Biophysical Chemistry, 46 (1993) 187–192
Elsevier Science Publishers B.V., Amsterdam

BIOCHE 01737

Biochemical oscillations: The opposite dynamical effects of competitive and noncompetitive enzyme inhibitors

C.D. Thron

Department of Pharmacology and Toxicology, Dartmouth Medical School, 7650 Remsen, Hanover, NH 03755-3835 (USA)

(Received 7 July 1992; accepted in revised form 27 October 1992)

Abstract

In a potentially oscillatory biochemical system, competitive and noncompetitive inhibition of an enzyme have opposite effects on the steady-state reaction order with respect to substrate, and this may result in opposite effects on the dynamical stability of the steady state. This is illustrated by a mathematical model, in which oscillations induced by a decrease in the amount of an enzyme are abolished by addition of a competitive inhibitor of the same enzyme. The differing dynamical effects of competitive and noncompetitive enzyme inhibition may have important implications for biochemical regulatory mechanisms.

Keywords: Competitive enzyme inhibition; noncompetitive enzyme inhibition; bifurcation; dynamical disease

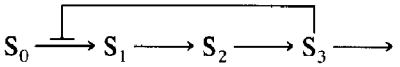
1. Introduction

Competitive and noncompetitive inhibitors of an enzyme in a biochemical system will ordinarily have qualitatively similar effects on the steady-state metabolite levels. However, some systems can undergo a qualitative change in the system dynamics, e.g. from a stable steady state to stable oscillations. Examples are the "mitotic clock" [1–4], intracellular Ca^{2+} dynamics [5–7], and various metabolic networks [8,9]. In such cases theoretical analysis [10] suggests that competitive and noncompetitive inhibitors might sometimes have opposite effects on the generation or suppression of oscillations. This communication presents a mathematical model which demonstrates this effect, and shows that a difference in mode of action which is little importance to the slowing of the enzymatic reaction may be of crucial importance to the dynamic stability of the system.

To emphasize the possible unexpected results of this effect, these ideas are illustrated by a paradoxical "cure" of a hypothetical "dynamical disease". A dynamical disease, as defined by Mackey and Glass [11,12] is a disease due to a qualitative change in the dynamics of some physiological system, leading to a change in temporal behavior (e.g. from steady-state to oscillatory or chaotic behavior).

2. Results

Consider a sequence of biochemical reactions in which the end-product inhibits the first reaction, forming a negative-feedback loop:



(1)

Assume that physiologically this system operates in a stable steady state, and that a pathophysiological state, or dynamical disease, appears if the steady state becomes unstable and the system goes into oscillations. A necessary condition for such instability of the steady state is [10]

$$\phi/\alpha_3 \geq 8, \tag{2}$$

where $-\phi$ is the reaction order of the first reaction with respect to the concentration S_3, and α_3 is the order of the reaction removing the substance S_3, with respect to its concentration S_3. (Throughout this paper we use the Roman S_i to refer to the substance and the italic S_i to denote its concentration.) Reaction order is defined here as follows: the order of a reaction of rate V, with respect to a chemical concentration S, is $(S/V)(\partial V/\partial S)$, or $\partial \log_e V/\partial \log_e S$. If the rate V is directly proportional to S^v then we have $\partial \log_e V/\partial \log_e S = v$; otherwise the reaction order is a function of S.

The inequality (2) implies that instability requires either a high feedback order ϕ or a low removal order α_3. The latter can be obtained if the reaction removing S_3 has saturable kinetics, e.g.

$$V = \frac{V_{max} S_3}{K_M + S_3}, \tag{3}$$

where V_{max} is the maximum velocity, K_M is the Michaelis–Menten constant, and S_3 is the substrate concentration. The order of reaction with respect to S_3 is

$$\alpha_3 = \left(\frac{S_3}{V}\right)\left(\frac{\partial V}{\partial S_3}\right) = \frac{K_M}{K_M + S_3} = 1 - \frac{V}{V_{max}}, \tag{4}$$

and it decreases from 1 to 0 as S_3 increases from 0 to ∞.

