# $Ca^{2+}$ Channel $\alpha_2$-$\delta$ Ligands for the Treatment of Neuropathic Pain

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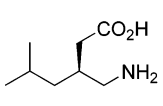
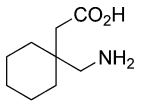
## Introduction

Pregabalin **1** was recently approved in the U.S. and Europe as an add-on therapy for epilepsy, as well as for the treatment of neuropathic pain (specifically, management of neuropathic pain associated with diabetic peripheral neuropathy and postherpetic neuralgia in the U.S. and peripheral neuropathic pain in Europe).[1,2] In addition, **1** recently gained approval in the EU for the treatment of generalized anxiety disorder. As a result of it's superior potency and pharmacokinetic profile[3,4] compared to gabapentin **2**, pregabalin has enjoyed rapid uptake by prescribing physicians for these conditions. Pregabalin is thought




**1**

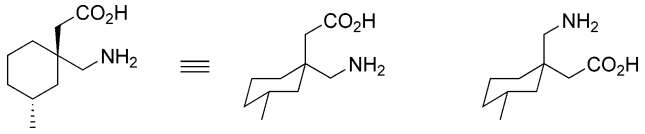



**2**

to access the central nervous system (CNS) via the system L amino acid transporter[5,6] and act via modulation of the $\alpha_2$-$\delta$ subunit of voltage-gated calcium channels.[7,8] Binding to the $\alpha_2$-$\delta$ subunit results in attenuation of calcium flux into the neuron,[9,10] which in turn inhibits the release of various neurotransmitters, including norepinephrine,[11] substance P,[12] and glutamate.[13] In addition, ruthenium red (a relatively nonselective calcium channel ligand) and magnesium chloride have been shown to attenuate not only [$^3$H]gabapentin binding to $\alpha_2$-$\delta$ in vitro[14] but also the antiallodynic effects of gabapentin in an in vivo incisional pain model.[15] Pregabalin is remarkably silent at all other known receptors (with the exception of being a substrate for the system L transmembrane amino acid transporter), and as a consequence, the $\alpha_2$-$\delta$ hypothesis is gaining traction as the accepted MOA of pregabalin. Some excellent reviews categorizing the available evidence to elucidate the mechanism of gabapentin in neuropathic pain have recently appeared.[16−19] The purpose of this Miniperspective is to showcase the medicinal chemistry efforts employed to obtain structurally diverse compounds with both reported affinity for the $\alpha_2$-$\delta$ subunit and in vivo activity in various neuropathic pain paradigms. Experiments to suggest that $\alpha_2$-$\delta$ ligands act to suppress the development and maintenance of central sensitization, as well as a discussion of the implications of $\alpha_2$-$\delta$ subtype localization and function, will also be presented.

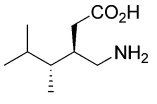
## Medicinal Chemistry To Identify $\alpha_2$-$\delta$ Ligand Compounds

***γ*-Amino Acids.** Several reports have appeared describing $\gamma$-amino acids that show affinity for the $\alpha_2$-$\delta$ subunit of voltage-gated calcium channels.[20] Not surprisingly, many share the core amino acid structural motif inherent to pregabalin. It has been reported that introduction of an appropriately positioned methyl group on the gabapentin backbone as in **3** resulted in enhanced potency for $\alpha_2$-$\delta$ relative to **2**.[21] By use of the ring methyl group to fix the conformation, it was determined that the axial carboxylate was required for $\alpha_2$-$\delta$ binding.[22] Additional in vivo studies showed that **3**, but not the $\alpha_2$-$\delta$ inactive (1*R*,3*R*) diastereomer **4**, blocked the maintenance of spinal nerve ligation and streptozocin-induced static and dynamic allodynia, supporting $\alpha_2$-$\delta$ as the MOA for the analgesic activity.[23] Recent studies by Merck comparing compounds **3** and **4** with gabapentin in the spinal nerve ligation model reproduced the results of those previously reported. Interestingly, data derived from acute challenge models of formalin, carrageenan-induced thermal hyperalgesia, and warm water tail withdrawal led those researchers to conclude that the role of $\alpha_2$-$\delta$ in the pharmacological action of gabapentin was "ambiguous".[24] It should be noted, however, that clinical relevance of such acute models is unclear particularly in relation to the chronic neuropathic disease state.

