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EXCITABILITY WITH MULTIPLE THRESHOLDS

A NEW MODE OF DYNAMIC BEHAVIOR ANALYZED IN A REGULATED BIOCHEMICAL SYSTEM

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We analyze in a biochemical model the phenomenon of excitability in which suprathreshold perturbations of a stable steady state are amplified in a pulsatory manner. The two-variable model is that of an autocatalytic enzyme reaction with recycling of product into the substrate. This model was previously studied for the coexistence between two stable periodic regimes (birhythmicity). We show that the multiplicity of dynamic behavioral modes extends to the phenomenon of excitability. Whereas excitable behavior is generally characterized by a single threshold for excitation, two distinct thresholds may coexist in this model. Moreover, in these conditions, two different plateaux are obtained for the response amplitude when the stimulus is gradually increased. By means of phase plane analysis we explain the origin of multiple thresholds for excitability and predict the conditions for their occurrence. Implications of the phenomenon for excitable cells, in particular for neurons, are discussed.

1. Introduction

Excitability is the phenomenon in which a system that operates in a stable steady state is capable of amplifying in a pulsatory manner suprathreshold perturbations. Excitable behavior is closely associated with the capability of undergoing sustained oscillations, as exemplified by chemical systems such as the Belousov-Zhabotinsky or Briggs-Rauscher reactions [1,2] or, in biochemistry, by the cyclic AMP signalling system of the slime mold *Dictyostelium discoideum* [3–5]. The properties of excitability and oscillations are also shared by nerve and muscle cells [6,7].

The analysis of chemical and biological models has revealed in recent years that nonlinear systems often possess multiple modes of behavior which are simultaneously stable in a given set of conditions [8]. Thus, in the situation of bistability, a system is capable of operating in either one of two stable steady states (see refs. 9–11 for biochemical examples). In the corresponding situation of birhythmicity [12], depending on initial conditions, the system evolves into either one of two stable periodic regimes. Both types of phenomena have been predicted on theoretical grounds and observed experimentally. (Although birhythmicity has been observed in chemical reactions [13,14], it has not yet been found in biological systems.)

Here we show that this multiplicity of behavioral modes extends to the phenomenon of excitability. By analyzing a two-variable biochemical model previously studied for birhythmicity [15], we find conditions in which this system is capable of amplifying perturbations beyond two distinct thresholds and of producing two different types of pulsatory response before returning to the same stable steady state. We account for these multiple thresholds for excitability by phase plane analysis,

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and discuss the phenomenon with respect to the dynamics of excitable cells.

2. Model and phase plane analysis

In order to understand the origin of multi-threshold excitability, it is particularly suitable to analyze a simple two-variable model that is amenable to phase plane analysis. An appropriate model of this sort is shown in fig. 1. It consists in a reaction catalyzed by an allosteric enzyme activated by the reaction product, with recycling of product into substrate. The time evolution of this system is governed by the following two kinetic equations [15]:

$$\frac{d\alpha}{dt} = v + \frac{\sigma_i \gamma^n}{K^n + \gamma^n} - \sigma_M \phi(\alpha, \gamma)$$

$$\frac{d\gamma}{dt} = q\sigma_M \phi(\alpha, \gamma) - k_s \gamma - \frac{q\sigma_i \gamma^n}{K^n + \gamma^n} \qquad (1)$$

with

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

where α and γ denote the normalized substrate and product concentrations, v and σ_M signify the normalized constant input of substrate and maximum rate of substrate to product enzymatic conversion, and k_s is the apparent first-order constant for the removal of product. The two parameters σ_i and K relate to product recycling and denote, respectively, the maximum rate and the threshold constant, i.e., the concentration of γ yielding half-maximum rate of recycling when the latter process is described by a Hill equation with a Hill coefficient n. Finally, L is the allosteric constant of the product-activated enzyme, and q the ratio of the Michaelis constant of the substrate divided through the dissociation constant of the product (see ref. 15 for further details).

