Drug Research

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From Basic Science to Blockbuster Drug: The Discovery of Lyrica

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Many great discoveries occur when they are least expected. These discoveries are most satisfying when they derive from studies of basic science. That is how the new blockbuster drug for the treatment of various neuropathic pains, epilepsy, and generalized anxiety disorder, Lyrica (pregabalin), was discovered. This essay describes the discovery and features of this new drug.

One of the early projects I initiated when I started my independent career at Northwestern University was the design and mechanism of new inactivators of the pyridoxal 5′-phosphate (PLP)-dependent enzyme γ-aminobutyric acid aminotransferase (GABA-AT).[1] GABA-AT is the enzyme responsible for the degradation of the inhibitory neurotransmitter, GABA, leading to its conversion to the excitatory neurotransmitter L-glutamate.[2] Compounds that inhibit this enzyme have anticonvulsant activity as well as exhibit activity against Huntington's disease, Alzheimer's disease, Parkinson's disease, and drug addiction.[3]

Epilepsy, broadly defined as any disease characterized by recurring convulsive seizures, has been known for many millennia.[4] There are numerous etiologies for epilepsy because it is not a single disease; consequently, 1–2% of the world population has some form of epilepsy.[5] Of those afflicted with this disease, 30–40% do not respond to multiple anticonvulsant drugs.[6] There are many causes for seizures, but one of them is an imbalance in the concentration of GABA relative to L-glutamate. When GABA levels in the brain diminish, seizures can result. Injection of GABA directly into the brain can terminate the seizure, but administration of GABA, either orally or intravenously, has no effect because GABA, a hydrophilic charged molecule, does not cross the blood–brain barrier (BBB),[7] a membrane that protects the brain from chemicals in the blood while still allowing essential metabolic function. The BBB comprises very tightly packed endothelial cells, which provide the walls of the blood vessels perfusing the brain; this higher density of cells restricts passage of unwanted substances from the bloodstream into the brain. One approach to increase the GABA concentration in the brain is to design a compound that can cross the BBB and inhibit GABA-AT, the only enzyme that degrades brain GABA. This prevents the breakdown of GABA, and its concentration rises, resulting in an anticonvulsant effect.

Because of the importance of increasing brain GABA levels in central nervous system (CNS) disorders, my group, in the years 1981–1988, designed a series of mechanism-based inactivators[8] for GABA-AT.[9] It became apparent, however, that to progress toward the design of a new anticonvulsant agent, it would be necessary to prepare compounds that were *selective* inhibitors of GABA-AT (to raise GABA levels) without inhibiting L-glutamic acid decarboxylase (GAD), the PLP-dependent enzyme that converts the excitatory neurotransmitter, L-glutamate, to the inhibitory neurotransmitter, GABA (Scheme 1).

Inhibition of GAD would *decrease* the concentration of GABA, the opposite of the desired effect. Furthermore, for brain penetration, which would be required for an anticonvulsant drug, increased lipophilicity would be important. Consequently, in 1988 I asked Dr. Ryszard Andruszkiewicz, a visiting scholar from the Technical University of Gdańsk, to synthesize a series of 3-alkyl-GABA and 3-alkylglutamate analogues, then to measure their inhibition of GABA-AT and GAD to determine if we could identify more lipophilic analogues that selectively bound to the former and not the latter enzyme. Dr. Andruskiewicz proceeded to synthesize fourteen 3-alkyl-GABA analogues (including four stereoisomers) (Scheme 2), 4-methyl-GABA (and its two enantiomers)[10] and seven 3-alkylglutamate analogues.[11] All of the GABA analogues were substrates for GABA-AT.[12] As the substituent size increased, so did the Michaelis constant K_m; the V_{max}/K_m value for the 3-methyl analogue was a little larger than that for GABA, but the V_{max}/K_m values for the remaining analogues were progressively smaller (V_{max} is the maximum velocity of the enzymatic reaction). The unexpected surprise came when these compounds were tested as inhibitors for GAD. Not only was none of them an inhibitor, but all of them were found to *activate* GAD, that is, the addition of compound produced an *increased* rate of GABA formation (Figure 1)![11,13] This had not previously been observed. My immediate thought was to have these compounds tested as anticonvulsant agents because they might provide a new