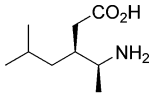



**3** **4**

Similar stereospecific introduction of a methyl group onto the pregabalin backbone to furnish **5** resulted in a 4-fold increase in $\alpha_2$-$\delta$ potency relative to **1**.[25] The affinity for the leucine transporter, known to be important for absorption in the small intestine and uptake into the CNS, was also conserved through this structural modification. As such, **5** showed robust in vivo activity in models of neuropathic pain, epilepsy, and anxiety. However, when the methyl substituent was imparted to the pregabalin structure in the position $\alpha$ to the amine as in **6**, complete erosion of the affinity for system L was observed.




**5**




**6**

Although potent $\alpha_2$-$\delta$ binding affinity was demonstrated for **6**, no in vivo anticonvulsant, analgesic, or anxiolytic-like activity was observed for this compound. As a consequence, this led the authors to postulate that a combination of $\alpha_2$-$\delta$ binding and transport by system L was required for obtaining compounds in this series that were active in vivo. An alignment of the stereochemically endowed structure of **5** with (1*S*,3*R*)-3-methylgabapentin **3** reveals their structural similarity and provides a glimpse into the preferred chemical space for $\gamma$-amino acid $\alpha_2$-$\delta$ ligands (Figure 1).

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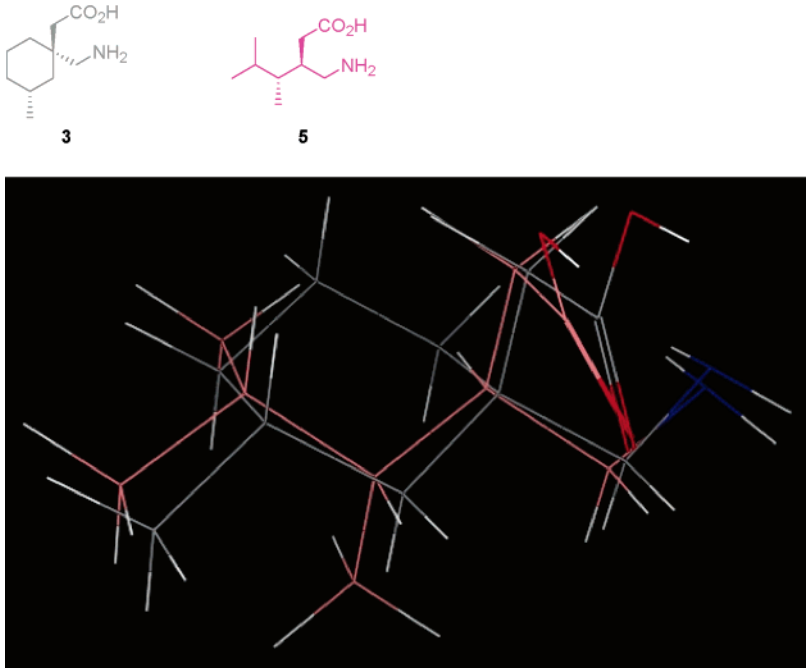
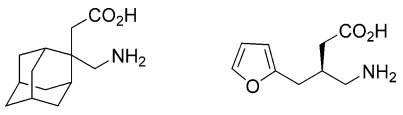


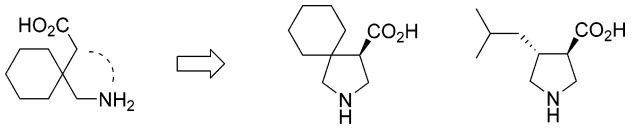
**Figure 1.** Overlay of $\gamma$-amino acids **3** and **5**. Compound **3** is in gray, and compound **5** is in pink.

