ waveform was rectangular as in Fig. 5. The abscissa is the percent change of $q(t)$ from the baseline value, $q_0 = 100$. The almost flat response profile is an essential quality of receivers of frequency encoded information; it allows the output of the receiver to be independent of the signal strength. The arrow shows the change needed to attain the value of q^*, and the square shows the resulting amplitude of the spontaneous oscillations. The modulation function had a period of 100 and a duty cycle of 0.2.

even when the *peak* of the oscillation is lower than the steady signal. In other words, small amplitude Ca^{2+} oscillations are more effective in exciting a PCOD than a high steady Ca^{2+} signal.

Fig. 6 also shows that periodic *negative* modulation of q can also elicit large amplitude cAMP oscillations. The origin of these large cAMP oscillations can be understood using Fig. 3 *C*, but this time let the β-nullcline for $q = 100$ be solid and let the dotted β-nullcline be that for $q = 95$. Starting from A, when q drops to 95 the state will follow a leftward and upward trajectory to C. Provided q remains at 95 long enough for the state to approach C, then when q jumps back up to 100 the state will move rightward and downward, clearing the hump of the solid β-nullcline and generate a large spike. This result means that large amplitude cAMP oscillations may also be generated in some cells having type I or type III adenylyl cyclase, which are *activated* by Ca^{2+} (Choi et al., 1992; Wu et al., 1993).

The second important property of a PCOD is that the cAMP oscillation amplitude is almost independent of the modulation amplitude beyond a critical value (~3% in this particular case). If information is encoded in the Ca^{2+} oscillation frequency, then unambiguous interpretation of the message requires that the output of the detector be independent of the signal strength. This independence arises because the output of the PCOD is determined by its own internal dynamics; the Ca^{2+} signal only triggers the generation of the large cAMP pulse. The PCOD behaves as a high input impedance device, which draws little power from the signal source.

Frequency response of a PCOD

Fig. 7 shows the amplitude and the normalized period of the cAMP oscillations as functions of the period of rectangular

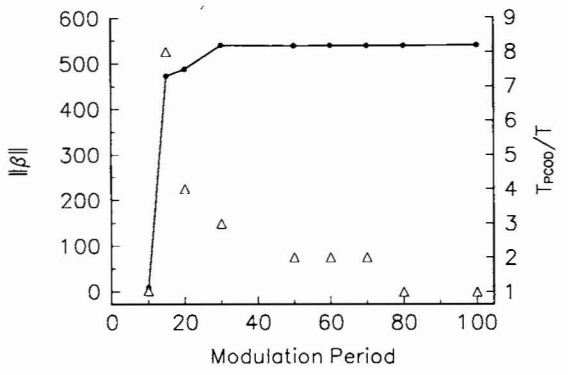

FIGURE 7 Amplitude (*circles*) and normalized period (*triangles*) of cAMP oscillations as functions of the $q(t)$ modulation period. The $q(t)$ waveform was similar to that in Fig. 5, except for the modulation period. The flat amplitude response for $T > 15$ shows that PCODs are poor frequency *discriminators*, unlike, say, a tuned tank circuit. The normalized period is the period of the cAMP oscillations divided by the modulation period, T_{PCOD}/T. The period of the PCOD is the smallest integer multiple of T that is greater than the time required for the PCOD to return to the SS after a suprathreshold perturbation, a time of ~80.