Other things being equal, a decrease in V_{max}, caused either by a noncompetitive inhibitor or a simple decrease in the amount of enzyme, will cause a backup of S_3, and the resulting increase in S_3 will cause a decrease in the reaction order α_3 (eq. 4). In effect, the reduced amount of functional enzyme must operate at higher saturation in order to handle the metabolic load. A large enough decrease in α_3 will establish the inequality (2), permitting the appearance of stable oscillations.

On the other hand, a competitive inhibitor of the same enzyme will decrease the reaction rate V without changing V_{max}, thereby increasing α_3 (eq. 4), possibly invalidating the inequality (2) and abolishing any oscillations.

As a specific model for purposes of illustration, we take the system with rate equations

$$\begin{aligned} dS_1/dt &= \frac{p_1 S_0}{p_2 + S_3} - p_3 S_1 \\ dS_2/dt &= p_3 S_1 - p_4 S_2 \\ dS_3/dt &= p_4 S_2 - \frac{V_{max} S_3}{K_M + S_3}. \end{aligned} \tag{5}$$

The feedback order ϕ is $S_3/(p_2 + S_3)$; and since this lies between 0 and 1, it follows from the inequality (2) that α_3 must be considerably less than 1 if instability is to appear. That is, the reaction removing S_3 must operate near saturation in the steady state.

In the presence of a competitive inhibitor I, K_M in the last equation is replaced by

$$K_M\left(1 + \frac{I}{K_I}\right). \tag{5a}$$

Figure 1 shows a numerical example. A suitable choice of parameters gives a stable steady state. A reduction in V_{max} (e.g. either by a noncompetitive antagonist or by reduction of the amount of enzyme) causes the steady state to become unstable—that is, produces a dynamical disease. After stable oscillations are established, an increase in K_M (e.g. by a competitive inhibitor in accordance with expression 5a) restores stability. The final steady state is not the same as the original, and in order for this to be a "cure" we must assume that this altered steady state is physiologically tolerable, whereas the oscillatory state is not.

A fuller picture is given by Routh–Hurwitz analysis of the Jacobian matrix of the system,

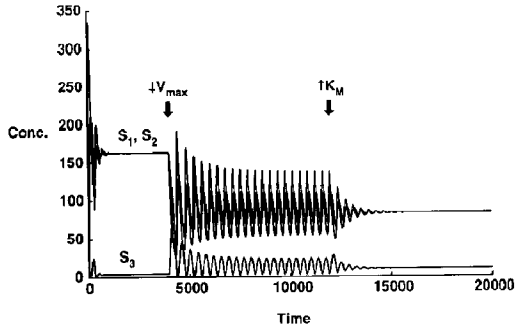


Fig. 1. Paradoxical cure of a dynamical disease: oscillations induced by loss or noncompetitive inhibition of an enzyme are abolished by addition of a competitive inhibitor of the deficient enzyme. The curves are computed from eqs. (5). The trace with the largest oscillations is S_1. At the start, parameter values are: $p_1S_0 = 9$, $p_2 = 1$, $p_3 = p_4 = 0.01$, $V_{max} = 2$, $K_M = 1$. The initial state is $S_1 = S_2 = S_3 = 0$. After a few heavily damped oscillations the system reaches a steady state (the "normal" condition) with $S_1 = S_2 = 163.636$, $S_3 = 4.5$. At 4000 time units, V_{max} is abruptly decreased to 1, simulating loss or noncompetitive inhibition of half of the enzyme which metabolizes S_3, and the system goes into stable oscillations (the "sick" condition). In this condition, the unstable steady state is $S_1 = S_2 = 90$, $S_3 = 9$. At 12000 time units, K_M is abruptly increased to 2, simulating addition of a competitive inhibitor of the deficient enzyme, in a concentration sufficient to occupy half the active sites not occupied by substrate. Oscillations are abolished, and a new steady state (the "cured" condition) is established with $S_1 = S_2 = 83.0952$, $S_3 = 9.83095$. Numerical integration was done by a 4th-order Runge–Kutta procedure with a step size of 1/64 time unit.

which gives as a necessary condition for instability of the steady state

$$\frac{\phi}{\alpha_3} - \sum \delta^2 \geq 8, \tag{6}$$

where $\Sigma\delta^2$ is nonnegative and is equal to 0 if and only if all the diagonal elements of the Jacobian matrix are equal [10]. With strict inequality, (6) is a sufficient condition for instability of the steady state.