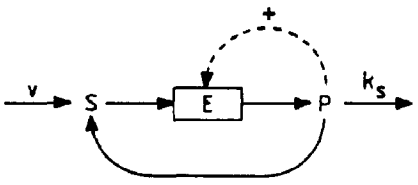

Fig. 1. Model of an allosteric enzyme reaction with activation by the product (P) and recycling of product into substrate (S). This model, previously analyzed [15] for the coexistence between two stable periodic regimes, also exhibits excitability with one or two thresholds for excitation.

When $\sigma_i = 0$, i.e., in the absence of product recycling, eqs. 1 provide a simple model for periodic behavior which yields good agreement with most experiments performed on glycolytic oscillations in yeast extracts [16]. The latter oscillations are indeed produced by phosphofructokinase whose autocatalytic regulation is analogous to that illustrated in fig. 1. The model without product recycling was also analyzed with respect to excitable behavior [17,18]. Both excitable and oscillatory behavior are readily accounted for by phase plane analysis, as first envisaged by Fitzhugh [19] in his theoretical study of the dynamic properties of the nerve membrane.

In the phase plane (α, γ) formed by the substrate and product concentrations, two curves are of particular significance: these are the nullclines $d\alpha/dt = 0$ and $d\gamma/dt = 0$. These curves are obtained by equating the right-hand side of eqs. 1 to zero. The steady state of the system lies at the intersection of the two nullclines. Of primary importance for excitable and oscillatory behavior is the fact that the sigmoid nullcline $d\gamma/dt = 0$ possesses a region of negative slope $(d\alpha/d\gamma) < 0$ for sufficiently large values of the allosteric constant L and of the ratio $q\sigma/k_s$. When the steady state is located in a region of sufficiently negative slope on this nullcline, such that $d\alpha/d\gamma < -(1/q)$, this state is unstable and the system evolves toward a limit cycle enclosing the unstable steady state [15,17,18]. Such a limit cycle corresponds to sustained oscillations in the metabolite concentrations.

When the steady state is located near the maximum on the sigmoid nullcline, just to the left of the oscillatory domain (see fig. 2a), this state is stable. Instantaneous horizontal displacements to the right, which correspond to the addition of pulses of product γ, show that the system is then excitable as it amplifies in a pulsatory manner

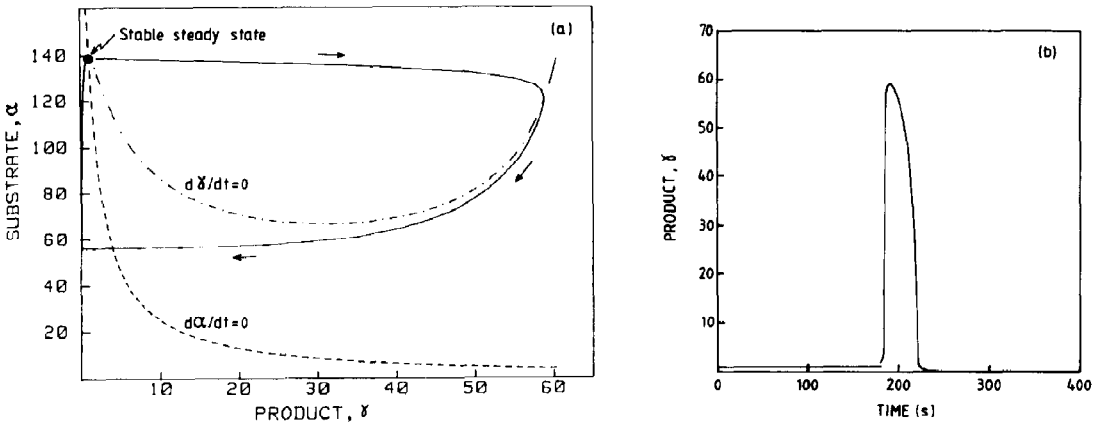

Fig. 2. Excitability with a single threshold. In the absence of product recycling (i.e., for $\sigma_i = 0$ in eqs. 1), the system of fig. 1 amplifies perturbations of the steady state once they exceed a unique threshold. The phase plane trajectory and time course following a suprathreshold perturbation are shown in (a) and (b), respectively. Parameter values: $L = 5 \times 10^6$, $k_s = 1\ s^{-1}$, $q = 20$, $v = 0.05\ s^{-1}$, $\sigma_M = 3.25\ s^{-1}$. The steady state $\alpha_0 = 139.26$, $\gamma_0 = 1$ is perturbed by setting initially $\gamma \equiv \gamma_i = 2$.

perturbations above a threshold (fig. 2b). This threshold is determined by the position of the steady state on the nullcline and, hence, by the parameters of the system [17,18]. As in a model for the cyclic AMP signalling system of *D. discoideum* [5,20], this analysis reveals the existence of a single threshold for excitation.