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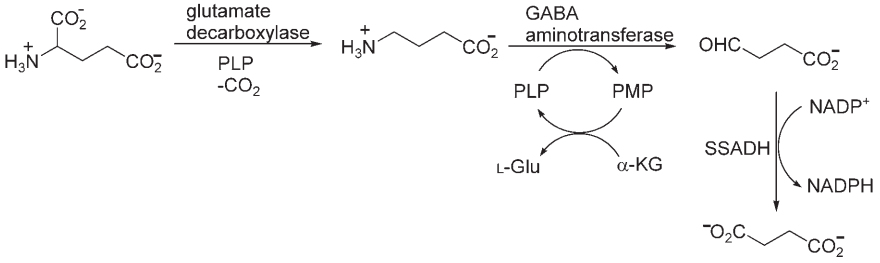
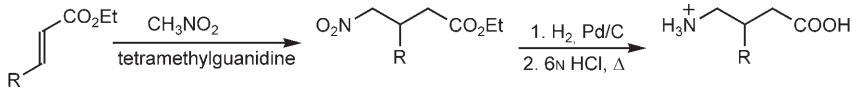


Scheme 1. Interconversion of excitatory and inhibitory neurotransmission. α-KG: α-ketoglutarate; PMP: pyridoxamine 5′-phosphate.



R = Me, Et, *n*Pr, *i*Pr, *n*Bu, *s*Bu, *i*Bu, *t*Bu

Scheme 2. Synthesis of 3-substituted GABA analogues

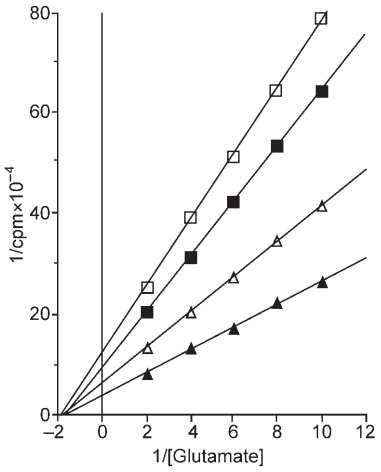


Figure 1. Activation of L-glutamic acid decarboxylase by (*R*)-3-methyl-GABA.[13] The GAD assay was run in the absence (□) and presence [0.25 mM (■), 1.0 mM (△), 2.5 mM (▲)] of (*R*)-3-methyl-GABA.

mechanism for anticonvulsant activity, namely, activation of GAD, leading to an increase in brain GABA levels.

An invention disclosure was submitted to the Northwestern University Technology Transfer Program in 1989, companies were contacted about potential interest in the technology, and two positive responses were received, one from Upjohn Pharmaceuticals and one from Parke–Davis Pharmaceuticals. Upjohn wanted to test only our "best" compound; Parke–Davis would test all of the analogues. The best GAD activator was the 3-methyl-GABA analogue (actually the *R* isomer was more potent than the *S* isomer), so that was selected for anticonvulsant studies in mice at Upjohn. Later that year Upjohn scientists reported that it was only a weak anticonvulsant agent, so they discontinued interest in this series. Parke–Davis, however, called in 1990 to invite me to give a seminar and to discuss the compounds further. It turned out that the ability of all of these compounds to prevent tonic extensor seizures in mice was good at 100 mg kg^{-1}, but at a dose of 14.4 mg kg^{-1} of 3-isobutyl-GABA, nearly complete protection of the mice from electroshock-induced seizures was observed (Table 1).[13]