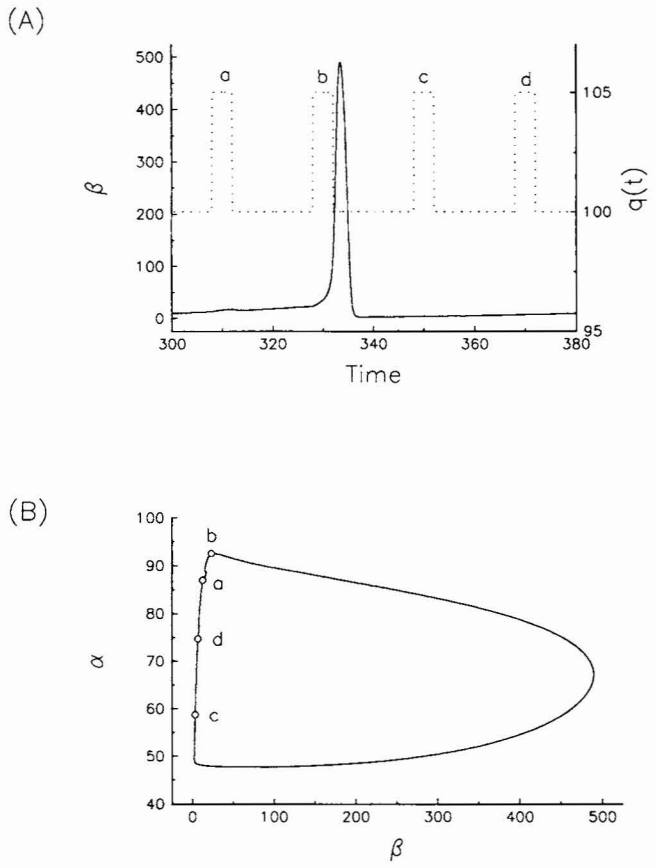

FIGURE 8 The subharmonic response, $T_{PCOD}/T > 1$, can be understood by examining the phase of the system (α, β) where the modulation pulse occurs. *A* shows that $q(t)$ pulses at times c and d have little effect, while the pulse at a has a small effect and a large spike is generated at b. The circles in *B* show the phase where the pulses occurred. Pulses a and b occur when the phase system is near the SS, which is where the system is most sensitive to perturbations. Pulses c and d occur when the system is insensitive to perturbations, analogous to the relative refractory period in nerve axons.

modulation of $q(t)$. The normalized period is the period of the cAMP oscillations divided by the modulation period, T_{PCOD}/T. δ and DC were fixed to 5 and 0.2. The amplitude is almost constant (≈500) down to $T \approx 15$ then drops to less than 10 for $T = 10$. Large amplitude spikes occur for T between 10 and 15, but the oscillations are not periodic and appear to be chaotic. We also found chaoticlike behavior when modulation parameters took on values in the transition regions of Figures 6 and 9.

This amplitude-period plot or tuning curve highlights a fundamental difference between PCODs and some other devices, such as a tuned tank circuit, that can also be used to detect oscillatory signals. A tank circuit responds most avidly to signals whose frequencies are close to its natural frequency, so its tuning curve has a more or less bell shape; the sharper the bell shape, the greater the frequency discrimination. PCODs, by contrast, respond equally well to almost all frequencies, so they make poor frequency discriminators. We shall see later that this property of PCODs allows Ca^{2+} oscillations to behave as cellular timekeepers.

The period of the cAMP oscillations is an integral multiple of the Ca^{2+} oscillation period. When the Ca^{2+} oscillation period is long ($T \approx 100$), T_{PCOD} equals T because the cAMP system has time to return to near its SS before the next Ca^{2+} spike arrives to trigger another cAMP pulse. For intermediate modulation period lengths ($T \approx 75$ down to $T \approx 14$) the cAMP oscillation period is longer than the modulation period. This subharmonic response ($T_{PCOD}/T > 1$) can be readily understood with the help of Fig. 8. Fig. 8 *A* shows the $q(t)$ modulation over one period of the cAMP oscillation. The modulation period $T = 20$ is one-fourth of T_{PCOD}. The solution trajectory in the phase plane is shown in Fig. 8 *B*. The circles show where the modulation pulses occurred; the letters correspond to the pulse labels in Fig. 8 *A*. Pulses c and d occur during phases of the cycle far from the SS point, which is where the cAMP system is most sensitive to perturbations. Accordingly, these $q(t)$ pulses have no perceptible effect on the trajectory. Only when the system is near the SS do small $q(t)$ pulses have appreciable effect; pulse a excites a small response and pulse b initiates a full cAMP spike. Thus the period of the cAMP oscillations is the smallest integer multiple of the Ca^{2+} oscillation period that is greater than the time required for the cAMP system to return to the SS after a suprathreshold perturbation, a time of ~80 in this case.

To understand why large amplitude oscillations disappear when T is small ($T \approx 10$) we return to Fig. 3 *C*. When q jumps from 100 to 105 the system starts to move from C, rightward on the trajectory CC′. But because $\tau (= T \times DC)$ is so small, when q drops back to 100 the state has not moved beyond C′, and it becomes corralled by the dotted β-nullcline. The energy analogy provides an alternative explanation. With the energy profile in Fig. 1 *C* the ball begins to move, but because the modulation period is short the ball does not move appreciably before the energy profile switches back to that in

Fig. 1 *A* where it begins to relax back to the stable SS. Thus the ball is simply jostled back and forth at the modulation period.

