ABSTRACT
Given their vital roles in mediating neuronal excitability, it is not surprising that voltage-gated calcium channels have been implicated in contributing to the development and maintenance of pain processes. Gabapentin, a synthetic analog of the neurotransmitter γ-aminobutyric acid (GABA), is a clinically effective anticonvulsant recently shown to be efficacious also for a variety of neuropathic pain conditions. While its mechanism of action is incompletely understood, current evidence suggests that gabapentin does not directly interact with GABA receptors but rather modulates the activity of high threshold calcium channels. Although gabapentin binds to the calcium channel α2δ ancillary subunit, it remains to be determined whether this interaction solely accounts for its therapeutic effects.

Key words: neuropathic pain; calcium channel; GABA analog

INTRODUCTION
Calcium influx through voltage-gated calcium channels has been demonstrated to initiate neurotransmitter release, mediate electrical excitability, activate a variety of second messenger pathways, as well as mediate neuronal outgrowth and gene expression. Calcium channels are also critical in mediating both the development and maintenance of the neuronal sensitization processes associated with neuropathic pain and provide attractive candidates for the development of analgesic drugs [reviewed in Vanegas and Schaible, 2000].

Pharmacological and electrophysiological studies have shown that at least five distinct classes of calcium channels are expressed in most neurons [reviewed by Stea et al., 1994; Catterall, 2000]. Calcium channels that first activate with strong depolarization (high voltage-activated, HVA) can be classified into L-type, N-type, and P/Q-type. L-type channels can be distinguished by their sensitivity to several classes of small organic molecules that include the dihydropyridines (DHPs), phenylalkylamines, and benzothiazepines. In contrast, N-type and P/Q-type channels are high-affinity targets for certain peptide toxins produced by venous spiders and marine snails: N-type channels are blocked by the α-conopeptides α-conotoxin GVIA (CTx-GVIA) and MVIIA (CTx-MVIIA) isolated from Conus geographus and Conus magus, respectively, while P/Q-type channels are resistant to CTx-GVIA and CTx-MVIIA but are sensitive to the funnel web spider peptide, α-agatoxin IVA (Aga-IVA). A fourth general class of neuronal calcium channel called T-type or low voltage-activated (LVA) displays voltage-dependent and kinetic properties distinct from HVA channels although they are not well characterized pharmacologically. Finally, a fifth class of relatively undefined calcium current, sometimes called R-type, dis-
plays various properties shared by both HVA and LVA channels.

Neuronal HVA calcium channels are composed of a large (> 200 kDa) pore-forming α subunit that is the target of identified pharmacological agents, a cytoplasmically localized ~50–70 kDa β subunit that tightly binds the α subunit and modulates channel biophysical properties, and an ~170 kDa αδ subunit [reviewed by Steyaert et al., 1994; Catterall, 2000]. Functional LVA calcium channels are encoded by an α subunit alone although the exact biochemical composition of this class of channels remains to be described. At the molecular level, nine different α subunits expressed in the nervous system have been identified and shown to encode the major classes of native calcium currents (Table 1).

Which types of neuronal calcium channels are likely to prove most useful as therapeutic targets for pain intervention? From a physiological perspective, it would seem that those channel types directly involved in neurotransmitter release would be the most favored candidates. A number of different studies indicate that L-type channels are unlikely to be directly involved in pain signaling processes. For example, examination of the cellular and subcellular distributions of the αC and αD subunits indicates these L-type channels are predominantly localized postsynaptically on cell bodies and proximal dendrites [Hell et al., 1993]. Furthermore, there is little evidence that L-type channels contribute to neurotransmitter release at either central or peripheral synapses [reviewed by Dunlap et al., 1995]. Finally, studies examining the effects of L-type calcium channel blockers in a variety of animal models indicate no significant effect of these agents on mechanical- or thermal-induced acute pain, on inflammatory pain, or on neuropathic pain conditions [Chaplan et al., 1994; McMahon and Yaksh, 1994; Sluka, 1997].

In contrast to L-type channels, the αA/P/Q-type and αB N-type channels are highly localized to presynaptic terminals on many central neurons [Westenbroek et al., 1992, 1995]. Application of peptide toxins to selectively inhibit N-type and P/Q-type channels blocks a significant portion of neurotransmission and indicates that these two channels together account for a large fraction of neurotransmitter release in the mammalian CNS [reviewed by Dunlap et al., 1995]. Furthermore, intrathecal administration of CTX-GVIA or CTX-MVIA to block N-type channels shows a strong effect on inflammatory pain, post-surgical pain, thermal hyperalgesia, and mechanical allodynia [McMahon and Yaksh, 1994; Bowersox et al., 1996; Sluka, 1998; Wang et al., 1998]. Administration of Aga-IVA to block P/Q-type channels has been shown to inhibit the late phase formalin response and inflammatory pain but to have no significant effect on mechanical allodynia or thermal hyperalgesia [Chaplan et al., 1994; McMahon and Yaksh, 1994; Sluka, 1997, 1998].