Additional $\gamma$-amino acid $\alpha_2$-$\delta$ ligands that have been disclosed include an interesting adamantyl-containing structure **7** (Ad-GABA).[26] The in vivo analgesic activity of **7** in the mouse hot plate test of pain sensibility was in fact weaker than gabapentin. It is of particular note that previous attempts to prepare **7** resulted in the formation of a lactam intermediate that could not be hydrolyzed to the corresponding amino acid.[21] However, Kolocouris et al. were able to circumvent formation of the obstinate lactam via hydrogenation of the cyano acid under acidic conditions. The propensity of such spiro-substituted $\gamma$-amino acids to lactamize (including gabapentin) prompted an investigation into carboxylic acid replacements (vide infra). More recently, amino acids containing heterocyclic side chains such as furan in compound **8** were shown to have moderate affinity for $\alpha_2$-$\delta$.[27]



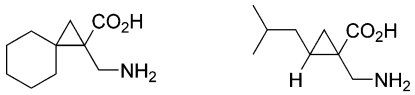
**7** **8**

***β*-Amino Acids.** When a methylene unit constraint was imparted to the gabapentin core structure between the position $\alpha$ to the carboxylate and the amine, the resultant $\beta$-amino acid **9** was found to have potent affinity for $\alpha_2$-$\delta$.[28] As observed previously with $\gamma$-amino acids, stereochemistry proved to be critical as the (*S*)-isomer of **9** was inactive at $\alpha_2$-$\delta$. A similar strategy was reported for the preparation of $\beta$-proline analogues of pregabalin such as **10**.[29]



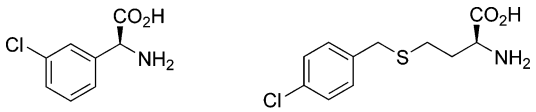
**2** **9** **10**

When constraint was introduced to gabapentin or pregabalin via cyclopropanation, a new class of $\alpha_2$-$\delta$ ligands represented by **11** and **12** containing a $\beta$-amino acid substructure was obtained.[30] As had been observed previously, the relative stereochemistry about the cyclopropane ring was important for $\alpha_2$-$\delta$ binding. For instance, in the case of **12**, the cis orientation of the alkyl substituent and the carboxylate was 10-fold more potent at $\alpha_2$-$\delta$ than the trans isomer. However, none of the cyclopropane amino acids prepared showed any affinity for the system L transporter. As a consequence, in vivo anticonvulsant activity was observed when compound **11** was delivered directly into the brain (intracerebroventricular administration) but not when it was dosed orally.



**11** **12**

**α-Amino Acids.** Abbott and others have identified $\alpha$-amino acids with affinity for $\alpha_2$-$\delta$.[31] Representative examples of potent structures from their investigations included the chlorinated phenylglycine **13** and (*S*)-benzylhomocysteine **14**. They found

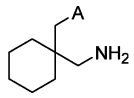


**13** **14**

that the des-chloro analogues of **13** and **14** displayed similar efficacy compared to gabapentin **2** in the rat complete Freund's adjuvant (CFA) model of inflammatory pain. In general, these authors observed that $\alpha_2$-$\delta$ binding affinity did not correlate well to efficacy in either the rat CFA model or spinal nerve ligation model.[32] They went on to speculate that since comparable brain levels were attained with several of their analogues, poor CNS access alone was not sufficient to explain their findings.

