Enzyme Kinetics and Inhibition in Neurotransmitter Metabolism

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Received August 31, 2024
Accepted November 30, 2024
Electronic access December 15, 2024

In this review, the major steps of the neurotransmitter lifecycle are explored through the kinetics of the enzymes that facilitate them, with an emphasis on how inhibitors can affect these kinetics. To develop effective treatment plans for neuropsychiatric illnesses, it is important to understand the precise mechanisms by which inhibitors impact enzyme activity and, in turn, neurotransmitter levels. To obtain data on enzyme kinetics and inhibition mechanisms, a thorough review methodology is used to analyze original research articles, review papers, and experimental studies. The literature was surveyed to identify research articles that specifically characterized the relevant enzymes' kinetic properties through in vitro and in vivo studies. Additionally, we reviewed articles that investigated these enzymes' inhibition and characterized the drug molecules' kinetic properties and their effect on their targets This presents how inhibitors of the enzymes that metabolize neurotransmitters can dramatically change their kinetics, which can impact signaling and neurotransmitter availability. Although using inhibitors to modify enzyme activity may have therapeutic advantages, side effects, and interactions must be carefully considered. This work emphasizes the potential of targeted inhibition as a therapeutic strategy and the significance of enzyme kinetics in neurotransmitter metabolism. It is advised that more research be done to determine the long-term consequences of enzyme inhibition and how it affects mental and neurological health. While comparing standard enzyme kinetics to inhibitor kinetics, certain inhibitors are more effective than others, as they exhibit tighter binding and lower IC_{50} values, which reflects their greater potency. In contrast to the native substrate, these selective inhibitors significantly reduce the enzyme's activity, while less effective inhibitors fail to achieve this degree of inhibition.

Introduction

Neurotransmitters (NTs) are chemical messengers that enable neurons to send signals to each other throughout the body to help it perform its everyday functions like moving limbs and sensing the environment[1]. The body uses a variety of neurotransmitters, such as acetylcholine for contracting muscles, learning, and memory[2] and for regulating sleep, mood, digestion, and blood clotting. Understanding neurotransmitters' role and metabolism is essential to comprehending the complex mechanisms that control the neurophysiological system. The synthesis, release, reuptake, and degradation of neurotransmitter molecules are major steps in the life cycle of neurotransmitters and are important for the regulation and proper function of neurotransmitters[3] The synthesis of neurotransmitters is the process by which primary metabolic molecules, such as amino acids, are converted to neurotransmitters. Synthesis pathways can involve multiple enzymatic steps, which can all be regulated and influence the levels of neurotransmitter /[4] The discharge of neurotransmitters from neurons occurs in response to an external stimulus known as neurotransmitter release. The release of neurotransmitters occurs at synapses, which are locations where various nerve impulses are transmitted[5] The reabsorption of certain neurotransmitters by a neurotransmitter transporter is known as neurotransmitter reuptake. Because it facilitates neurotransmitter recycling and regulates the concentration of neurotransmitters in the synapse, reuptake plays a crucial role in synaptic physiology by influencing the duration of signals originating from neurotransmitter release[4] Degradation of neurotransmitters occurs when an enzyme modifies or breaks them down so that their receptors cannot bind them. This prevents signaling from occurring and allows the product to be recycled back into the neurotransmitter metabolism[6] Disturbances in the homeostasis of neurotransmitters resulting from abnormalities and deficiencies in metabolism can cause altered neurotransmission-mediated signaling, which can be correlated with several different neurological and neurodegenerative diseases including schizophrenia, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease (AD)[7] Drugs can likewise affect the homeostasis of neurotransmitters and alter how neurons communicate with each other by interacting with enzymes and proteins involved in neurotransmitter metabolism[8]. Because drugs can specifically alter the various steps in the neurotransmitter life cycle, they can be utilized in the treatment of these diseases and disorders. Therefore, a deeper understanding of the activity of enzymes in the neurotransmitter lifecycle, and how they interact with their substrates and these drugs, is important for developing treatments[8] Given the important role that enzymes play in neurotransmitter metabolism, understanding the kinetics of these enzymes becomes crucial. Altering the kinetics of enzyme-