3. Excitability with multiple thresholds

In the presence of product recycling, i.e., for $\sigma_i > 0$, the model acquires new modes of dynamic behavior. We have previously analyzed one such emerging property, namely, that of birhythmicity [15]: with the additional input of substrate that originates from recycling, the system becomes capable of switching back and forth between two stable periodic regimes upon appropriate perturbation.

We now wish to describe a second emerging property which relates to excitable behavior. For appropriate values of the recycling parameters σ_i and K (see below), the steady state is stable and excitable when the value of the substrate input is taken just below that giving rise to sustained oscillations. A small, subthreshold increase in γ is not amplified, in contrast with a slightly larger, suprathreshold perturbation (see response to stimuli (1) and (2) in fig. 3a). As the stimulus rises, the maximum value γ_M of the response reaches a plateau. Further increase in the perturbation fails at first to elicit any amplification. When the perturbation exceeds a second, higher threshold, however, the system amplifies it in a pulsatory manner before returning to the same stable steady state (see response to stimuli (3) and (4) in fig. 3a).

The maximum of the peak in γ is shown in fig. 3b as a function of the initial displacement from steady state as measured by the initial value γ_i. Two sharp thresholds, each followed by a plateau in the response amplitude, can be distinguished around $\gamma_i = 1.722$ and $\gamma_i = 11.675$ in the case considered in fig. 3. The linearly rising part of the curve preceding and following the second threshold corresponds to the absence of amplification, when $\gamma_M = \gamma_i$. This multiplicity of thresholds gives the response curve a typical staircase appearance. The plateau reached in response to stimuli exceeding the high threshold lies well above the plateau reached for stimuli just above the low threshold.

Phase plane analysis provides an explanation for the origin of multithreshold excitability. As in the case of birhythmicity [15], this phenomenon depends on the existence of two regions of negative slope $d\alpha/d\gamma$ on the sigmoid nullcline $d\gamma/dt$