**Carboxylate Bioisosteres.** As mentioned earlier, the propensity for the spiro-substituted $\gamma$-amino acids to cyclize to the corresponding lactams spawned an investigation into the replacement of the carboxylate function with a bioisostere. By

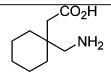
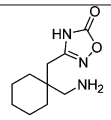
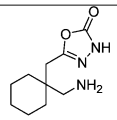
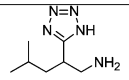
**Table 1.** SAR of Gabapentin Carboxylate Replacements



| Compound | A | $\alpha_2$-$\delta$ $IC_{50}$ (nM)[a] |
|---|---|---|
| **2** | $CO_2H$ | 87 |
| **15** |  | 100 |
| **16** |  | >10,000 |
| **17** |  | 210 |
| **18** |  | 4,220 |
| **19** |  | >10,000 |

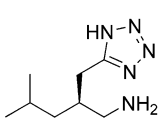
[a] $IC_{50}$ is the concentration (nM) producing half-maximal inhibition of the specific binding of [$^3$H]gabapentin binding to pig brain membranes (see ref 7).

**Table 2.** pKa of Gabapentin and Pregabalin Carboxylate Replacements

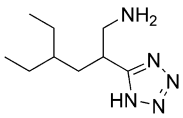
| Compound | Structure | $\alpha_2$-$\delta$ $IC_{50}$ (nM) | $pKa_1$ |
|---|---|---|---|
| **2** |  | 87 | 4.10 |
| **17** |  | 210 | 5.24 |
| **19** |  | >10,000 | 7.18 |
| **20** |  | 170 | 3.74 |

use of conventional replacements, only the tetrazole[33] and oxadiazolone[34] replacements of the gabapentin carboxylate were found to bind to $\alpha_2$-$\delta$ with comparable affinity.[35] Extending this finding, several additional heterocycles were prepared from gabapentin to probe the SAR (Table 1).[36] Radioligand binding $IC_{50}$ values were determined using inhibition of specific binding of [$^3$H]gabapentin to partially purified membranes from pig forebrain.[7] When the acidic hydrogen atom in **15** was replaced by a methyl group as in **16**, binding to $\alpha_2$-$\delta$ was abolished. The importance of acidity of the bioisostere is underscored in Table 2. For instance, simple rearrangement of the oxadiazolone heteroatoms as in **19** results in a heterocycle that is no longer acidic, thereby diminishing affinity for $\alpha_2$-$\delta$.

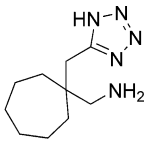
When the acid moiety of pregabalin was replaced with a tetrazole ring (cf. **21**), binding to $\alpha_2$-$\delta$ was diminished with respect to **1**.[35] However, mindful that certain truncated amino acids had shown potency for $\alpha_2$-$\delta$, a series of $\beta$-aminotetrazoles similar to **20** (Table 2) were prepared by one of two highly convergent routes.[37] The most potent compound to emerge from the series was the 2-ethylbutyl substituted analogue **22**, which also demonstrated robust in vivo anticonvulsant activity. $\gamma$-Aminotetrazole **23** ($\alpha_2$-$\delta$ $IC_{50}$ = 100 nM)[35] was further profiled in neuropathic and incisional pain models, which will be discussed below.



**21**

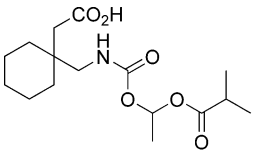


**22**

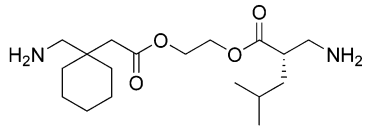


**23**

**Prodrugs.** Compound **24** (XP13512), a carbamate prodrug of gabapentin that is a substrate for several absorption pathways other than system L, was designed by Xenoport with the aim of achieving improved bioavailability and dose proportionality compared to gabapentin prior to liberation of the parent amino acid via esterases.[38] Now in clinical development by Xenoport, **24** has demonstrated 17-fold higher gabapentin exposure in rats and a 34-fold increase in monkeys.[39] Other less sophisticated diester prodrug "dimers" of gabapentin and pregabalin (cf. **25**) reversed tactile allodynia in the rat chronic constriction injury (CCI) model.[40]