Table 1 shows a numerical dissection of the left-hand side of inequality (6) for the three conditions illustrated in Fig. 1. As the condition changes from "normal" to "sick" to "cured", the changes in ϕ are small, and the changes in ϕ/α_3 are mainly due to the changes in α_3. As expected, decreasing V_{max} decreases α_3, and increasing K_M increases α_3. The values of ϕ/α_3 in the "normal" and "cured" conditions are both less than 8—low enough to guarantee stability of the steady state regardless of the value of $\Sigma\delta^2$. However, the steady-state instability of the "sick" condition requires not only that ϕ/α_3 be large, but also that it not be counteracted by a large value of $\Sigma\delta^2$. In fact, the decrease in $\Sigma\delta^2$, in going from the "normal" to the "sick" condition, accounts for more of the change in $(\phi/\alpha_3) - \Sigma\delta^2$ than does the change in ϕ/α_3.

For a more complete picture of the unstable region of parameter space, we can first reduce the number of parameters by taking only the case where $p_3 = p_4$ and normalizing parameters and concentrations to eliminate the parameters p_1S_0 and p_2, i.e.

$$p_3' = p_3 p_2^2/(p_1S_0), \qquad V_{max}' = V_{max}\, p_2/(p_1S_0), \tag{7}$$

$$K_M' = K_M/p_2, \qquad S_i' = S_i/p_2 \qquad (i = 1, 2, 3),$$

with time rescaled to $\tau = (p_1S_0/p_2^2)t$. The inequality (6), expressed in these terms (for details, see ref. [10]) is then

$$\frac{S_3'(K_M' + S_3')}{(1 + S_3')K_M'} - 2\left[u - \frac{1}{u}\right]^2 \geq 8, \tag{8}$$

Table 1

Values of parameters under the different conditions in Fig. 1

Condition	Parameter						
	V_{max}	K_M	ϕ	α_3	ϕ/α_3	$\Sigma\delta^2$	$(\phi/\alpha_3) - \Sigma\delta^2$
Normal	2	1	0.818	0.182	4.5	9.53	−5.03
Sick	1	1	0.9	0.1	9	0	9
Cured	1	2	0.908	0.169	5.37	0.26	5.11

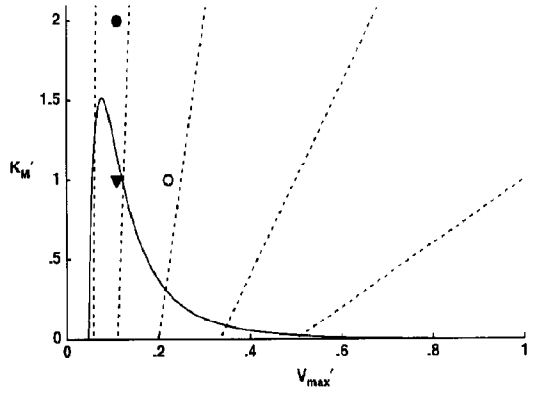


Fig. 2. Stable and unstable regions of the (V'_{max}, K'_M) plane for the normalized (eqs. 7) system of Fig. 1. The unstable region lies below the curve defined by assuming equality in (8). The method of computing this curve is given in the Appendix. The points (○), (▼), and (●) correspond to the "normal", "sick", and "cured" conditions, respectively, of Fig. 1. The dashed straight lines are the loci of constant steady-state S'_3, i.e. solutions of eq. (10) with constant S'_3. From left to right, they have $S'_3 = 16$, 8, 4, 2 and 1. (These are also lines of constant $\phi = S'_3/(1+S'_3)$). A coordinated change in V'_{max} and K'_M along one of these lines makes no change in the steady-state values of S'_1, S'_2, or S'_3, but may produce or abolish instability. The unstable region looks very small for $V'_{max} > 0.4$; but on a logarithmic scale of K'_M this region would extend downward infinitely.

where

$$u = (K'_M + S'_3)\sqrt{\frac{p'_3}{V'_{max}K'_M}}\,; \tag{9}$$

and the steady state condition, obtained by adding eqs. (5) and equating to zero, is

$$\frac{1}{1+S'_3} = \frac{V'_{max}S'_3}{K'_M + S'_3}. \tag{10}$$

Equations (8)–(10) define the unstable region of the (V'_{max}, K'_M) plane for any given p'_3 (Fig. 2). The paradoxical effect noted here reflects the fact that the boundary between the stable and unstable regions slopes downward from left to right over much of its length. Over this region, competitive and noncompetitive inhibitors have opposite dynamical effects, the former causing an upward shift (an increase in K'_M) from the unstable to the stable region, and the latter causing a leftward shift (decrease in V'_{max}) from the stable to the unstable region.