Mutations in calcium channel α subunit genes in animals also provide important clues to those that are potential therapeutic targets for pain intervention. For example, mice lacking a functional αA/P/Q-type gene exhibit severe muscle spasms and ataxia and eventually die by 3–4 weeks postnatal [Jun et al., 2000; Fletcher et al., 2001]. Taken together with evidence that spontaneous αA gene mouse mutants exhibit ataxia and seizures, these data suggest that although the P/Q-type channel contributes to some types of pain processes, the highly efficacious block of this channel in humans could be fraught with significant side effects issues.

### TABLE 1. Native and Molecular Classification Scheme of Neuronal Calcium Channels

<table>
<thead>
<tr>
<th>Native class</th>
<th>cDNA</th>
<th>Gene name</th>
<th>ω-AGA IVA</th>
<th>ω-CTX GVIA</th>
<th>Dihydopyridines</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/Q-type</td>
<td>α1A</td>
<td>CaV2.1</td>
<td>✓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N-type</td>
<td>α1B</td>
<td>CaV2.2</td>
<td>—</td>
<td>✓</td>
<td>—</td>
</tr>
<tr>
<td>L-type</td>
<td>α1C</td>
<td>CaV1.2</td>
<td>—</td>
<td>—</td>
<td>✓</td>
</tr>
<tr>
<td>T-type</td>
<td>α1D</td>
<td>CaV1.3</td>
<td>—</td>
<td>—</td>
<td>✓</td>
</tr>
<tr>
<td>Novel, R-typea</td>
<td>α1E</td>
<td>CaV2.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>L-typeb</td>
<td>α1F</td>
<td>CaV1.4</td>
<td>—</td>
<td>—</td>
<td>✓</td>
</tr>
<tr>
<td>T-type</td>
<td>α1G</td>
<td>CaV3.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>T-type</td>
<td>α1H</td>
<td>CaV3.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>T-type</td>
<td>α1I</td>
<td>CaV3.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

aThe α1E subunit has been variously reported to encode a novel type of calcium with properties shared between both low and high threshold calcium channels [Soong et al., 1993; Bourinet et al., 1996] or a type of high threshold channel resistant to DHPs, ω-agatoxin-IIVA, and ω-conotoxin-GVIA and called R-type (for “residual”). That the tarantula toxin, SNX-482, blocks exogenously expressed α1E currents [Newcombe et al., 1998] but is only partially effective on native cerebellar R-type currents [Tottene et al., 2000], suggests that α1E does not encode a significant portion of the R-type current as originally defined [Randall and Tsien, 1995]. Furthermore, mice deficient for the α1E gene retain a substantial cerebellar R-type current [Wilson et al., 2000], again refuting the notion that the α1E channel accounts for a significant portion of native “R-type” currents. Overall, while the exact native calcium current encoded by the α1E channel remains to be precisely defined, it is likely that R-type currents actually reflect a heterogeneous mixture of currents. This mixture likely consists of α1E currents together with other currents resulting from either yet to be identified calcium channel subtypes and/or from the incomplete block of the known high threshold calcium channels by DHPs and peptide toxins.

bAlthough the functional expression of the α1F subunit has yet to be demonstrated, this channel is designated as L-type based upon conservation of amino acid residues critical for dihydropyridine and phenylalkylamine binding. ✓ indicates sensitivity to the pharmacological agent.
Knock-out of the \( \alpha_{1E} \) calcium channel in mice shows a very different phenotype compared to animals null for the \( \alpha_{1B} \) channel. Interestingly, while the \( \alpha_{1E} \) channel is widely expressed in the CNS and other tissues, homozygous \( \alpha_{1E} \) null mice survive to adulthood, reproduce, and are apparently behaviorally normal [Saegusa et al., 2000; Wilson et al., 2000]. Of particular interest, mutant mice exhibit an increased resistance to formalin-induced pain suggesting an involvement of the \( \alpha_{1E} \) calcium channel in transmitting and/or the development of somatic inflammatory pain [Saegusa et al., 2000].

Mice null for the \( \alpha_{1B} \) N-type calcium channel have also recently been reported [Saegusa et al., 2001; Ino et al., 2001]. Somewhat surprisingly given their significant contribution to neurotransmission, \( \alpha_{1B} \) null mice survive to adulthood and reproduce, although there are some effects on the sympathetic nervous system. Importantly, these mice also exhibit a significantly reduced sensitivity to neuropathic and inflammatory pain.