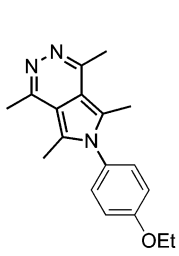


**24**

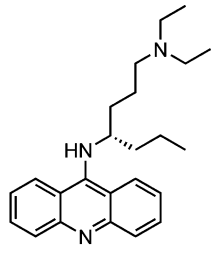


**25**

**Non-Amino Acid Structures.** Finally, through high-throughput screening, Merck has identified several $\alpha_2$-$\delta$ ligands that do not contain an amino acid core. Hence, compound **26** dosed ip demonstrated slightly more potent activity than gabapentin in the rat Chung model of neuropathic pain.[41] Additional derivatives of **26** were prepared using parallel chemistry techniques.[42] The effort also resulted in the discovery of a potent acridine analogue **27** that upon tritiation showed high specific binding to $\alpha_2$-$\delta$ using gabapentin as a cold displacer.[43] A tritiated analogue of **23** afforded a similar result.[44] Pyrrolopthalazine **28** was prepared and tritiated as before and also shown to bind to $\alpha_2$-$\delta$.[45] Not surprisingly, given the proclivity of $\alpha_2$-$\delta$ to stereodifferentiation, the (*R*)-isomer of **28** was inactive at $\alpha_2$-$\delta$.



**26**



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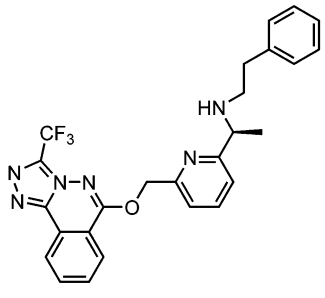

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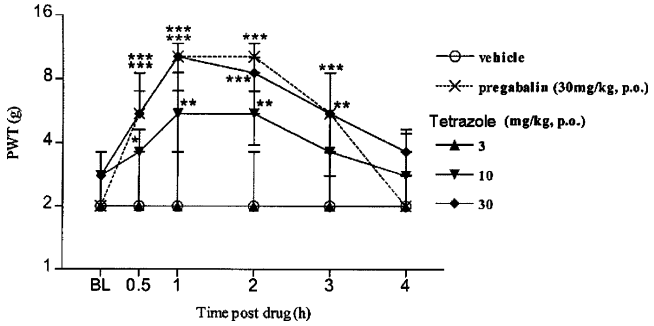


**Figure 2.** Blockade of static allodynia by tetrazole **23** in rat CCI model. Results are expressed as median force (g) required to induce a withdrawal of 8−10 animals per group (vertical bars represent upper and lower quartiles), (∗) $P < 0.05$, (∗∗) $P < 0.01$, (∗∗∗) $P < 0.001$ significantly different (Kruskal−Wallis followed by Mann−Whitney *U* test), comparing ipsilateral paw of drug-treated groups to ipsilateral paw of vehicle-treated group at each time point.

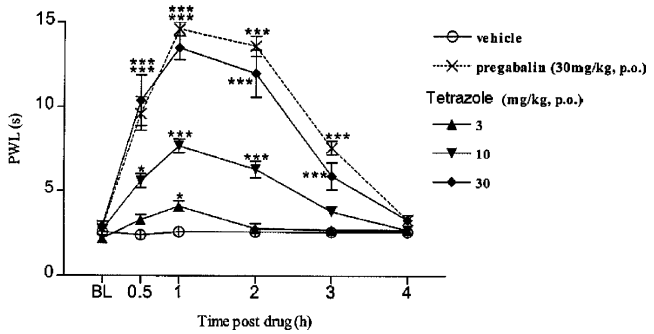


**Figure 3.** Blockade of dynamic allodynia by tetrazole **23** in rat CCI model. Results are expressed as the mean PWL (*s*) of 8−10 animals per group (vertical bars represent ± SEM), (∗) $P < 0.05$, (∗∗) $P < 0.01$, (∗∗∗) $P < 0.001$ significantly different (ANOVA followed by Dunnett's *t* test), comparing ipsilateral paw of drug-treated groups to ipsilateral paw of vehicle-treated group at each time point.