The (*S*)-(+)-isomer of 3-isobutyl-GABA, later renamed pregabalin, then given the trade name Lyrica, was one of the most potent anticonvulsant agents they had tested. All of the compounds exhibited dose-dependent prevention of tonic extensor seizures in mice without ataxia (an unsteady and clumsy motion of the limbs and torso, a common side effect of anticonvulsant agents).[11] A license agreement was signed between Parke–Davis Pharmaceuticals (actually with Warner–Lambert, the parent company) and Northwestern University, and a patent was applied for toward the end of 1990. This was followed by a patent option agreement in 1991. Animal pharmacokinetic and metabolism experiments were carried out over six months in 1992, followed by animal toxicology studies for two years at Parke–Davis. Syntheses were developed to obtain large quantities of the (*S*)-(+)-isomer of 3-isobutyl-GABA[14] because it was the *S* isomer that was found to contain almost all of the compound's activity (Scheme 3).[15]

The IND (investigational new drug) application (required to allow human testing) was filed at the end of 1995. Phase I clinical trials lasted 2.5 years, then Phase II/III clinical trials were carried out between 1999 and 2003; over 100 different clinical trials with more than 10000 patients were initiated for epilepsy, neuropathic pain, and generalized anxiety disorder. In 2000 Pfizer purchased Warner–Lambert, which gave them exclusive rights to continue the development of Lyrica. In October 2003, the new drug application (NDA) for marketing approval was filed with the Food and Drug Administration (FDA) by Pfizer. Lyrica was approved by the European Union for medical use in July 2004 (it went on the European

Table 1: Anticonvulsant effect of 3-substituted GABA analogues[13]

3-Substituent	Dose [mg kg^{-1}]	Effect[a]	3-Substituent	Dose [mg kg^{-1}]	Effect[a]
(*R*,*S*)-methyl	100	3/5	*n*-butyl	100	2/10
(*R*)-methyl	100	5/10	isobutyl	14.4	9/10
(*S*)-methyl	100	5/10	*sec*-butyl	30	2/10
3,3-dimethyl	100	8/10	*tert*-butyl	100	5/10
ethyl	100	5/5	neopentyl	100	4/10
n-propyl	100	3/10	isopentyl	100	0/10
isopropyl	100	6/10			

[a] Number of protected/number of tested individuals. The compounds were tested for anticonvulsant activity in male CF-1 mice (20–28 g) by intravenous administration followed 120 min later with low-intensity corneal electroshock at 17 mA base-to-peak sinusoidal current for 0.2 s. Anticonvulsant activity was determined by prevention of tonic extensor seizures of the hind limbs from electroshock application.

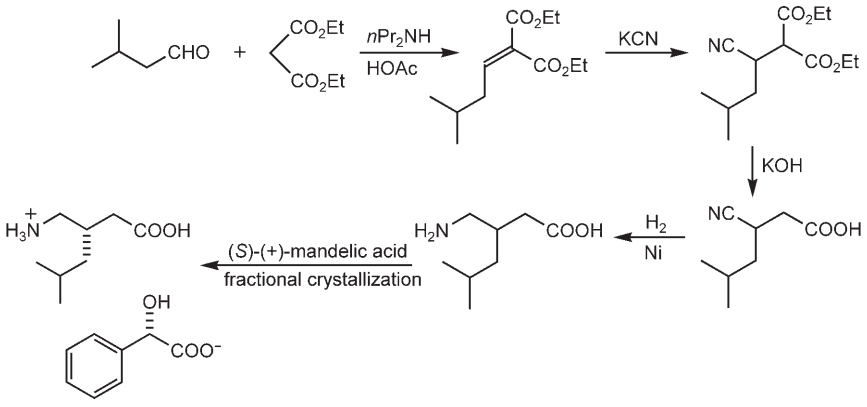
market in September of that year), and by the FDA in December 2004 (it appeared on the U. S. market in September 2005). In its first full year of sales (2006), Lyrica attained the status of a blockbuster drug with $1.2 billion in global sales.