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catalyzed reactions can disrupt neurotransmitter homeostasis, influencing everything from synthesis to degradation. Insights into these kinetic parameters can help clarify how changes in enzyme activity contribute to neurophysiological disorders and interactions with inhibitors The fundamental way of modeling and understanding enzyme kinetics was first defined by Michaelis and Menten over 100 years ago and provides a platform for understanding enzymes. The Michaelis-Menten equation defines that the rate of an enzyme-catalyzed reaction depends on the concentration of the enzyme and its substrate[9] There are three important constants when studying enzyme kinetics: kcat, V_{max}, and K_M. kcat is the turnover rate, the number of substrate molecules that are being converted to a product by a single enzyme molecule.[9] . The Michaelis constant, K_M, is the substrate concentration at which the enzymes achieve half of the maximum velocity of the enzyme. V_{max} (maximum velocity) is the highest theoretical rate for an enzyme-catalyzed reaction[9]. Measuring these kinetic parameters allows researchers to observe altered enzyme activity, such as reduced turnover rates or changes in substrate binding affinity, which can indicate the effects of inhibitors or mutations on enzyme function. In addition to their native or natural substrate, enzymes can also bind to other molecules. Inhibitors are molecules that can alter enzymes' natural kinetics. Enzyme inhibitors are molecules that interact in some way, either permanently or temporarily, with enzymes to either decrease the rate of an enzyme-catalyzed reaction or stop enzymes from functioning normally[10]. The inhibition constant (Ki) is a measure of the affinity of an inhibitor for its target enzyme, the concentration at which the inhibitor achieves half maximum inhibition, and it can be compared to the Michaelis constant (K_M) to evaluate how effectively an inhibitor competes with the substrate TheIC_{50} is one of the parameters measuring how different inhibitors interact with their target enzyme;IC_{50} is the concentration of an inhibitor required to decrease the rate of an enzymatic reaction by 50%[11]. This parameter is often used in conjunction with kcat, the turnover rate, to assess how well an inhibitor affects the enzyme's catalytic efficiency and overall activity Competitive, uncompetitive, and mixed-type are the three major modalities of enzyme-inhibitor interaction. Competitive inhibition refers to the mutually exclusive binding of the inhibitor and substrate, which is generally same-site binding. When an inhibitor preferentially binds to an enzyme form primed by substrate binding, this is known as uncompetitive inhibition. In a mixed-type inhibition instance, the inhibitor and substrate may attach to the enzyme separately, with the inhibitor's concentration influencing the substrate's ability to bind either favorably or unfavorably[11]. Using inhibitors as drugs to alter the kinetics of specific enzymes is a common way to treat diseases. Drugs such as antacids can alter enzymes by increasing the pH and changing the kinetics of these enzymes[12]. Specifically for neurotransmitters, many inhibitors act upon the enzymes involved in the neurotransmitter lifecycle. This review will discuss and further define the steps of neurotransmitter metabolism and some of the enzymes involved, specifically how the altered activity of these enzymes can cause different effects in the body, and examine some of the ways these targets can be drugged. Gaining insight into some of the most influential enzymes involved in neurotransmitter recycling, how altering their activity can cause effects and which strategies and targets in medicine are useful for treating diseases are the objectives of this review. The most effective targets for inhibition, the importance of specific versus nonspecific inhibition, the importance of the mechanisms by which the enzymes can be targeted and inhibited, and how the challenges of specific enzymes affect the outcomes have been identified and discussed in this review.

Results

Synthesis

There are multiple enzymes involved in synthesizing neurotransmitters. Some well-known and studied enzymes involved in this process are tryptophan hydroxylase 2 (TPH2) and choline acetyltransferase (ChAT), which are involved synthesizing the neurotransmitters serotonin and acetylcholine, respectively.

Tryptophanhydroxylase.(TPH2) is involved in synthesizing the neurotransmitter serotonin found in vertebrates. This enzyme is the rate-determining step in the pathway for producing serotonin and therefore has the greatest impact on the levels of this neurotransmitter, making it the best target for drugging. TPH2 is an isozyme of tryptophan hydroxylase; an isozyme is one of two or more enzymes with identical functions but different structures.TPH2 regulates serotonin levels and appears in the brain's serotonergic neurons ((Palleria, C. et al. Pharmacokinetic drug-drug interaction and their implication in clinical management (2013).)). Tidemand et al. evaluated the kinetics of the catalytic domain of the human tryptophan hydroxylase (chTPH). The kinetic parameters for chTPH1 and chTPH2 are presented in

Table 1. Kinetic Parameters of Tryptophan Hydroxylase 2

Enzyme	Kcat (min^{-1})	K_M (μM)
chTPH2	258±20	13.1±1.3
chTPH1	108 ± 10	13.1±1.3 μM

The K_M values of wild-type chTPH1 and chTPH2 were identical, but chTPH2 was shown to have a 2-fold higher kcat than chTPH1. Additionally, only chTPH1 showed substrate inhibition. These kinetic parameters have a good correlation with the values previously reported by Windahl et al. and Moran et al[13,14]

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Para-chlorophenylalanine (pCPA) and para-ethynylphenylalanine are both inhibitors of TPH2. pCPA is the most wideldy used TPH2 inhibitor, with over 300 different citations of its inhibiton of TPH2. The Ki reported was 300 uM in a rat brain TPH2 assay[15] In vivo administration of pCPA leads to a prolonged reduction in brain levels of 5-HT in laboratory rodents and a single administration of pCPA reduces TPH2 protein density in the rat brain. This drug has notable limitations, including a delayed onset of effect , the need for relatively high doses, and limited efficacy. Roughly 10 injections of pCPA (300 mg/kg, ip) are required to achieve an 80% reduction in brain 5-HT levels. Additionally, pCPA lacks specificity, inhibiting both TPH2 and TPH1[15]. To address these limitations of pCPA, the para-position chloride was replaced with an ethynyl functional group, resulting in the synthesis of a new drug: para-ethynylphenylalanine (pEPA). In vitro, pEPA Ki was reported 32.6 uM, and pEPA was reported that a single administration of pEPA (30 mg/kg, ip) significantly reduces 5-HT levels and TPH2 activity in the rat brain for 24 hours[15]. pEPA had a lower Ki value than pCPA with pEPA Ki = 32.6 uM and pCPA Ki = 300 uM. This indicates that pEPA hads a higher affinity for its target than pCPA, as reflected by its lower Ki value.

