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Review article

Slow-binding inhibition of cholinesterases, pharmacological and toxicological relevance

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ABSTRACT

Slow-binding inhibition (SBI) of enzymes is characterized by slow establishment of enzyme-inhibitor equilibrium. Cholinesterases (ChEs) display slow onset of inhibition with certain inhibitors. After a survey of SBI mechanisms, SBI of ChEs is examined. SBI results either from simple slow interaction, induced-fit, or slow conformational selection. In some cases, the slow equilibrium is followed by an irreversible chemical step. This later was observed for the interaction of ChEs with certain irreversible inhibitors. Because slow-binding inhibitors present pharmacological advantages over classical reversible inhibitors (e.g. long target-residence times, resulting in prolonged efficacy with minimal unwanted side effects), slow-binding inhibitors of ChEs are promising new drugs for treatment of Alzheimer disease, myasthenia, and neuroprotection. SBI is also of toxicological importance; it may play a role in mechanisms of resistance and protection against poisoning by irreversible agents.

Contents

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; ChE, cholinesterase; CSP, cresyl saligenin phosphate; DFP, diisopropylfluorophosphate; DTI, delay time for inhibition; PAS, peripheral anionic site; PD, pharmacodynamics; PK, pharmacokinetics; OP, organophosphorus compound; SBI, slow-binding inhibition; TBI, tight-binding inhibition; TMTFA, *m*-(N,N,N-trimethylammonio)trifluoroacetophenone.

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1. Introduction

Cholinesterases are α/β serine hydrolases. Their catalytic triad (Ser-His-Glu) is located at the bottom of a deep gorge (20 Å depth). Acetylcholinesterase (AChE, EC. 3.1.1.7) has an important physiological function in terminating the action of the neurotransmitter acetylcholine in the central cholinergic system, ganglia and at neuromuscular junctions [1]. AChE has also non-cholinergic functions in cell development and embryogenesis [2,3]. AChE is involved in pathogenesis of the Alzheimer disease in promoting formation of β-amyloid fibrils [2]. Inhibitors of AChE have been used for the palliative treatment of Alzheimer disease, glaucoma and myasthenia. Irreversible inhibition of AChE by organophosphorus compounds and carbamates causes a major cholinergic syndrome, responsible for the acute toxicity of these compounds [4]. The related enzyme, butyrylcholinesterase (BChE, EC. 3.1.1.8) has no known physiological function, though it has recently been found to hydrolyze ghrelin [5]. Otherwise, BChE is of toxicological and pharmacological importance. It acts as an endogenous bioscavenger against numerous esters used as drugs, pesticides, or banned chemical warfare agents [6]. Certain potent reversible inhibitors bind slowly to the active center of both ChEs. Slow onset inhibition determines kinetic complexities in terms of possible mechanisms of protection against external toxicants, pharmacological uses of slow-binding inhibitors and design of new drugs with long residence time on targets and short residence time in the bloodstream. Although there are several recent reviews on SBI of enzymes in general, this article—the first one about SBI of ChEs—highlights the pharmaco-toxicological importance of SBI of these enzymes.

2. Slow-binding inhibition of enzymes

In general, reversible inhibition of enzyme is a very fast process. As for enzyme-substrate interaction, enzyme-inhibitor equilibrium establishes within microseconds. Thus, classical reversible inhibitors are characterized by rapid on/off rates. However, a number of enzymes do not respond instantly to reversible inhibitors. In such cases, there is a slow onset of inhibition. This type of reversible inhibition is called slow-binding inhibition.

Slow interactions in enzymology were popularized by Frieden [7] and Kurganov [8] who introduced the concept of hysteretic enzymes, i.e. enzymes that slowly respond to a rapid change in concentration of substrate or effector. Later, slow-binding inhibition (SBI) and tight-binding (TBI) mechanisms of enzymes were thoroughly formalized and experimentally investigated [9–14]. The pharmacological interest of these inhibitors made the subject of a recent book [15].