More generally, it can be seen that a competitive inhibitor never causes instability, and in fact a competitive inhibitor will always produce stability if given in a high enough concentration. On the other hand, a further loss of enzyme, beyond that which produces instability, eventually restores stability, though only at the price of increasing the steady state S_3 to almost four times the level in the "normal" condition. This stabilization is due to an increase in $\Sigma\delta^2$ in the inequality (6).

It may also be noted that if K'_M and V'_{max} are varied together in such a way as to preserve the steady-state relation (10) with a constant S'_3, then the steady-state stability can be altered without changing any of the steady-state concentrations S'_i. Lines showing several such loci are shown in Fig. 2.

3. Discussion

The implication of all this for biochemical and physiological regulatory mechanisms is that enzyme regulation by a competitive or allosteric inhibitor affecting substrate affinity may have dynamical consequences quite different from those of regulation by a noncompetitive inhibitor or by a kinase–phosphatase system affecting V_{max}. Of course a kinase–phosphatase system with rapid turnover may be effectively competitive in character; while on the other hand a nominally competitive inhibitor with slow equilibration kinetics might be effectively noncompetitive. A very strongly bound competitive inhibitor may also be effectively noncompetitive, if the local free inhibitor concentration is so low that the association and dissociation reactions become essentially diffusion-limited [12].

It would also seem to follow that, since the competitive or noncompetitive character of inhibition may depend on the rate of equilibration of the inhibitory reaction, regulation of that equilibration rate (without necessarily shifting the equilibrium point) would be a means of turning on and off oscillations of the system (5). However, this question has not yet been carefully investigated, either theoretically or in numerical simulations.

Another implication is the possibility of some very puzzling experimental observations. One might find, for example, two highly specific inhibitors of the same enzyme which had opposite effects on system behavior, because of their different rates of equilibration. Usually one would be aware that a qualitative change in system dynamics was involved; but in a very large system, such as an intact animal, the change in dynamics might occur in an unobserved part of the system; and in that case one might not be forewarned of the need to consider the dynamic as well as the static aspects of enzyme inhibitor action.

It is an interesting question whether dynamic considerations like those discussed here for enzyme inhibition can play an equally important part in the actions of drugs on receptors or ion channels. If so, a major reevaluation might be in order for at least some parts of the pharmacology of receptor- and channel-specific agents in the central nervous system.

In theoretical investigations of biochemical systems, it is common to choose a plausible particular mathematical model, such as eqs. (5), and study its properties. The approach here has been different, in that eqs. (5) are merely illustrative, whereas the fundamentally important principles have been established in inequalities (2) and (6) in terms of reaction orders that might be those of any number of particular models of the system 1. This analysis in terms of reaction orders exploits the important insights of Higgins [14]. Not only is it more general than any particular model, it is simpler. The overall effects of K'_M and V'_{max} on the Routh–Hurwitz conditions (8)–(10) are difficult if not impossible to appreciate by inspection of those equations; but the effect of α_3 in inequalities (2) and (6) is practically impossible to misunderstand. Likewise, the importance of inhibitor equilibration rate might be quite hard to see in the equations for a detailed particular model with explicit representation of inhibitor reaction rates (though it would of course appear in numerical simulation); but here we have this result, at least qualitatively, with very little trouble. Other applications of this approach have been similarly effective [4].