Table 2. Inhibitor values of TPH2

Inhibitor	Ki (uM)
Para-chlorophenylalanine	300
para-ethynyl phenylalanine	32.6

Choline acetyltransferase. Another enzyme that is involved in the synthesis of another neurotransmitter is ChAT. ChAT is the most important enzyme that participates in acetylcholine synthesis and is currently the most accurate indicator for tracking the functional state of cholinergic neurons in the central and peripheral nervous systems. In many cholinergic neurons, ChAT is widely distributed intraneurally and can be found in cell bodies, dendrites, axons, and axon terminals[16]. Ryan et al. used ChAT from rat cerebrum and bovine caudate nuclei to determine the K_M which are shown in **Table 3**. The Michaelis constants for choline were calculated by secondary replots of the initial velocity data[17].

Table 3. Kinetic Parameters of ChAToline Acetyltransferase

Substrate	Cell	K_M (μM)
choline	rat cerebrum	1010
choline	bovine caudate nuclei	714
acetyl-CoA (AcCoA)	rat cerebrum	46.5
acetyl-CoA (AcCoA)	bovine caudate nuclei	16.5

Omeprazole, lansoprazole, and pantoprazole are some inhibitors of ChAT[18]. These molecules, which are also proton-pump inhibitors (PPIs), selectively inhibit ChAT inhibiting neuronal signaling within the brain, possibly be related to the increased risk of dementia[19].Baidya et al. shared that it can be challenging to interpretIC_{50} values in some situations because they can change based on factors like the concentration of the enzyme substrates. As a result, the authors carried out thorough enzyme-inhibition kinetics analyses that took into account various substrate and medication concentrations. In this manner, it was possible to ascertain the most dependable inhibition constants (Ki) and how PPIs function as ChAT inhibitors. Omeprazole, lansoprazole, and pantoprazole exhibitedIC_{50} values of 0.1, 1.5, and 5.3 μ**M**.[18]. The estimated Ki for omeprazole ranged between 0.07 and 0.14 μM. Non-linear enzyme-ligand kinetic analyses found a high probability (>99%) to suggest that this PPI functions as a mixed-competitive reversible inhibitor of ChAT with choline concentration. This mode of operation had the significant consequence of ensuring that omeprazole binds to the enzyme with high affinity, irrespective of the enzyme's state. It also implied that as would be expected from a compound that functions as a full competitive inhμMibitor, the endogenous in vivo choline concentration may only slightly impair this compound's inhibition of ChAT. The Ki for omeprazole was lower than the reported K_M of rat ChAT (1010 μM), and for bovine ChAT (714 μM) the Ki was around 1000 times smaller than the K_M. A smaller K value suggests that omeprazole was found to be more effective at binding to the enzyme than its native substrate For ChAT, rabeprazole's Ki value was 0.0175 μM and tenatoprazole was 0.018 μM. Furthermore, both exhibited non-competitive reversible ChAT inhibitory properties. A non-competitive mode of action suggests that rabeprazole and tenatoprazole can inhibit ChAT regardless of the in vivo concentration of its substrate, choline. They both have a similar high affinity for the free and choline-bound enzymes[19].

Table 4. Inhibitor values of ChAT

Inhibitors	Type of Inhibitor	IC_{50} (μM)	Ki (μM)
Omeprazole	Irreversible/selective	0.1	-
Lansoprazole	Irreversible	1.5	-
Pantoprazole	Irreversible	0.14	0.07 - 0.14
Rabeprazole	Reversible	-	0.0175
Tenatiorazole	Reversible	-	0.018

Rabeprazole's and tenatoprazole's Ki values were very similar with only a 0.005 difference. Similar to omeprazole their Ki values were lower than ChAT K_M values showing that all inhibitors have a greater binding affinity. But Rabeprazole had the smallest Ki value and omeprazole had the largest at 0.07 to 0.14 μM. Comparing these inhibitors, Rabeprazole was the most effective at binding to the enzyme.

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Reuptake

The reuptake of neurotransmitters is an essential component of neurotransmitter metabolism and is mediated by a variety of enzymes. The serotonin transporter (SERT) and the dopamine transporter (DAT) are both crucial enzymes that are responsible for the reuptake of these important neurotransmitters.

Dopamine transporter. (DAT) is an enzyme responsible for dopamine reuptake and is part of a superfamily of neurotransmitter transporters dependent on Na^{+}/Cl^{-}[20]. DAT is located within the membrane of various nerve cells in the brain and drives the reuptake of extracellular transmitters into presynaptic neurons, which in turn regulates the temporal and spatial dynamics of dopamine (DA) neurotransmission[20]. Abnormal DA levels are linked to several disorders, including Parkinson's disease, depression, and bipolar disorder, suggesting that DAT may have a role in the genesis of these conditions[20]. Ross previously described the kinetics of DAT in vitro in the mouse striatum which are shown in **Table 5**. (Ross et al. 1991). Other authors have used these kinetic parameters and the methods for their determination to study the effect of various inhibitors on DAT and how they impact its activity.