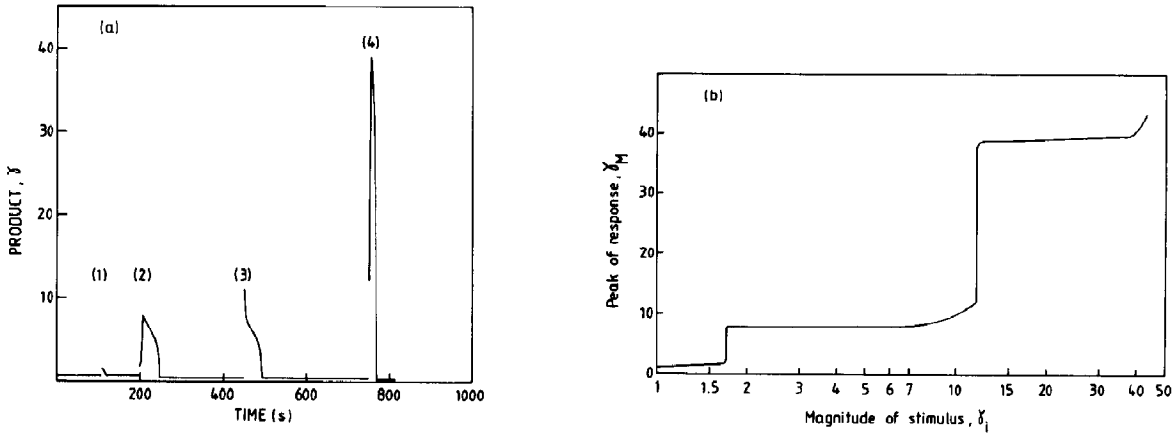

Fig. 3. Excitability with multiple thresholds. The time course of the response to four increasing stimuli is shown in (a) whereas the full dose-response curve is shown in (b). Two sharp thresholds can be distinguished around $\gamma_i = 1.722$ and $\gamma_i = 11.675$, each of which is followed by a different plateau in the response amplitude. The curves are established by numerical integration of eqs. 1 for $v = 0.04$ s^{-1}, $\sigma_M = 4\ s^{-1}$, $k_s = 3\ s^{-1}$, $q = 50$, $\sigma_i = 1\ s^{-1}$, $n = 4$, $K = 8.7$. The stable steady state $\alpha_0 = 134.405$, $\gamma_0 = 0.667$ is perturbed in (a) by setting the initial value γ_i equal to 1.6, 1.8, 11, and 12 in cases (1)–(4), respectively.

$= 0$ (see fig. 4). Whereas a single region of negative slope is apparent when $\sigma_i = 0$ (fig. 2), product recycling induces a 'bump' in the sigmoid nullcline

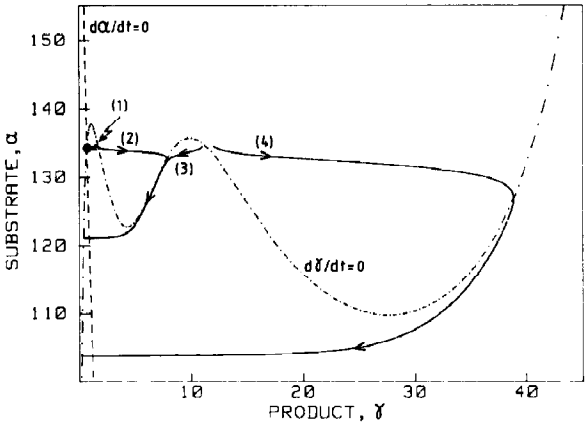

Fig. 4. Explanation of multithreshold excitability by phase plane analysis. The trajectories followed by the system of fig. 1 in response to the four stimuli in fig. 3a are shown, together with the nullclines $d\alpha/dt = 0$ and $d\gamma/dt = 0$. Multiple thresholds for excitability arise when the steady state (●), located at the intersection of the two nullclines, lies in a horizontal range in which the $d\gamma/dt = 0$ nullcline possesses two regions of negative slope. This behavior originates from the fact that the product concentration tends to increase (decrease) whenever the system is above (below) this nullcline in the phase plane.

around $\gamma = K$ (when autocatalysis is effective, any increase in γ at steady state produces an increase in substrate consumption and, hence, a decrease in the steady-state level of α; this decrease is counterbalanced by synthesis of substrate from product once γ exceeds K). For sufficiently large values of σ_i, product recycling thus creates a second local maximum on the nullcline and, hence, two regions of negative slope.

How such a nullcline structure affects excitability is shown in fig. 4. The trajectories following the four increasing stimuli of fig. 3a are indicated. When the steady state is located on the left limb of the sigmoid nullcline, the dynamics of the system is dictated by the position of the initial value of γ – which measures the magnitude of the stimulus – relative to the two portions of the curve where the slope $d\alpha/d\gamma$ is negative. These portions of the nullcline indeed determine two thresholds, each of which separates a region of amplification of the perturbation ($d\gamma/dt > 0$) from a region of immediate decay ($d\gamma/dt < 0$) since the sign of the derivative of γ changes upon crossing the nullcline $d\gamma/dt = 0$.

The magnitude of the plateau reached for suprathreshold stimuli is dictated by the position of the rising part of the nullcline that is attained by the trajectories upon amplification of the initial