### Effect of $\alpha_2$-$\delta$ Ligands on Central Sensitization

Neuropathic pain is a consequence of disease, trauma, or dysfunction of the central or peripheral nervous systems. It is characterized by the presence of spontaneous and evoked pains including allodynia and hyperalgesia. The disease is poorly treated by nonsteroidal anti-inflammatory drugs, and even opioids have limited efficacy; thus, new mechanisms such as the modulation of calcium channels represent an attractive target for drug discovery.[46] Neuropathic pain is thought to be produced by spontaneous abnormal (ectopic) activity following injury to a nerve, which may persist even though any external tissue injury has resolved. The resultant discharges from peripheral nerves result in increased spinal neurotransmitter release which in turn increases the excitation of the spinal dorsal horn and creates a phenomenon known as windup (or summation), leading to hyperexcitability and central sensitization[47] of the dorsal horn. The sensitized spinal cord contains increased levels of various neuronal proteins including $\alpha_2$-$\delta$.[48] Physiologically, the outcome of these neuronal changes is the development and maintenance of neuropathic pain.

Tetrazole $\alpha_2$-$\delta$ ligand **23**[35] was investigated in the chronic constriction injury model of neuropathic pain and demonstrated efficacy similar to that of pregabalin **1**, which was used as a control (Figures 2 and 3).[49,50] To assess the effects of **23** on central sensitization, the compound was tested in the rat footpad incisional model.[51] Plantaris muscle (sole) paw incision surgery was performed, and paw withdrawal threshold to von Frey hairs (PWT) was re-examined 1 h after surgery. Tetrazole **23** was

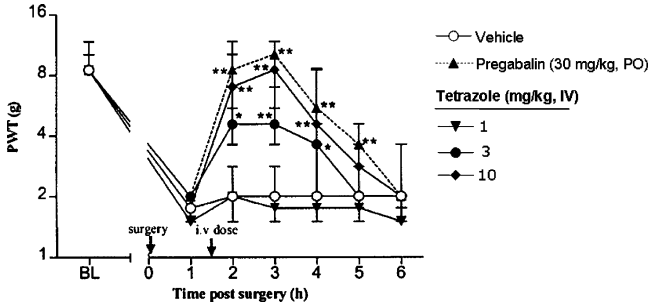


**Figure 4.** Blockade of static allodynia by tetrazole **23** in rat footpad incision model. Results are expressed as median force (g) required to induce a withdrawal of 8−10 animals per group (vertical bars represent upper and lower quartiles), (∗) $P < 0.05$, (∗∗) $P < 0.01$, significantly different (Kruskal−Wallis followed by Mann−Whitney *U* test), comparing ipsilateral paw of drug-treated groups to ipsilateral paw of vehicle-treated group at each time point.

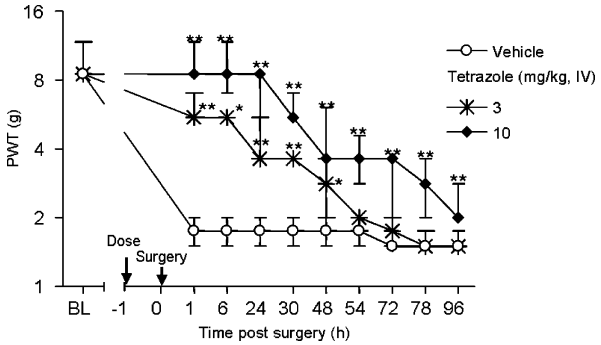


**Figure 5.** Blockade of static allodynia in the incisional model by tetrazole **23** administered prior to surgery. Results are expressed as median force (g) required to induce a withdrawal of 8−10 animals per group (vertical bars represent upper and lower quartiles), (∗) $P < 0.05$, (∗∗) $P < 0.01$, significantly different (Kruskal−Wallis followed by Mann−Whitney *U* test), comparing ipsilateral paw of drug-treated groups to ipsilateral paw of vehicle-treated group at each time point.