Table 5. Kinetic Parameters of DAT Transporter

Enzyme	K_M (μM)	V_{max} (μM/g tissue/min)
Dopamine Transporter	0.209 +- 0.02 μM	0.007 ± 0.00129

Cocaine and methamphetamine are both inhibitors of DAT[21,22]. Cocaine can increase dopamine signaling by blocking DAT. Methamphetamine increases synaptic dopamine levels many times above baseline by inhibiting DAT-mediated dopamine uptake and stimulating dopamine efflux. Methamphetamine also decreases wound healing and increases susceptibility to infections by targeting peripheral immune cells that express DAT[22]. Jones et al. investigated the dopamine release and reuptake by DAT in the mouse striatum and the effect of amphetamine on its kinetics. Using electrical stimulation on these brain slices evoked increased measured DA concentration, which rapidly returned to regular steady state constant. The results of these studies are reported in Table 6. This data indicated that dopamine transport rates were significantly lowered by amphetamine[23]. Han and Gu, compard the potency of Amphetamine on DAT when inhibiting a mouse brain and found that the Ki value was approximately 0.6 μM

Table 6. Amphetamine Inhibition of DAT

DAT concerning Amphetamine	V_{max} (μM/s)	K_M (μM)	[DA]p (μM)
Before Amphetamine	3.5	0.16	9
30 minutes After 10 μM Amphertamine	3.5	8	2.7

The function of DAT can be described by a simple transporter model and in general, drug interactions can be explained by a few basic concepts. DAT can move dopamine bidirectionally, but outward transport mainly depends on the availability of inwardly oriented transporters. The role of transporters is to move the neurotransmitter from the synapse to inside the cell. However, these enzymes can also move neurotransmitters from inside the cell to out if they are already oriented inside the cell. Amphetamine can cause DAT to release rather than uptake dopamine by binding to the transporter in the synapse and bringing it inside the cell. Once DAT is oriented inside the cell, the transporter can return dopamine to the synapse. Amphetamine is a broad-spectrum inhibitor and can inhibit many enzymes that are part of the reuptake step in the metabolism of neurotransmitters. Conversely, cocaine inhibits DAT by blocking the transporter completely, which is a different mechanism from amphetamine, which inverts the direction of transport[24]. Ferris et al. researched the effect of self-administered cocaine on rat brains and found that the application of cocaine on DAT greatly increased the apparent K_M value of DAT. The results of these studies are reported in **Table 7**.[24].

Table 7. Cocaine Inhibition of DAT

DAT concerning Cocaine	K_M (μM)
Native binding of DAT	0.2

A larger K_M value indicated that dopamine wasn't binding as well with the transporter in the presence of cocaine. Cocaine was found to reduce the binding of substrate to the transporter, and the more often the rat subjects self-administered the cocaine the less of an effect it had. Cocaine's ability to inhibit the enzyme was reduced over time, which is important in drug addiction wherein subjects then do more cocaine to achieve the same effect. It was also found that cocaine did not affect the ability of amphetamine to then inhibit the enzyme, which shows these drugs affect DAT by two different mechanisms. Before cocaine inhibited DAT, the enzyme K_M value was 0.2μM. After the inhibition of cocaine, the DAT K_M value increased to 28.75 ± 3.14 μM, a greater than 100-fold increase. Before the inhibition of amphetamine, the enzyme K_M value was 0.16 μM. After the inhibition of cocaine, the DAT K_M value increased to 8 μM. Cocaine significantly increased the DAT K_M value, indicating a stronger inhibitory effect on DAT affinity compared to amphetamine. Therefore, cocaine appears to more drastically impact DAT function, potentially leading to more pronounced alterations in dopamine signaling.

Serotonin transporter. Another enzyme that is part of the reuptake of a different neurotransmitter is serotonin transporter (SERT), which reuptakes serotonin. SERT is an essential membrane protien exhibited in neurons of the raphe nuclei in the brain. The main function of SERT is the quick clearing of serotonin from the synaptic cleft, and causing the termination of the chemical signal transduction in serotonergic neurons. Additionally, SERT has an important role in functions such as sleeping, mood, and appetite. SERT is the primary target

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for selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs), prescribed for depression and anxiety disorders[25] Kilic et al. have previously examined the kinetics of human serotonin transporter by measuring the transmigration rate over time in two cell lines, human-derived HeLa cells and primate-derived COS-7 cells which are presented in **Table 8**.[26].

Table 8. Kinetic Parameters of SERT

Type of cell line	K_M (μM)	V_{max} (pmol/min/mg)	Average Transportation Rate (pmol/min/mg)
Human-derived HeLa cells	1.28 ± 0.19	1.73 ± 0.06	0.131 ± 0.01
Primate-derived COS-7 cells	1.04 ± 0.51	1.37 ± 0.56	0.416 ± .007

Some inhibitors of SERT are selective serotonin uptake inhibitors (SSRIs) such as S-citalopram (S-CIT), sertraline, fluoxetine, and paroxetine. SSRIs are used to treat different types of disorders like depression and anxiety[25]. The inhibitory kinetics of some of these inhibitors are outlined in **Table 9**. Citalopram hydrobromide is a selective serotonin reuptake inhibitor. The FDA primarily indicates citalopram hydrobromide for the treatment of adult depression. By increasing serotonergic activity in the central nervous system, citalopram hydrobromide has an antidepressant effect. Citalopram hydrobromide was found to have anIC_{50} of 0.0018 μM[27]. The K_M found for SERT previously was 1.28 ± 0.19 μM for the HeLa BS cell line and the K_M = 1.04 ± 0.51 μM for COS-7. The Ki of Citalopram hydrobromide is orders of magnitude smaller than the K_M of SERT for serotonin, which indicates a strong binding affinity between the inhibitor and the enzyme and shows that the inhibitor has a higher affinity for SERT than the substrate. Fluoxetine, a racemic mixture of the stereoisomers R- and S-fluoxetine, is another selective serotonin reuptake inhibitor (SSRI) that regulators have approved for use in psychiatry for various clinical uses[28,29].