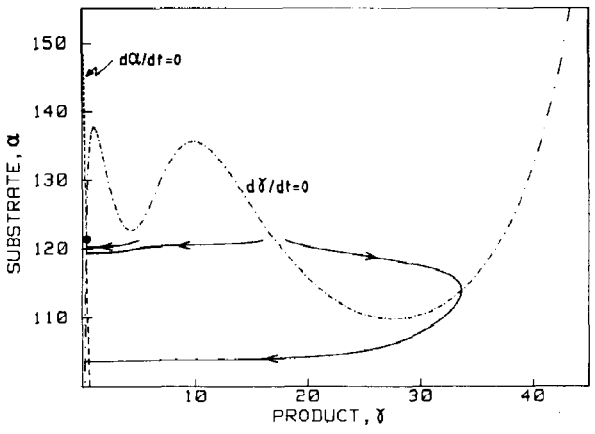

Fig. 5. Excitability with a single threshold obtains when the stable steady state lies below the range in which the nullcline $d\gamma/dt = 0$ possesses two regions of negative slope. Parameter values are as in figs. 3 and 4, except $v = 0.022\ s^{-1}$. The steady state (●) $\alpha_0 = 121.188$, $\gamma_0 = 0.367$ is perturbed by setting $\gamma_i = 5$, 16, and 18 successively.

perturbation (cf. trajectories (2) and (4) in fig. 4). Indeed, γ begins to decrease after reaching its maximum value γ_M once the trajectory crosses the nullcline $d\gamma/dt = 0$. For the same reason, values of γ_i that bring the system below the sigmoid nullcline (e.g., $\gamma_i = 11$ or $\gamma_i > 40$) do not produce any amplification, so that $\gamma_M = \gamma_i$ (see figs. 3a and 4).

From fig. 4 we may predict that the relative values of the two thresholds and of the two plateaux can be modulated simply by moving the middle local maximum on the sigmoid nullcline. This maximum, being due to product recycling, will be shifted upward (downward) by increasing (decreasing) the maximum recycling rate σ_i. Moreover, it will be shifted from left to right by increasing the threshold constant K. As a consequence, if the second maximum on the nullcline is much lower than the first maximum, so that it lies below the stable steady state of fig. 4, a single threshold for excitability will be observed. Similarly, if the second maximum is displaced to the right by an increase in K, then the plateau reached for stimuli just above the low threshold will increase and approach the upper plateau.

Phase plane analysis also predicts that excitability with multiple thresholds will only occur when the excitable steady state is located in a range of α values where the nullcline $d\gamma/dt = 0$ possesses more than one region of negative slope (see fig. 5). Finally, a single threshold for excitation is observed when the steady state is stable but located in the second region of positive slope that separates the two oscillatory domains. A phenomenon of hard excitation can then be observed [15] (fig. 6). Upon subthreshold perturbation, the system returns to the stable steady state. When the perturbation exceeds the threshold, the system evolves toward a stable limit cycle corresponding to sustained oscillations.

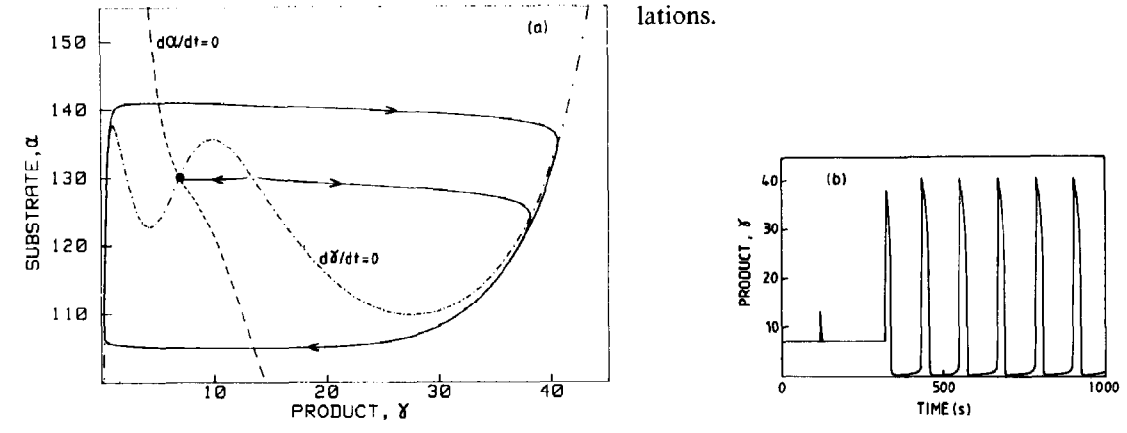

Fig. 6. Hard excitation. For a subthreshold perturbation ($\gamma_i = 13$), the system returns to the stable steady state. When the perturbation exceeds a threshold ($\gamma_i = 14$), the system evolves to a stable limit cycle (a) corresponding to sustained oscillations (b). The curves are obtained by perturbing the stable steady state (●) $\alpha_0 = 130.05$, $\gamma_0 = 7$ for the parameter values of figs. 3–5, with $v = 0.42\ s^{-1}$.