You may be thinking that Upjohn should be "kicking" itself for the shortsightedness of testing just one compound of a series, and losing the opportunity to acquire the compound that became Lyrica. Ironically, the two companies that initially competed for testing the GABA analogues, Upjohn and Parke–Davis, were both bought by Pfizer (Warner–Lambert/Parke–Davis in 2000; Pharmacia/Upjohn in 2002), so it would not have mattered which eventually decided to continue testing the compound; Lyrica still would have been acquired by Pfizer.

This would have been an outstanding example of how the study of fundamental science was rationally developed into an important commercial product, except for one unexpected finding: the anticonvulsant activity of Lyrica is not related to its ability to activate GAD! Further studies showed that there is no correlation between activation of GAD by the 3-alkyl-GABA analogues and their anticonvulsant activity. Prior to the testing of our 3-alkyl-GABA analogues, Parke–Davis Pharmaceuticals had identified a related compound for the treatment of epilepsy called gabapentin (trade name later became Neurontin). We showed that this compound also activated GAD,[13] albeit to a lesser degree than the 3-alkyl-GABA analogues. However, in 1996, scientists at Parke–Davis Pharmaceuticals discovered that gabapentin selectively binds to the $\alpha_2\delta$ subunit of voltage-gated calcium channels.[16] This binding attenuates Ca^{2+} flux into the neuron,[17] which inhibits release of substance P and glutamate from excitatory amino acid nerve terminals.[18] The (*S*)-(+)-isomer of 3-isobutyl-GABA (pregabalin, Lyrica) was subsequently shown to have the same mechanism of action as gabapentin.[19] This mechanism of action represents another ironic twist of fate related to Lyrica: The 3-alkyl-GABA analogues were designed to selectively inhibit GABA-AT, but were found to activate GAD; either mechanism would raise the concentration of the inhibitory neurotransmitter GABA. Instead, Lyrica's mechanism of action turned out to be the blockage of the release of the excitatory neurotransmitter L-glutamate. In effect, lowering the concentration of the excitatory neurotransmitter accomplishes the same objective as raising the concentration of the inhibitory neurotransmitter.

The reason that 3-isobutyl-GABA is so much more potent than the other 3-alkyl-GABA analogues is because it is a substrate for the System L transporter[20] and is actively transported into the brain. The other analogues are not substrates for this transporter and have poor blood–brain barrier penetration. This is reasonable because the System L transporter carries L-leucine into the brain; L-leucine is 2-isobutylglycine, so Lyrica is a suitable mimic of L-leucine.

Although Lyrica was initially designed as a more lipophilic GABA analogue, it is inactive at all GABA receptors tested, does not affect GABA uptake or degradation, and does not change brain GABA levels.[21] The pharmacokinetic properties of Lyrica are what make this drug even more appealing.[22] It is 90% orally bioavailable[23] (90% of the administered compound is absorbed and reaches the site of action), and less than 2% is metabolized; it is excreted completely unchanged.[24] Fur-



Scheme 3. Large-scale synthesis of (*S*)-(+)-3-isobutyl-GABA

thermore, the rate of elimination from the body (half-life is 6 h) is independent of dose and frequency of administration, so there is no concern of variation of drug half-life among individuals taking different doses and at different frequencies. Unlike its predecessor, Neurontin, the pharmacokinetics of Lyrica is linear and highly predictable.[25] Therefore, if the dose is increased, the efficacy is increased and the rate of absorption, distribution, metabolism, and excretion (ADME) are likewise increased. Because of its well-behaved pharmacokinetics, there is no need to take Lyrica with food.[26] Other important properties of this drug are that in clinical trials it was found that the onset of its efficacy can be as early as day two, unlike the usual week or more for CNS drugs to take effect. Lyrica also does not bind to plasma proteins[27] and does not induce or inhibit liver enzymes, particularly cytochrome P450 s.[28] Because of this, drug–drug interactions (when administration of one drug has an effect on metabolism, excretion, and efficacy of another drug) have not been observed.[29]