Table 9. Inhibitor values of SERT

Inhibitor	Ki (μM)	IC50 (μM)
Citalopram hydrobromide	0.001939	0.0018
Fluoxetine	0.19119	5.992
Amphetamine	20 - 40	—

Fluoxetine was observed to have a Ki = 0.19119 μM[30]. and anIC_{50} of 5.992 μM. Similarly to citalopram hydrobromide, fluoxetine had a smaller Ki than the K_M of SERT which shows that it was better at binding to the enzyme than the native substrate serotonin. Fluoxetine was shown to have a higherIC_{50} than citalopram hydrobromide, which means that citalopram hydrobromide was more effective at inhibiting SERT as a lowerIC_{50} indicates a higher potency. Amphetamine also inhibits SERT, in addition to DAT, where the Ki value reported by Dawn D Han 1, Howard H Gu, was 20 to 40 μM. While amphetamine inhibits both the serotonin transporter (SERT) and the dopamine transporter (DAT), it displays significantly higher affinity for DAT. The Ki value for amphetamine's inhibition of DAT is approximately 0.6 μM, which was around 30 to 60 times more than its Ki value for SERT inhibition (20 to 40 μM), as reported by Han and Gu. This difference suggests that amphetamine's primary effect is likely mediated through DAT inhibition, with a comparatively weaker impact on SERT.[21]. Citalopram had a lower Ki value than Fluoxetine with Citalopram Ki = 0.001939 μM and Fluoxetine Ki = 0.19119 μM. This indicates that citalopram had a higher affinity for its target than fluoxetine, as reflected by its lower Ki value. Consequently, citalopram was likely to be a more potent inhibitor in this context than fluoxetine. Fluoxetine was shown to have a higherIC_{50} than citalopram hydrobromide, which means that citalopram hydrobromide was more effective at inhibiting SERT as a lowerIC_{50} indicates a higher potency.

Degradation

Degradation of neurotransmitters is the final step and an important part of neurotransmitter metabolism. Multiple different enzymes are responsible for the degradation of different neurotransmitters. Monoamine oxidase A (MAO-A) and AChE are two of the most significant enzymes.

Monoamine oxidase A. MAO-A is an important enzyme that is part of the degradation of multiple different monoaminergic neurotransmitters and plays an important role in their neurotransmission regulation[31,32]. MAO is an FAD-containing enzyme that facilitates the α-carbon oxidation of monoamines, such as serotonin, norepinephrine, and dopamine, which are all broken down by MAO-A[33]. MAO-A has an important role in brain development, as well as regulating mood, sleep, and appetite[34]. Together with its isoenzyme MAO-A, monoamine oxidase-B (MAO-B) is a FAD-dependent mitochondrial enzyme that catalyzes the oxidative deamination of structurally diverse amines[35]. MAO-A contains multiple binding sites for a variety of substrates required for its activity, and its catalytic mechanism proceeds through multiple different steps throughout its reaction. As MAO-A acts on a variety of different neurotransmitters the kinetic parameters will vary for each substrate, which are outlined in **Table 10**[36].

Table 10. Kinetic Parameters of MAO-A on multiple substrates

Substrate	K_M(μM)	V_{max} (pmol min^{-1} mg protein^{-1}),	V_{max}/K_M (μmol M^{-1} min^{-1} mg protein^{-1})
Adrenaline	125 ± 42	379 ± 54	3.03 ± 1.11
dopamine	212 ± 33	680 ± 123	3.21 ± .77
5-hydroxytryptamine	137 ± 24	228 ± 31	1.66 ± 0.37 46