The limitation of this approach is that it leaves many things undetermined. It makes no specific numerical predictions. It does not determine the steady-state values of the S_i, and since many of the reaction orders are concentration-dependent they too are numerically undetermined (though they may be restricted to a certain range). The overall effect of a specific parameter change in a particular model is often hard to predict because (as can be seen in Table 1) it may affect several reaction orders, both directly (as a change in K_M affects α_3, eq. 4) and indirectly (as the same change in K_M, causing a change in the steady-state S_3, affects ϕ). Moreover, factors other than reaction orders (e.g. $\Sigma\delta^2$ in eq. 6) may be affected. Finally, the relative impact of all these factors on system stability may depend upon the actual parameter values and the steady-state S_3. To someone trying to fit real data these limitations would appear to render this approach useless. The value of this analysis, however, is that it gives some insight into how the system works qualitatively, and brings out some important features such as, in this case, the competitive or noncompetitive character of inhibition. This helps to organize one's computational approach to particular models, helps locate the interesting (and uninteresting) regions of parameter space, suggests ways to control steady-state stability, and suggests what are the important things to look for in biological experiments.

Appendix

Computation of the stability–instability boundary in Fig. 2

Simple elimination of S'_3, and u from eqs. (8)–(10) yields extremely complicated expressions in K'_M, V'_{max}; therefore the curve was plotted from parametric equations in S'_3. The first step was to obtain an equation containing only S'_3 and u, Eliminate V'_{max} between (9) and (10) to get

$$K'_M = \frac{p'_3(1+S'_3)S'^2_3}{u^2 - p'_3(1+S'_3)S'_3}. \qquad \text{(A1)}$$

Substitute in (8) to get

$$\frac{u^2}{(1+S_3')^2 p_3'} - 2\left[u - \frac{1}{u}\right]^2 \geq 8. \tag{A2}$$

Take the equal sign for the stability/instability border, and transpose the second term to the right-hand side, square it out, and collect terms:

$$\frac{u^2}{(1+S_3')^2 p_3'} = 2\left[u + \frac{1}{u}\right]^2, \tag{A3}$$

and

$$\frac{1}{(1+S_3')^2 p_3'} = 2\left[1 + \frac{1}{u^2}\right]^2. \tag{A4}$$

Solve for

$$\frac{p_3'}{u^2} = \frac{\sqrt{p_3'/2}}{(1+S_3')} - p_3' = v. \tag{A5}$$

This has no positive solution (there is no instability) if $S_3' \geq (1/\sqrt{2p_3'}) - 1$. Now divide both the numerator and the denominator of eq. (A1) by u^2 and substitute for p_3'/u^2 from eq. (A5):

$$K_M' = \frac{v(1+S_3')S_3'^2}{1 - v(1+S_3')S_3'} = \frac{S_3'(1-w)}{w}. \tag{A6}$$

where

$$w = 1 - v(1+S_3')S_3'. \tag{A7}$$

Equation (A6), with w and v given as functions of S_3' by eqs. (A7) and (A5), gives K_M' as a function of S_3'. Substituting eq. (A6) in eq. (10) and solving gives the corresponding parametric equation for V_{max}':

$$V_{max}' = \frac{1}{w(1+S_3')}. \tag{A8}$$

To draw the curves in Fig. 2, S_3' is varied between 0 and $(1/\sqrt{2p_3'}) - 1$, and v, w, K_M', and V_{max}' are computed from (A5)–(A8).

References

1 R. Norel and Z. Agur, Science 251 (1991) 1076.
2 J.J. Tyson, Proc. Natl.. Acad. Sci. USA 88 (1991) 7328.
3 A. Goldbeter, Proc. Natl.. Acad. Sci. USA 88 (1991) 9107.
4 C.D. Thron, Science 254 (1991) 122.
5 K.S.R. Cuthbertson and T.R. Chay, Cell Calcium 12 (1991) 97.
6 G. Dupont, M.J. Berridge and A. Goldbeter, Cell Calcium 12 (1991) 73.
7 T. Meyer and L. Stryer, Annu. Rev. Biophys. Biophys. Chem. 20 (1991) 153.
8 A. Goldbeter and G. Dupont, Biophys. Chem. 37 (1990) 341.
9 Y. Termonia and J. Ross, Proc. Natl.. Acad. Sci. USA 79 (1982) 2878.
10 C.D. Thron, Bull. Math. Biol. 53 (1991) 383.
11 M.C. Mackey and L. Glass, Science 197 (1977) 287.
12 L. Glass and M.C. Mackey, From Clocks to Chaos (Princeton University Press, Princeton, NJ, 1988).
13 C.D. Thron and D.R. Waud, J. Pharmacol. 160 (1968) 91.
14 J. Higgins, Ind. Eng. Chem. 59 (1967) 18.