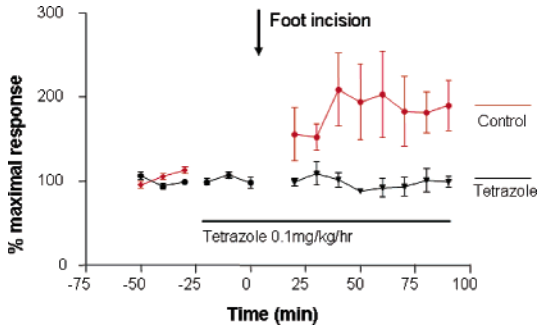


**Figure 6.** Effect of tetrazole **23** on electrophysiological activity in incision model.

administered as an iv bolus directly followed by an iv infusion, and PWTs were re-examined up to 6 h after surgery. Similar effects were observed with both **23** and pregabalin, namely, a reversal of allodynia out to 5 h (Figure 4). When tetrazole **23** was dosed *prior* to surgery, however, the analgesic effect lasted 3−4 days, indicating interference in the development of central sensitization (Figure 5).

Gabapentin has been shown to suppress ectopic discharges resulting from peripheral nerve injury.[52] The effect of tetrazole **23** on the responses of spinal WDR (wide dynamic range) neurons to a 0.6 g mechanical stimuli following plantar surgery of the hind paw was examined next (Figure 6). The outcome was indeed prevention of sensitization by the infusion of 0.1 mg/kg/h of tetrazole **23**. Clinically, it has been shown that

premedication with both pregabalin and gabapentin resulted in a significant mitigation of postsurgical pain response.[53,54]

### Studies with R217A Mutant Mice (Deficient in Drug Binding to $\alpha_2$-$\delta$ Type 1) in Neuropathic Pain Paradigms

Of the four known subtypes of $\alpha_2$-$\delta$, only $\alpha_2\delta$-1 and $\alpha_2\delta$-2, but not $\alpha_2\delta$-3 or $\alpha_2\delta$-4, bind pregabalin and gabapentin.[55,56] In an attempt to elucidate the pharmacological role of the pregabalin-binding subtypes, a single amino acid substitution of the arginine residue at position 217 on the $\alpha_2\delta$-1 protein with alanine (R217A) reduced [$^3$H]gabapentin binding in vitro with recombitant mammalian cells.[57] On the basis of this finding, a strain of genetically modified mice was produced that were homozygous for the mutation.[58] Saturation binding and autoradiography studies in R217A mice demonstrated a pronounced decrease in [$^3$H]gabapentin binding to areas where $\alpha_2\delta$-1 is preferentially expressed (neocortex, hippocampus, basolateral amygdala, and spinal cord) but little change in binding to cerebellum and brainstem, which are dominated by $\alpha_2\delta$-2.[59] Additionally, analgesia from pregabalin, but not from opiates or tricyclic antidepressants, was abolished in the R217A knock in mice in the CCI and formalin models, indicating that binding to the $\alpha_2\delta$-1 subtype is required for activity against pain states.[60,61]