Response of the PCOD to sinusoidal parameter modulation

We have been using a rectangular modulation function; now assume that $q(t)$ varies sinusoidally between 100 and 105. Fig. 9 shows the amplitude and T_{PCOD}/T as a function of the modulation period T. The response is similar to that shown in Fig. 7 except that now the PCOD no longer responds to long period modulation.

The generation of large amplitude oscillations at moderate frequencies and the disappearance of large amplitude oscillations at high frequencies in this case can be understood by using the same reasoning used as when q underwent rectangular modulation. The disappearance of large amplitude oscillations at low frequencies is new and points out another property of the signal required for parametric excitation.

With low frequency rectangular modulation, although the period between pulses is long, the transition from $q = 100$ to $q = 105$ is instantaneous. Because τ is long in this case, the state in Fig. 3 *C* has time to move beyond C′ before q returns to 100 and the system fires. With sinusoidal modulation the β-nullcline changes continuously between that for $q = 100$ and $q = 105$. If the change is slow enough, the state has time to relax to the slowly moving SS. Thus the system is always close to the SS so no firing occurs. Thus the oscillations are small, simply reflecting the movement of the SS and its period equals the modulation period.

Thus for a signal to elicit large amplitude oscillations, the rate of change of the signal must be faster than the PCOD's relaxation rate and slower than its excitation rate (inverse of the time for the state to go between C and C′ in Fig. 3 *C*). What this means is that the dynamics of the PCOD effectively filters out both high frequency noise (small τ) and slow variations of Ca^{2+}.

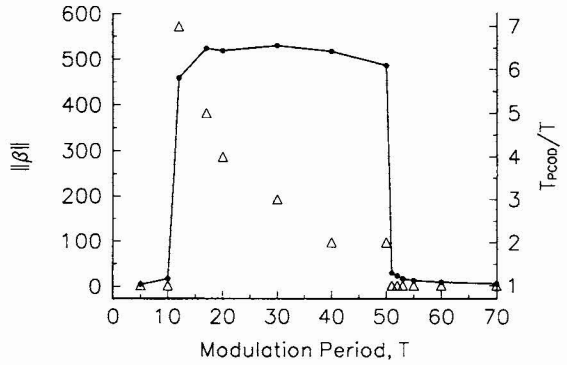

FIGURE 9 This figure is similar to Fig. 7, except $q(t)$ is sinusoidally modulated between 100 and 105. The system responds similarly to sinusoidal and rectangular $q(t)$ modulation except when the modulation period is large. See text for explanation.

The PCOD can extract information from a noisy signal

The ability of a PCOD to filter out high frequency noise becomes clear when we add Gaussian noise to the clean rectangular modulation function shown in Fig. 10 *A*. The solid curve in Fig. 10 *B* is the response to the clean rectangular modulation function in Fig. 10 *A*. The dotted curve in Fig. 10 *B* is the response to the noisy modulation function. At this scale the differences are imperceptible; the inset shows the effect of the noise when the system is near the SS. We used a long ($T = 150$) modulation period so the system could spend a long time near the SS where it is most susceptible to noise, thereby maximizing the chance of getting a spurious firing from a noise spike.

DISCUSSION

One of the main functions of Ca^{2+} in cells is to carry information of external events to the intracellular biochemical machinery. Early Ca^{2+} measurements in a population of cells indicated that a change in an external signal (neurotransmitter, hormone, drug, etc.) caused a shift in the steady level