2.1. Definition

With classical reversible inhibitors, the enzyme-inhibitor equilibrium sets up very rapidly.

$$E + I \leftrightarrows EI$$

Under steady state (ss) conditions where the total enzyme concentration is much less than the total inhibitor concentration, $[E_T] \ll [I_T]$, the inhibition constant (K_i) is:

$$K_i = \frac{[E_F][I_T]}{[EI]} \quad (1)$$

where $[E_F]$ is the free enzyme concentration at equilibrium, and $[I_T]$ the total inhibitor concentration. For SBI, slow establishment of the equilibrium between E, I and EI can be of the order of seconds or minutes, and even days, depending on $[I_T]$. In fact, the rate of establishment of equilibrium depends not only on the relative magnitudes of $[E_T]$ and $[I_T]$, but also on K_i. Classical reversible inhibitors behave like tight binding inhibitors *if* $[E_T] \approx K_i$ [14].

If the affinity of the inhibitor is so high that K_i is close to the total enzyme concentration $[E_T]$, then $[E_T]$ and $[I_T]$ are of the same order, inhibition is called tight-binding inhibition (TBI). Eq. (1) does not apply because of depletion in inhibitor concentration. For TBI, the rate of equilibrium between E, I and EI is fast. In the case of slow TBI, the establishment of equilibrium is slow even though $[E_T] \approx [I_T] \approx K_i$.

Therefore, there is no sharp demarcation between classical inhibitors, SBI and TBI, rather there is a continuum of affinity. Tightest TBI, down to femtomolar range (10^{-15} M), is observed for transition state analogues [16].

2.2. Detection of slow-binding inhibition

SBI is detected from progress curves of the enzyme catalytic reaction in the presence of inhibitor. Inhibition reaches steady state (ss) after a slow mono-exponential phase. The integrated rate equation relates the increase in released product of reaction (P) as a function of time is (Eq. (2), Fig. 1):

$$[P]_t = v_{ss}t + \frac{(v_i - v_{ss})(1 - \exp(-k_{obs}t))}{k_{obs}} \quad (2)$$

In the absence of inhibitor (**1**), there is a linear increase of P concentration with time: $[P] = v_{ss}t$. In the presence of a SBI, after rapid mixing of enzyme, substrate and inhibitor, the steady state is established after a lag time, τ (**3**). The lag time is the reciprocal of the first-order rate constant (k_{obs}) associated with establishment of the steady state. If the enzyme and inhibitor are pre-incubated in the absence of substrate for a certain time, the complex EI is formed. Then, when the substrate is added, a slow displacement of the equilibrium occurs, characterized by a lag, before reaching the steady state (**4**). In case of TBI, Eq. (2) becomes [17]:

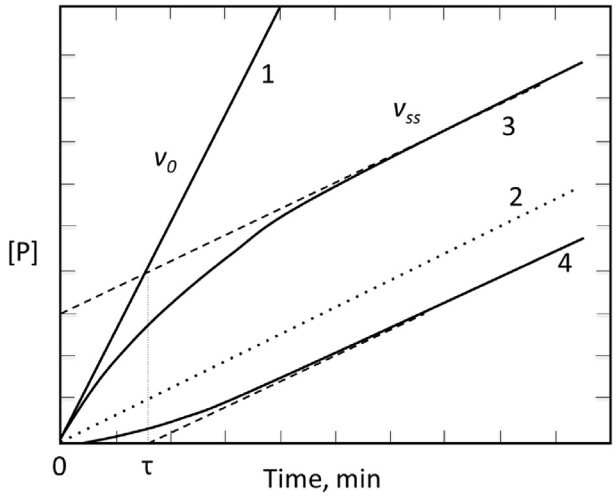

Fig. 1. Progress curves for enzyme inhibition by reversible inhibitors: **1**, enzyme velocity in the absence of inhibitor (v_0); **2**, inhibition by a classical reversible inhibitor; **3**, inhibition by a slow-binding inhibitor: reaction starts after rapid mixing of enzyme and inhibitor; **4**, inhibition by a slow-binding inhibitor: enzyme and inhibitor are pre-incubated before starting reaction. These plots pertain to the case where there substrate depletion is negligible, and hence velocity changes are due to the enzyme-inhibitor interactions. The lag time (τ) is the reciprocal of the first-order rate constant (k_{obs}).