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Some inhibitors of MAO-A are phenelzine, tranylcypromine, and isocarboxazid[36]. As MAO-A is responsible for removing norepinephrine, serotonin, and dopamine from the brain, MAOIs work non-selectively to decrease the degradation of these neurotransmitters, allowing them to have a more prolonged effect on their target receptors[37]. Many different classes of drugs inhibit MAO-A through a variety of mechanisms. There are four main types of inhibition observed for MAO: reversible, irreversible, mechanism-based irreversible, and poor substrate. Each type of inhibition affects the enzyme differently, with different results. A subclass of MAOIs known as reversible inhibitors, selectively and reversibly inhibit the MAO-A enzyme. These reversible inhibitors primarily work by competitive inhibition. D-amphetamine is one of the most basic reversible inhibitors of MAO. It is used to treat attention deficit hyperactivity disorder (ADHD), although its pharmacology and clinical effects are complicated. It was has been shown to inhibit both MAO and monoamine reuptake systems. With a Ki value of 15 μM, it was observed to be an effective reversible competitive inhibitor of MAO-A[38,38]. while the inhibition is mixed for MAO B. When comparing the Ki value of D-amphetamine to the K_M values from the different substrates, the K_M values ranged from 125 to 212 μM, where the Ki of D-amphetamine is 15 μM. This Ki is substantially smaller than the reported K_M values showing that D-amphetamine is more effective at binding with MAO-A than any of the above substrates listed. The IC_{50} is approximately ten times higher with MAO B because both reduced and oxidized MAO B are present during turnover. In contrast to reversible inhibition, irreversible inhibition of an enzyme results in the enzyme's permanent deactivation. To regain activity, MAO-inhibitor adducts must be eliminated and freshly synthesized MAO has to take their place. Following irreversible inhibition, MAO activity in rat brains was found to recover over a nine-day half-life[36]. One of the main reasons irreversible MAOIs are effective medications is because of the slow regeneration of MAO in vivo. Suicide, time-dependent, enzyme-activated, covalent, and mechanism-based inhibitors are all terms used to describe different irreversible MAO inhibitors[35]. The seven requirements for the designation—time dependence of inactivation, observation of saturation kinetics, substrate protection, irreversibility, fixed stoichiometry, involvement of an enzyme-catalyzed step, and inactivation before release of active species— have been described in detail by Silverman[39] Propargylamines, cyclopropylamines, hydrazines, aminoacetamides, and aryloxazolidinones are among the classes of irreversible MAO inhibitors. The propargylamines are a particularly well-studied class of irreversible MAOIs. Different propargylamine inhibitors can be distinguished from one another not only by how well they bind to MAO but also by how quickly they render the enzyme inactive. Thus, the rate at which distinct propargylamines inactivate MAO A or MAO B provides information about the inhibitor's selectivity[40]. Clorgyline's affinity for MAO-A is three orders of magnitude (>1000x) greater than that of the B-form, and its rate of inactivation (kinact) for MAO-A is 0.76 min^{-1}, compared to 0.006 for MAO-B, which accounts for the inhibitor's strong MAO-A selectivity., In contrast, L-deprenyl only demonstrates a 40-fold difference in the affinity of the two forms, and the rates of inactivation by L-deprenyl for MAO A and B are 0.14 and > 0.99 min^{-1}, respectively, which significantly increases the inhibitor selectivity towards the B-form. Further, Pargyline is primarily non-selective because it only slightly increases the affinity for MAO B, but the inactivation rates for both forms are extremely close. In a single molecule, the new generation of propargylamine multi-target inhibitors for the treatment of Alzheimer's disease contains moieties that inhibit both cholinesterase and MAO. One member of this class, ASS234, inhibits membrane-bound MAO-A with Ki and kinact values of 0.053 μM and 0.133 min^{-1}, respectively[41]. Clorgyline is an irreversible potent and selective monoamine oxidase (MAO) inhbitor, used for research purposes, particularly in inhibiting the growth of tumor cells and reducing the aggressiveness of prostate cancer[42] Clorgyline was reported to have Ki values of 0.054 and 58 μM andIC_{50} values of 0.0012 and 1.9 μM for MAO-A and MAO-B, respectively[43]

Table 11. Inhibitor values of MAO-A/B

Inhibitors	Kinact (1/min)		Ki (μM)
	MAO-A	MAO-B	—
propargylamines	0.76	0.006	—
L-deprenyl	0.14	> 0.99	—
ASS234	0.133	–	0.053
D-amphetamine	–	—	15

The Ki of ASS234 was smaller than the reported K_M values of MAO-A, similar to D-amphetamine where the inhibitors were more effective at binding with MAO-A than the original substrates. Comparing the two inhibitors, the Ki for propargylamine was 0.053 μM and the Ki of D-amphetamine was 15 μM. Propargylamine had a smaller Ki value which indicates that it had a stronger binding affinity with MAO-A than D-amphetamine[41].

Acetylcholinesterase. Another enzyme that participates in the degradation of a different neurotransmitter is AChE. AChE is a cholinergic enzyme that degrades acetylcholine (ACh), a naturally occurring neurotransmitter. AChE is found in postsynaptic neuromuscular junctions, muscles, and nerves. The main role of AChE is to cease neuronal communication and signaling between synapses to avert ACh dispersal and activation of nearby receptors . AChE also plays a role in neural development besides its common function of terminating neural transmission. The activity of ACho has been reported for many different organisms against the substrate acetylthiocholine. Some of these are displayed in Table 12. Pohanka et al. determined the kinetics of the electric eel AChE, using two

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different substrates: acetylthiocholine and indoxyl acetate. The values were determined to be, K_M as 206 μM and the V_{max} as 4.97×10^{-7} mol/s for the acetylthiocholine[44]. This study was done to assess the inhibition by the inhibitor paraoxon and subsequent reactivation by several different oxime inhibitors

Table 12. Kinetic Parameters of AChE in Different Organisms

Organism/cell type	K_M (μM)	V_{max} (mol/s)
Rat	2650	–
House fly	1800	–
Electric Eel	206	4.97 × 10-7

AChE inhibitors, also known as anti-cholinesterases, prevent the cholinesterase enzyme from degrading ACh, thereby increasing the level and duration of neurotransmitter action. There are various inhibitors of AChE, such as nerve agents like sarin, and pesticides such as paraoxon and parathion[45]. AChE inhibitors can be classified into two groups based on their method of execution: irreversible and reversible. An essential function of reversible AChE inhibitors is the pharmacological control of the enzyme activity. These compounds, which have different functional groups have been used as an antidote for anticholinergic overdose as well as in the diagnosis and/or treatment of several diseases, including Alzheimer's disease (AD), postoperative ileus, and glaucoma[46]. A selective and reversible inhibitor of AChE, donepezil binds to the peripheral anionic site to produce both symptomatic and causative effects in the treatment of AD by delaying the buildup of amyloid plaque. The primary therapeutic application of donepezil is in the palliative management of mild to moderate AD. Moreover, according to certain clinical trials, donepezil also enhances cognitive function in individuals exhibiting severe symptoms of AD. Donepezil was found to inhibit AChE with anIC_{50} value of 53.6 ± 4.0 ng/mL. Since the brain synthesizes AChE slowly, irreversible inhibitors increase therapeutic benefits and lower the risk of peripheral toxicity. According to clinical studies, irreversible AChE inhibitors can be very successful and accepted[47].