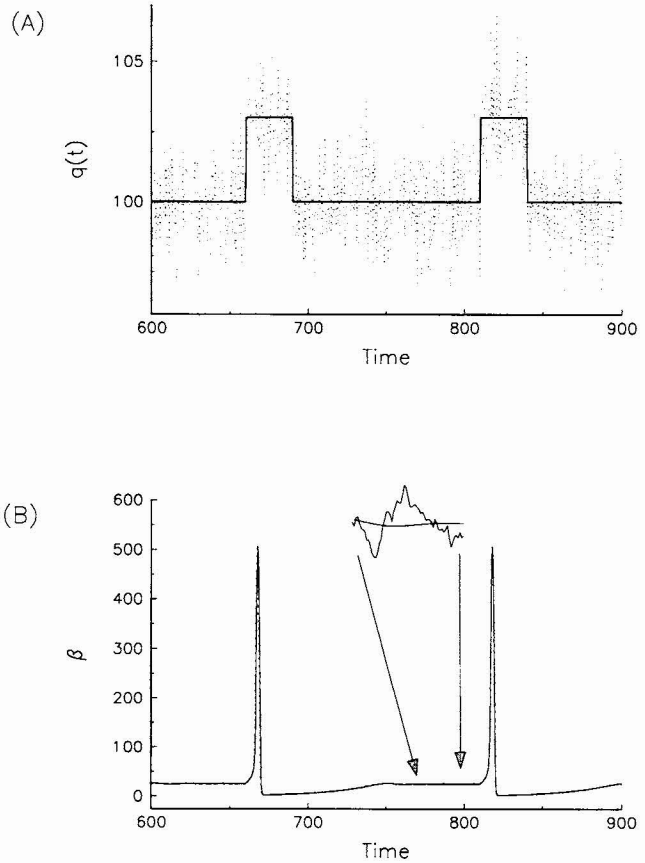

FIGURE 10 PCODs are virtually immune from high-frequency noise spikes. *A* shows a clean $q(t)$ signal (*solid curve*) and one corrupted by high-frequency noise (*dotted curve*). B shows that the PCOD responses to the noisy (*dotted*) and clean (*solid*) signals are virtually identical. The inset in *C* shows the small effect of the noise.

of intracellular Ca^{2+}. In other words these experiments suggested that information was encoded in the amplitude of the Ca^{2+} signal. Ensemble averaging, however, concealed a more dynamic and complex Ca^{2+} response to extracellular signals (Wilson et al. 1987). Later Ca^{2+} measurements in single cells revealed that Ca^{2+} oscillations are a common response to extracellular signals. See Berridge and Dupont (1994) for a short compendium of cells showing Ca^{2+} oscillations.

The use of oscillating Ca^{2+} signals may be Nature's solution to the constraints of using Ca^{2+} as a signaling molecule. Rasmussen and Barrett (1984) call Ca^{2+} a minatory signal because it kills at high concentrations. Thus cells have evolved a constellation of mechanisms to keep Ca^{2+} low ($\approx$100 nM at rest). But by letting Ca^{2+} rise moderately in response to external signals Nature has transformed a lethal toxin into one of the most versatile second messengers.

Since the resting Ca^{2+} concentration is so low, only a small Ca^{2+} influx is needed to cause a large fractional change in the cytosolic Ca^{2+} concentration. This makes Ca^{2+} signaling very economical inasmuch as only a small amount of energy is needed to remove the added Ca^{2+} from the cytosol and restore the resting state. The price of economy, however, is noise. Random opening of Ca^{2+} channels, unrelated to external signals, can also cause large fractional changes in the Ca^{2+} concentration and potentially wreak havoc on an amplitude based Ca^{2+} signal.

Frequency coding of information offers a greater immunity from noise corruption (Rapp et al., 1981). To take advantage of frequency coded information there must be receivers that can respond specifically to an oscillatory signal. Li and Goldbeter (1989) developed a receiver based on a model for receptor desensitization. Their model, like a tank oscillator, responds preferentially to a limited range of agonist oscillation frequencies. The frequency specificity arises from the interplay of the kinetics of receptor desensitization and the periodically changing kinetics of binding of agonist to receptor.

The PCODs are another class of reactions that can respond to oscillatory Ca^{2+} signals and function as receivers. This is potentially a very large class because many oscillators can be made into excitable systems by altering some parameters. Rapp (1979) lists over 125 cellular oscillators, and many more (including the Ca^{2+} oscillators) have been discovered in the intervening 15 years. It is quite possible that many of these oscillators have kinetic parameters that are modulated by Ca^{2+} and might function as PCODs.

One system that normally operates as a PCOD—though it is not thought of as such—is the Ca^{2+} control system in heart cells. The rhythmic membrane depolarization (the external signal) initiated by the sinoatrial node causes a periodic intracellular Ca^{2+} pulse. This Ca^{2+} pulse is received by the sarcoplasmic reticulum (SR), which triggers a regenerative Ca^{2+} release. It is the SR's ability to regeneratively release Ca^{2+}, known as calcium-induced calcium release, that confers excitability on the Ca^{2+} control system. The released Ca^{2+} starts a sequence of reactions resulting in muscle contraction. Ca^{2+}-ATPases on the SR membrane pump the Ca^{2+} back into the SR returning the system to the SS and ready for the next Ca^{2+} pulse. In this way the Ca^{2+} control system acting as a PCOD is entrained to the cardiac pacemaker, producing a correctly timed contraction. Our initial studies used a model for Ca^{2+} control in heart cells as a PCOD that gave results similar to those for the cAMP system. The decision to use the cAMP instead of the Ca^{2+} control system was made simply to avoid the confusion of having Ca^{2+} as both signal and output.