4. Discussion

Whereas most chemical and biological systems evolve towards a single steady state or periodic regime in a given set of conditions, theoretical and experimental studies have shown that in nonlinear systems, multiple, simultaneously stable regimes may coexist. The most common type of such multiplicity relates to the coexistence between two stable regimes which may be stationary (bistability), periodic (birhythmicity), or of both types (hard excitation). Here we have shown that such multiplicity extends to another mode of dynamic behavior, excitability.

Excitability is a dynamic property associated with sustained oscillations. Both phenomena occur in closely related conditions. An oscillatory system will often become excitable for slightly different parameter values, and vice versa. Excitable systems amplify in a pulsatory manner perturbations that exceed a sharp threshold. Generally this threshold is unique under given conditions, so that the dose-response curve is a sigmoid characterized by a steep, quasivertical rise followed by a plateau [5,17,18,20]. The present study of a simple two-variable model indicates that some excitable systems may admit more than one threshold for excitation. Phase plane analysis explains the origin of the phenomenon and allows one to predict the conditions under which it will occur. The maximum number of thresholds for excitability is equal to the number of regions of negative slope on one of the nullclines of the system.

With multiple thresholds, the excitability response curve shows a series of staircase transitions (fig. 3b). Such a phenomenon could be of physiological significance in extending the range of sensitivity of an excitable system with respect to the controlling stimulus (a similar argument has been presented in a context unrelated to excitability, namely, that of multiple thresholds that may arise in the kinetics of protein covalent modification; see fig. 10 of ref. 21, which presents a striking resemblance to fig. 3b). The fact that the response amplitude can reach two distinct levels also enlarges the action spectrum of the excitable system, given that different target cells can be activated (or inhibited) depending on their own threshold for response.

Phase plane analysis shows that the multiplicity of thresholds for excitation is associated with the existence of multiple oscillatory domains and birhythmicity [15]. As the latter phenomenon has been predicted in a model for the cyclic AMP signalling system of the slime mold *D. discoideum* [22], the experimental search for multiple excitability thresholds could be carried out in *D. discoideum* suspensions. Such experiments have already indicated the existence of one threshold for excitability in response to cyclic AMP pulses [4].

Another biochemical system in which the phenomenon might occur is the glycolytic pathway. This metabolic system is known to operate in a periodic manner in yeast cells as well as in yeast and muscle extracts [23,24]. In glycolysis, ADP is the product and a positive effector of phosphofructokinase, and is recycled into the substrate ATP; ADP thus plays the role of product P in fig. 1. Excitability with respect to ADP (or AMP) pulses could be investigated in glycolyzing yeast extracts, for substrate injection rates just below those that produce sustained oscillations [17,18]. Such experiments would permit one to determine whether the glycolytic system can amplify in a pulsatory manner biochemical perturbations, and whether the phenomenon is associated with the existence of multiple thresholds for excitation. Chemical oscillatory systems in which birhythmicity has been observed [13,14] could also be investigated with respect to the multiplicity of thresholds and plateaux in their excitable response.

Some observations in neurophysiology might be interpreted in terms of the present analysis. Distinct thresholds for the generation of action potentials of different amplitudes have been demonstrated in certain neurons, in the same experimental conditions. For example, Llinas and Yarom [25] have shown the existence of both a low and a large threshold for stimulation of neurons of the inferior olive. A similar phenomenon was demonstrated in thalamic cells [26]. The present study of a biochemical analog of excitable and oscillatory neuronal systems suggests an explanation for these observations. The existence of two distinct thresholds for excitability should be associated with phase portraits in which a nullcline should

possess more than one region of negative slope. As a result, these neuronal systems should also possess multiple instability domains in parameter space, in which the oscillations would be characterized by markedly different amplitudes and frequencies [27]. In support of this correlation, multiple oscillatory modes have been found in thalamic and olivary neurons [25,26].

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