A powerful AChE irreversible inhibitor, diazoxon is a toxic byproduct of the pesticide diazinon. When diazinon passes through the body, it may undergo hepatic oxidation to produce diazoxon, which inhibits AChE at peripheral and central nervous system nerve terminals. Acetylcholine builds up at receptors as a result of this inhibition, causing cholinergic reactions in the nervous system and neuromuscular junctions. These reactions may result in particularly apparent symptoms in the gastrointestinal, cardiovascular, and respiratory systems[48].

Table 13. Inhibitor values of AChE

Inhibitor	K_i (μM/L)	IC50 (ng/mL)
Donepezil	-	53.6 ± 4.0
Diaxonon	0.7899	0.053

Diaxonon'sIC_{50} for AChE was found to be 0.053 μM, and its K_i =0.7899 μM/L,. AChE was shown to have a K_M of 206 μM/L with the electrical eel, and when comparing the K_i of diazinon and the K_M of MAO-A, the K_i is smaller than the K_M, which indicates that diazinon binds better with the enzyme than does its substrate. The IC_{50} of Diaxonon is also very low which shows that Diaxonon is very effective at inhibiting the enzyme.

Discussion

Based on a survey of the various mechanisms of inhibition presented for neurotransmitter regulation, it was found that the enzymatic mechanisms of action could be associated with different mechanisms of inhibition. It was observed that irreversible inhibitors were effective with enzymes involved in the synthesis and degradation pathways because these processes rely on covalent interactions between the enzymes and substrates. This is exemplified by the significantly smaller IC50 value of diaxonon (irreversible) versus donepezil (reversible) for AChE. These enzymes that follow covalent mechanisms appear to be likely to be inhibited by reactive and irreversible inhibitors. In contrast, reuptake transporter enzymes were found to interact with reversible inhibitors, which temporarily bind to the transporter before detaching via non-covalent interactions.

Every inhibitor ultimately affects the concentration of neurotransmitters in the synapse, but the mechanisms by which this occurs can vary. In the case of synthesis and degradation enzymes, inhibitors primarily work by blocking the enzyme's function, preventing it from facilitating the chemical reactions it usually catalyzes. This inhibition of synthesizing enzymes results in decreased neurotransmitter concentration, while inhibition of degrading enzymes leads to an increase in their concentration. On the other hand, reuptake transporters can be targeted by inhibitors that may reverse the enzyme's direction rather than merely blocking its activity. This was found to be particularly relevant to the dopamine transporter, which can operate in two directions, offering different opportunities for inhibition to alter the direction of dopamine flux in the synapse.

It was also noted that certain enzymes involved in neurotransmitter metabolism were more selective than others, meaning that their inhibition could affect the flux of one or many different neurotransmitters It was found that serotonin reuptake transporter (SERT) inhibitors exhibited high specificity because SERT is responsible for only the reuptake of serotonin. Conversely, the dopamine transporter (DAT) moves and reuptakes multiple different molecules, making DAT inhibitors less specific compared to SERT inhibitors. DAT inhibitors affect a larger range of neurotransmitter targets due to the multiple molecules that DAT transports. This distinction suggests that enzyme target selection could be important for effective inhibition across different neurotransmitter systems.

Monoamine oxidases (MAOs), which degrade many

neurotransmitters are similar to DAT. Inhibiting MAOs can change the actions of multiple neurotransmitters, therefore its inhibitors are less specific for regulating a single neurotransmitter. This broad activity spectrum of MAOs shows the importance of modulating the enzyme's action across various neurotransmitter pathways. Many different inhibitors targeting enzymes function differently to inhibit the enzyme properly. Understanding how different enzymes work with neurotransmitters and inhibitor molecules is important to affect the neurotransmitter's concentration correctly. An analysis of K_M values across different enzymes reveals that choline acetyltransferase (ChAT) has a notably high K_M value, recorded at 1010 μM for the rat and 714 μM for bovine caudate. These values are significantly larger than those observed for other enzymes, indicating a potentially easier inhibition of ChAT. Interestingly, every inhibitor presented had a Ki value lower than the K_M, suggesting that ChAT might be more readily inhibited due to its high K_M values. This insight provides an intriguing perspective on enzyme kinetics and inhibition, particularly in the context of neurotransmitter synthesis and degradation. In general, the complex relationship between neurotransmitter systems' inhibitors and enzymes emphasizes how difficult pharmacological interventions can be. A thorough understanding of these interactions are necessary to enable us to effectively customize therapeutic strategies to modulate neurotransmitter concentrations. The findings of this study provide essential insights into the enzymatic regulation of different steps of neurotransmitter metabolism, contributing to our understanding of neurochemical balance and potential therapeutic interventions. This review brings together many different enzymes that participate in the steps of neurotransmitter metabolism, identifying reported enzymes' kinetic values and how inhibitors can alter those specific kinetics. More literature on enzymes in the neurotransmitter metabolism cycle and how different neurotransmitters can be affected by inhibitors within the cycle should be identified. Comparing different types of inhibitors and different enzymes can offer a pathway to refine different drugs for better outcomes. Identifying inhibitors and describing the enzymatic mechanisms underlying neurotransmitter regulation were the main goals of the study. By presenting kinetic analyses and investigating the effects of different inhibitors on important enzymes involved in neurotransmitter metabolism, the study effectively achieved these goals. Some key areas for future research have been identified, such as finding more specific MAO-A and MAO-B inhibitors to minimize side effects and enhance therapeutic efficacy for psychiatric and neurodegenerative disorders. This paper covered how different MAOIs were less specific because of their substrates including serotonin, norepinephrine, tyramine, and dopamine for MAO-A and MAO-B substrates dopamine and tyramine. Due to these differences in substrate profiles, identifying inhibitors specific to either MAO-A or MAO-B could be beneficial. Specifically, MAO-A was seen to have better activity on serotonin, so targeting MAO-A with a more selective inhibitor will affect the concentration of serotonin more, similar to MAO-B and dopamine. Designing inhibitors that are more selective for MAO-A or MAO-B could be useful to selectively regulate those neurotransmitters. Integrating kinetic data into personalized medicine approaches, considering individual variations in enzyme activity and inhibitor response for tailored treatment strategies is also encouraged as a target for further research. For instance, in The isoform-specific substrate inhibition mechanism of human tryptophan hydroxylase, chTRP1/chTRP2 the authors made different mutations to those enzymes to observe their kinetic data and how it was different from the original chTRP1/chTRP2 values. Gathering kinetic data on other mutated enzymes would be of interest.