PCODs are near ideal Ca^{2+} oscillation receivers

The use of Ca^{2+} as a signaling molecule imposes a number of demands on the receiver. Noise is inevitable and, as Fig. 10 shows, PCODs can accurately extract the information from even a badly corrupted signal.

The demand to keep cytosolic Ca^{2+} low imposes another constraint on the Ca^{2+} signaling system. Cells contain cytosolic Ca^{2+} buffers, Ca^{2+}-ATPases, and Ca^{2+} exchangers to maintain Ca^{2+} low. While keeping cytosolic Ca^{2+} low they also limit the range of Ca^{2+} signaling to $\sim$5 μm (Allbritton et al., 1992). Thus the magnitude of the Ca^{2+} signal decays rapidly as it propagates from the oscillation generator. This decay is particularly acute for high frequency Ca^{2+} oscillations because diffusion acts as a low-pass filter. (The magnitude of the gradient can be estimated from the frequency dependent spatial decay length, $l(\nu_j) \sim (D/(\pi\nu_j))^{1/2}$, where ν_j is the frequency of the jth Fourier component of the signal and $D \approx 2 \times 10^{-7}$ cm^2/s (Allbritton et al., 1992) is the Ca^{2+} diffusion coefficient. The amplitude of a 1-Hz rectangular Ca^{2+} signal with 10% duty cycle would decrease by 66% just 2 μm from the generator.) The output of a receiver of frequency encoded signals should not depend on the magnitude of the signal. Otherwise, the meaning of the message would depend on the distance between the signal source and receiver. A PCOD satisfies this requirement, as shown in Fig. 6, because its output is determined by its internal dynamics; the Ca^{2+} signal only triggers the detector.

Since Ca^{2+} is toxic at high concentrations it is beneficial to use the smallest practical Ca^{2+} signal. Fig. 6 shows that PCODs can respond more strongly to a small oscillatory Ca^{2+} signal than to a high steady Ca^{2+} signal even when the peak of the Ca^{2+} oscillation is lower than the steady signal. This property of PCODs, coupled with PCODs' noise immunity, allows the cell to use *small* Ca^{2+} oscillations for signaling.

Synchronization of different reaction pathways

PCODs are made to be nonoscillatory although they can be made to oscillate spontaneously, in this example by raising q above q_c. There is an important advantage, however, of being nonoscillatory and entrained to the Ca^{2+} oscillations. Suppose there are two reaction pathways, which can function as PCODs, $PCOD_1$ and $PCOD_2$, which produce outputs O_1 and O_2 that are substrates for another common reaction. Suppose Ca^{2+} is raised to a *steady* level that causes $PCOD_1$ and

$PCOD_2$ to oscillate spontaneously. (Note that now we are using the name $PCOD_1$ for the reaction pathway, not implying that it functions as a PCOD.) It is extremely unlikely that $PCOD_1$ and $PCOD_2$ would have exactly the same intrinsic oscillation frequency so their outputs would move out of phase with each other. Thus the common reaction using O_1 and O_2 would have the proper ratio of these two substrates only for brief instants.

Instead, now suppose that $PCOD_1$ and $PCOD_2$ are entrained to the Ca^{2+} oscillation. Then the oscillation frequency is not set by their internal dynamics but by the Ca^{2+} oscillations. Then the common reaction sees a fairly constant ratio of O_1 and O_2.

Here the Ca^{2+} oscillator behaves as a master timekeeper and the Ca^{2+} oscillations as the ticks of a clock providing temporal coordination between $PCOD_1$ and $PCOD_2$. As the external signal changes the frequency of the Ca^{2+} oscillations, the pace of these related reactions is also changed. Because PCODs have flat frequency responses (Fig. 7) the outputs of $PCOD_1$ and $PCOD_2$ would be constant despite changes in the Ca^{2+} oscillation frequency. Thus the common reaction would simply be driven faster or slower without seeing changes in the amplitude of the substrates O_1 and O_2. The efficiency and throughput of the common reaction may be enhanced by the temporal coordination of $PCOD_1$ and $PCOD_2$, thereby suggesting another possible advantage of an oscillatory rather than a steady Ca^{2+} signaling mechanism.

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