### Studies on Distribution of $\alpha_2\delta$-1 and $\alpha_2\delta$-2 Subtypes: Implications for Future Therapeutics

In a recent gene expression study utilizing an in situ hybridization method, Cole et al. systematically mapped $\alpha_2$-$\delta$ subunit mRNA-containing cells throughout the rat CNS and the dorsal root ganglia.[62] $\alpha_2\delta$-1, $\alpha_2\delta$-2, and $\alpha_2\delta$-3 mRNA-containing cells were found throughout the CNS and dorsal root ganglia in regions that are believed to be involved in pain mediation (e.g., dorsal horn of spinal cord, dorsal root ganglia, brainstem, and thalamus).[63] It is interesting to note that the expression of $\alpha_2\delta$-1 and $\alpha_2\delta$-2 mRNA was largely divergent within particular rat brain locations. For example, in regions such as the cortex, the pyramidal cell layers of hippocampus, and the basomedial nucleus of amygdala, the expression of $\alpha_2\delta$-1 was high whereas that of $\alpha_2\delta$-2 was low. In contrast, brain regions including the septum, reticular nucleus, and medial habenula of thalamus, laterodorsal tegmental nucleus, and arcuate nucleus of hypothalamus showed high levels of $\alpha_2\delta$-2 mRNA expression but low levels of $\alpha_2\delta$-1. Therefore, an individual $\alpha_2$-$\delta$ subtype may complex with different $\alpha$1 subunits of VGCCs[64] to provide differential regulation to the pore-forming $\alpha_1$ subunit in different brain structures or neuronal cell types. For example, $\alpha_2\delta$-2 was colocalized selectively on GABAergic interneurons in the cortex, hippocampus, the reticular nucleus of the thalamus and the Purkinje cells of cerebellum. The loss of $\alpha_2\delta$-2 protein in these cells could result in a decrease in GABAergic inhibition. The ataxia associated with the phenotype in ducky mouse, a spontaneous mutation in the Cacna2 ($\alpha$2$\delta$-2) gene[65] and in newly identified entla mutant,[66] may result from the loss of inhibitory influence in GABAergic circuitry, especially from Purkinje cells of the cerebellum. $\alpha_2\delta$-2 mRNA-containing cells were also found in high density in brain structures thought to play important roles in the regulation of normal sleep and awakening (e.g., interpeduncular nucleus, pedunculopontine nucleus, and laterodorsal tegmental nucleus) or arousal (e.g., nucleus incertus).[67−69] It has been previously shown that pregabalin was equipotent in binding to the $\alpha_2\delta$-1 and $\alpha_2\delta$-2 subtypes[70] and that the most common adverse events reported for pregabalin in clinical trials for neuropathic pain were sedation, dizziness, and ataxia.[71] On the basis of the distribution of $\alpha_2\delta$-2 neurons in the CNS, the above data suggest that a compound with selective affinity for either the $\alpha_2\delta$-1 or $\alpha_2\delta$-2 subtype may contain a unique therapeutic and side effect profile in comparison to a nonselective agent.

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### Autobiographies

**Mark J. Field** started his career in the pharmaceutical industry at Merck Sharp & Dohme Neuroscience Center (Harlow, U.K.) in 1986 working in Susan Iverson's behavioral pharmacology lab. In 1990 he joined the Parke-Davis (Warner-Lambert) Neuroscience Research Center in Cambridge, U.K., eventually managing the in vivo behavioral pharmacology group. During this time he completed his training at the University of London (Institute of Psychiatry). Mark moved to Pfizer Global Research and Development, Sandwich Laboratories, in 2001 working in the pain therapeutic area and is currently an Associate Director in the Translational Medicine group.

**Zheng Li** received a B.S. degree in Biophysics from the University of Science and Technology of China in 1984 and a Ph.D. in Neuroscience and Behavior from the University of Massachusetts at Amherst in 1996. He completed NSF-sponsored postdoctoral training with Professors Stephen S. Easter and Daniel Goldman at the University of Michigan, Ann Arbor. Currently Zheng is a Principal Scientist at Pfizer Global Research and Development, Michigan Laboratories, in the neuroscience therapeutic area.

**Jacob B. Schwarz** received a B.S. degree in Chemistry from Michigan State University in 1993, and a Ph.D. in Synthetic Organic Chemistry from Colorado State University in 1997 under the direction of Prof. Albert I. Meyers. After an NIH-sponsored postdoctoral fellowship in the laboratories of Prof. Samuel J. Danishefsky at Memorial Sloan-Kettering Cancer Center in New York City, he joined the Parke-Davis Division of the Warner-Lambert Company in Ann Arbor, MI. Currently Jacob is a Senior Principal Scientist at Pfizer Global Research and Development, Michigan Laboratories, in the neuroscience therapeutic area.

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