By presenting the kinetic data for the enzymes discussed and how their inhibitors altered these kinetics this paper shared a base for understanding the importance of enzyme kinetics and inhibition in neurotransmitter regulation Also explores the long-term effects of cocaine and methamphetamine on DAT to understand their contribution to addiction. Additionally, investigates the potential cross-reactivity of SSRIs with other neurotransmitter systems. As shown in this paper cocaine and methamphetamine had different effects on the kinetic values, but looking at a longer period how would the effects change and lead to addiction, with kinetic values?

There were limitations within this review. The study draws from kinetic data derived from various organisms, enzyme constructs, and assay conditions, potentially affecting the generalizability of findings. Species-specific differences in enzyme activity may not fully represent human physiological conditions. There was variability in substrate binding and inhibition across different studies highlighting the need for standardized experimental conditions to ensure consistent results.

In conclusion, the way enzymes manage neurotransmitters is crucial for keeping our brains balanced. As we learn more about these processes, trying to make big advances in treating neurological issues while understanding the variety of competitive, noncompetitive, and uncompetitive inhibitors, and how they work to inhibit specific enzymes and do their jobs. Every enzyme works differently with its substrate or inhibitor; knowing and learning how these interactions work is crucial to improving and influencing proper treatment. This research is not just about science; it's about understanding the core of how our brains work and how we feel. We should continue exploring this, as it could lead to new treatments that improve the lives of many people.

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Methods

For this review, several databases, including PubMed and Google Scholar, were utilized to locate relevant research. To achieve thorough search coverage, a strategic combination of keywords and phrases, such as "enzyme kinetics"and "kinetic values,"enabled the extraction of the desired parameters. Using Peer-reviewed articles, conference papers, and research that directly addressed the research question. Studies with weak methodology, duplicate articles across multiple databases, and articles for which the full text was not available were omitted. Further, only English-language studies were taken into account to avoid translation biases and guarantee consistency in the review process.

Numerous study designs, including observational studies, experimental studies, qualitative research, and systematic reviews, are discussed in this review. A more thorough understanding of the research topic is made possible by incorporating a variety of study designs as different methodologies can offer distinctive insights and perspectives. To quickly determine relevance, studies were first screened using keywords and abstracts. Only those that were highly relevant moved on to the full-text evaluation stage. This review attempted to offer a thorough, targeted, and methodologically sound analysis of the research topic by utilizing these selection criteria.

Relevant information was methodically gathered to facilitate analysis and synthesis, as part of the data extraction process. The planning and execution of this process were done with great care to guarantee uniformity, precision, and thoroughness. The relevance of each study to a particular subtopic or theme within the larger research area determined how it was categorized, carefully examining full-text articles to extract pertinent details. When relevant information was discovered, it was arranged on a document into distinct sections.

The results from the studies were interpreted and summarized in an organized manner using narrative synthesis. The main conclusions of each study were condensed into a narrative that shared the main ideas and advancements made in the field. To provide a deeper understanding of the study's implications, this step involved connecting the study's findings to preexisting theories and models. Numerous factors, including methodological strategies, and significant results, were taken into consideration when comparing and contrasting the studies. This made it easier to find patterns, conflicts, and original contributions in the literature.

The narrative was combined with qualitative insights to give the quantitative findings more depth and context. The narrative synthesis was structured to lead the reader through the development of research findings and their importance while maintaining a logical flow and coherence. In addition to making it easier to identify important trends and insights, this methodical approach also indicated areas that warrant further investigation, which deepened understanding of the subject matter.

Acknowledgments

I would like to express my sincere gratitude to my advisor, Michael Lichstrahl, for his continuous support and guidance throughout this research. I would also like to extend my deepest appreciation to my family and friends for their unwavering encouragement. good

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