**GABAPENTIN AS A NOVEL ANALGESIC**

Gabapentin, \( 1-(\alpha\text{-aminoethyl}) \) cyclohexaneacetic acid; Neurontin\textsuperscript{a}), is an anticonvulsant originally found to be active in a number of animal seizure models [Taylor et al., 1998]. Subsequent work has demonstrated that gabapentin is also successful at preventing hyperalgesia in a number of different animal pain models, including chronic constriction injury (CCI), heat hyperalgesia, inflammation, diabetic neuropathy, and static and dynamic mechanallodynia associated with postoperative pain [Taylor et al., 1998; Cesena and Calcult, 1999; Field et al., 1999; Cheng et al., 2000; Nicholson, 2000]. In humans, gabapentin exhibits clinically effective anti-hyperalgesic activity against a wide-ranging number of neuropathic pain conditions. Numerous open label case studies and three large double blind trials now provide supporting evidence for gabapentin as a useful alternative in the treatment of pain. Doses ranging from 300–2,400 mg/day were effective in treating diabetic neuropathy [Backonja et al., 1998], postherpetic neuralgia [Rowbotham et al., 1998], trigeminal neuralgia, migraine, and pain associated with cancer and multiple sclerosis [Di Trapini et al., 2000; Caraceni et al., 1999; Houckens et al., 1997; see also Magnus, 1999; Laird and Gidal, 2000; Nicholson, 2000]. Moreover, gabapentin has no major drug interactions, and has proved advantageous as an add-on treatment recommended for use in combination therapies [Caraceni et al., 1999]. Additional evidence also exists for the successful treatment of other indications including cocaine addiction and various movement and psychiatric disorders [Markowitz et al., 1997; also reviewed in Magnus, 1999]. Gabapentin as a treatment is particularly attractive in that it offers reasonable efficacy, yet is associated with a well-documented and favorable side effect profile [Ramsey, 1995], a property not usually associated with conventional neuropathic pain therapies. Gabapentin is neither protein bound nor metabolized in humans and is renally excreted as the unchanged parent compound.

Although gabapentin has now proved an effective treatment for a number of neuropathic pain conditions, its mechanism of action remains elusive. As a drug, gabapentin was originally designed as a structural analogue of the inhibitory neurotransmitter \( \gamma \)-aminobutyric acid (GABA). Nevertheless, work to date suggests that gabapentin does not bind to either \( \text{GABA}_A \) or \( \text{GABA}_B \) receptors, it is not converted metabolically into GABA and is neither a substrate nor an inhibitor of GABA transport [Taylor et al., 1998; McClelland et al., 2000]. Rather, an alternative site for gabapentin interaction was identified on the \( \alpha_{2\delta} \) subunit of HVA calcium channels [Gee et al., 1996]. Expression studies have characterized several modulatory effects associated with a functional interaction between the accessory \( \alpha_{2\delta} \) and the pore-forming \( \alpha_1 \) subunit of calcium channels [reviewed in Walker and De Waard, 1998]. As the only ligand identified to-date that interacts with this accessory \( \alpha_{2\delta} \) subunit, gabapentin may therefore provide a unique, alternative candidate for mediating voltage-dependent calcium influx. Indeed, the differing \( \alpha_{2\delta} \) binding affinities of stereo-selective analogues of gabapentin correlate well with their structure-activity relationships in animal models of pain [Field et al., 2000] and levels of the accessory \( \alpha_{2\delta} \) subunit have been shown to be up-regulated in dorsal horn tissue obtained from hyperalgesic rats [Philp et al., 1999a,b]. Levels of mRNA, protein, and \( [3H] \)-gabapentin binding sites were all shown to be increased and were accompanied by a corresponding increase in expression of the pore-forming \( \alpha_{1B} \) (N-type) subunit [Philp et al., 2000]. N-type calcium channels have already been shown to play an important role in mediating neuropathic pain [Vanegas and Schaible, 2000] and the potential for indirect modulation of channel function associated with gabapentin binding to the \( \alpha_{2\delta} \) subunit may prove an important means for mediating neuronal excitability and neuropathic pain.

Voltage-dependent calcium influx is associated with the activation of a number of critical intracellular pathways and contributes to the overall function and development of many different tissues. Given the ubiquitous and critical role played by calcium channels, use of channel inhibitors as potential therapeutic agents might be expected to produce potentially serious side effects. Yet, gabapentin exerts relatively specific analgesic actions with only minor contraindications. In addition, gabapentin is not effective in all patients [Laird and Gidal, 2000], a finding that may reflect a biovariability in its pharmacokinetic properties or target binding interactions of gabapentin. The recent identification of three different...
subtypes of αδ subunit provides the potential for subunit-specific interaction with gabapentin [Klugbauer et al., 1999; Su et al., 2000] and the tissue-specific distribution of different αδ subtypes may also contribute to its localized site of action. Moreover, gabapentin efficacy may require, not only the right combination of channel subunits, but distinct modulatory conditions associated with the presence of additional accessory proteins and/or activation of second messenger cascades [Sutton et al., 2000]. Experimental evidence supports this assumption, and is reflected in a mixed susceptibility of different calcium channel preparations to the action of gabapentin.