Clinical trials were extensive because of the multiple indications targeted for approval.[30] Patients with refractory partial-onset seizures (the indication that was the goal when the project began) who received pregabalin 150 to 600 mg per day (divided into two or three doses) concomitantly with antiepileptic drugs, had significantly fewer seizures than placebo recipients ($p \leq 0.0001$); patients with postherpetic neuralgia (shingles) or diabetic peripheral neuropathy (neuropathic pain from diabetes), who received pregabalin 300 to 600 mg per day (divided into two or three doses), had significantly greater improvement in relief of pain and pain-related sleep interference than placebo recipients ($p \leq 0.002$ and $p \leq 0.01$, respectively); and patients with generalized anxiety disorder or social anxiety disorder, who received pregabalin 200 to 600 mg per day (divided into two or three doses), had significantly greater reduction in mean scores on the Hamilton Anxiety Scale than placebo recipients ($p \leq 0.01$). In addition to Lyrica being the first FDA-approved treatment for neuropathic pain associated with both diabetic peripheral neuropathy and postherpetic neuralgia, it recently became the first approved treatment for fibromyalgia, a poorly diagnosed disorder characterized by widespread musculoskeletal pain, lowered pain threshold, disordered sleep, and fatigue, which affects an estimated 2% of the population, predominantly women. In clinical trials for fibromyalgia it was found that Lyrica at 450 mg per day significantly reduced the average severity of pain (50% improvement in pain at the end point) and caused significant improvements in sleep quality, fatigue, and global measures of health-related quality of life.[31]

Use of Lyrica, as with any drug, especially CNS drugs, is not without side effects; however, they are relatively minor considering the devastating pain and debilitating effects of the diseases that it treats. In premarketing controlled clinical trials of all populations combined, 14% of patients treated with Lyrica and 7% of patients treated with placebo discontinued use prematurely due to adverse reactions. The most common side effects were somnolence (sleepiness), dizziness, peripheral edema (accumulation of fluid, particularly in the legs), ataxia (unsteadiness), headache, blurred vision, difficulty concentrating, weight gain, and dry mouth.

Although the discovery of Lyrica had its beginnings in basic science, the eventual outcome was that its mechanism of action is unrelated to the initial observation that led to its testing. This is not uncommon. For example, the cholesterol-lowering drug ezetimibe (Zetia) was designed as an inhibitor of acyl-CoA cholesterol acyltransferase (ACAT)[32] because cholesterol is not directly absorbed, but must first be acylated prior to absorption from the intestines. ACAT is the enzyme that catalyzes the esterification of cholesterol by long-chain fatty acyl-coenzyme A, leading to its absorption; inhibition of ACAT should prevent cholesterol from being absorbed, which would allow consumption of cholesterol without it being utilized. However, no correlation was found between in vitro inhibition of ACAT and in vivo efficacy of Zetia. Although it appeared to inhibit the absorption of cholesterol from the intestines (which was the aim of inhibiting ACAT), it does so by binding to a cholesterol transporter, which then prevents the absorption of cholesterol. Just like Lyrica, which was targeted for an enzyme and was found to function by binding to another protein (the $\alpha_2\delta$ subunit of voltage-gated calcium channel), Zetia also was targeted for an enzyme, but serendipitously functions by binding to another protein (the cholesterol transporter). The key to the discovery of Lyrica and Zetia, however, was the development of a fundamental hypothesis, the design of experiments to test that hypothesis, making and interpreting observations, and the follow through on those observations, leading to the eventual drug discovery. The complexity of the human organism allows specific molecules to interact with multiple potential targets. Consequently, serendipity can be a powerful tool in medicinal chemistry.

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