In 1998, Stefani et al. were the first group to demonstrate that gabapentin inhibited voltage-dependent calcium channel currents recorded from cortical neurons, although the gabapentin-mediated reduction in current varied between different cell types [Stefani et al., 1998]. A survey of comparative work undertaken by a number of different groups also reflects the inconsistent nature of the gabapentin-sensitive calcium current. However, several independent studies have now shown that, given the right conditions, gabapentin can produce a reduction in calcium channel influx. Gabapentin reduces P/Q-type calcium current into synaptosomes and inhibits release of excitatory amino acids from neocortical and trigeminal nucleus slices [Fink et al., 2000; Maneuf and McKnight, 2000]. In these systems inhibition by gabapentin has been shown to be both stimulus-dependent and regionally selective [Dooley et al., 2000; Meder and Dooley, 2000; Maneuf and McKnight, 2000]. Early studies indicated that gabapentin had no effect on freshly dissociated dorsal root ganglia [DRG; Rock et al., 1993]. More recently, however, a decrease in current has been detected using both electrophysiological and calcium imaging techniques [Martin et al., 2000; McClelland et al., 2000; Sarantopoulos et al., 2000; Sutton et al., 2000]. Depression of presynaptic calcium currents participating in nociceptive synaptic transmission may well contribute to the antihyperalgesic action of gabapentin. Recordings taken from dorsal horn neurons in spinal cord slices from adult hyperalgesic rats or neonatal control slices have confirmed a presynaptic (DRG) site of action for gabapentin [Patel et al., 2000; Shiyoma et al., 2000]. Nevertheless, affects on spinal glutamatergic synaptic transmission are not consistently observed in the literature. Postsynaptic affects have also been demonstrated whereby gabapentin has been shown to variably modulate either NMDA- or AMPA-mediated synaptic transmission in freshly dissociated DRGs, control slices and in vivo [Rock et al., 1993; Chizh et al., 2000; Shiyoma et al., 2000]. Investigation of comparable effects in epileptic tissue has also revealed similar discrepancies in determining the mechanism of action of gabapentin.

Electrophysiological recordings have failed to detect any gabapentin-mediated change in the calcium channel currents recorded from hippocampal neurons taken from patients with temporal lobe epilepsy [Shumacher et al., 1998]. Nevertheless, gabapentin was shown to prevent increased duration of seizure discharge in the rat hippocampus in a similar manner to the L-type blocker, nimodipine [Stringer and Taylor, 2000]. There is now additional evidence for gabapentin exerting its influence by a number of alternative means, independent from a direct action at the calcium channel αδ subunit. Gabapentin can substitute as a substrate for the system L amino acid transporter [Stewart et al., 1993; Su et al., 1995], providing an effective route of entry for gabapentin into the intracellular environment. As a consequence, accumulating concentrations of gabapentin could interact with any number of different cytoplasmic proteins and second messenger cascades, indirectly influencing calcium influx and/or other neuronal functions. Although gabapentin does not appear to act directly with either GABA_A or GABA_B receptor isoforms [Rock et al., 1993; Taylor et al., 1998], gabapentin has been shown to increase in vitro activity of the GABA synthesizing enzyme, glutamic acid decarboxylase [GAD; Taylor et al., 1998], increase the turnover of GABA and non-vesicular release [Honmou et al., 1995a,b] and enhance levels of GABA in the brains of epileptic patients [Petroff et al., 1996]. Thus, the overall mechanism of action for gabapentin may well reflect a combined action mediated by both extra and intracellular targets that together provide a means for reducing overall neuronal excitability.

**SUMMARY**

Gabapentin is a calcium channel ligand currently prescribed for pain that exhibits atypical properties when compared to traditional calcium channel antagonists. The use of gabapentin to successfully treat a broad range of hyperalgesic conditions, combined with its well-tolerated side effect profile, suggests that the actions of this drug may be mediated via a distinct assortment of different calcium channel subunit combinations and/or additional mechanisms, rather than a global reduction in voltage-gated calcium influx per se. In the future, it may therefore be possible to develop specific calcium channel ligands, whose conditions of use are targeted to and limited by hyperexcitable disease-associated changes such as those typically encountered with neuropathic pain and epilepsy.

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