A summary of mechanistic hypotheses of gabapentin pharmacology

Charles P. Taylor a,*, Nicolas S. Gee d, Ti-Zhi Su b, Jeffery D. Kocsis e, Devin F. Welty c, Jason P. Brown d, David J. Dooley a, Philip Boden d, Lakhbir Singh d

a Department of Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48105, USA
b Department of Molecular Biology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48105, USA
c Department of Pharmacokinetics and Drug Metabolism, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48105, USA
d Parke-Davis Neuroscience Research Centre, Cambridge University Foresee Site, Robinson Way, Cambridge, CB2 2QB, UK
e Neuroscience and Regeneration Research Center A127A, Veterans Affairs Medical Center, Building 34, Room 123, 950 Campbell Ave., West Haven, CT 06516, USA

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Abstract

Although the cellular mechanisms of pharmacological actions of gabapentin (Neurontin®) remain incompletely described, several hypotheses have been proposed. It is possible that different mechanisms account for anticonvulsant, antinociceptive, anxiolytic and neuroprotective activity in animal models. Gabapentin is an amino acid, with a mechanism that differs from those of other anticonvulsant drugs such as phenytoin, carbamazepine or valproate. Radiotracer studies with [14C]gabapentin suggest that gabapentin is rapidly accessible to brain cell cytosol. Several hypotheses of cellular mechanisms have been proposed to explain the pharmacology of gabapentin: 1. Gabapentin crosses several membrane barriers in the body via a specific amino acid transporter (system L) and competes with leucine, isoleucine, valine and phenylalanine for transport. 2. Gabapentin increases the concentration and probably the rate of synthesis of GABA in brain, which may enhance non-vesicular GABA release during seizures. 3. Gabapentin binds with high affinity to a novel binding site in brain tissues that is associated with an auxiliary subunit of voltage-sensitive Ca2+ channels. Recent electrophysiology results suggest that gabapentin may modulate certain types of Ca2+ current. 4. Gabapentin reduces the release of several monoamine neurotransmitters. 5. Electrophysiology suggests that gabapentin inhibits voltage-activated Na+ channels, but other results contradict these findings. 6. Gabapentin increases serotonin concentrations in human whole blood, which may be relevant to neurobehavioral...
actions. Gabapentin prevents neuronal death in several models including those designed to mimic amyotrophic lateral sclerosis (ALS). This may occur by inhibition of glutamate synthesis by branched-chain amino acid aminotransferase (BCAA-t). © 1998 Elsevier Science B.V. All rights reserved.

1. Introduction

Gabapentin, 1-(aminomethyl)cyclohexaneacetic acid (Neurontin®) is a novel anticonvulsant drug that is active in a variety of animal seizure models (Taylor, 1995) and prevents partial seizures and generalized tonic-clonic seizures in several placebo-controlled clinical studies, both in add-on and monotherapy (Andrews et al., 1990; McLean et al., 1993; Marson et al., 1996; Beydoun et al., 1997; Burgey et al., 1997). Clinical use of gabapentin has been associated with several side effects, but it is generally well tolerated (Ramsey, 1995). Its pharmacokinetic profile, and use in combination with other medications has been described (McLean, 1995). Gabapentin was originally synthesized to treat spasticity and to reduce polysynaptic spinal reflexes. It is active in several animal models of spasticity.

Recently, it has been shown in animal models that gabapentin prevents nociceptive responses from hyperalgesia in animal models (Everhart et al., 1997; Field et al., 1997a,b; Gillin and Sorkin, 1997; Hunter et al., 1997; Hwang and Yaksh, 1997; Jun and Yaksh, 1997; Singh et al., 1996; Xiao and Bennett, 1997) and also has analgesic actions in clinical reports (Rosner et al., 1996; Backonja et al., 1997; Mellick and Mellick, 1997; McGraw and Kosek, 1997). Other studies suggest that gabapentin has anxiolytic-like effects in animal models (Singh et al., 1996). Furthermore, gabapentin treatment prevents motoneuron degeneration in an in vitro model of amyotrophic lateral sclerosis (ALS) (Rothstein and Kunc, 1995) and delays death in a transgenic animal model of ALS (Gurney et al., 1996). Results in a preliminary placebo-controlled clinical trial of gabapentin for treatment of ALS were not statistically significant (Miller et al., 1996), but suggested that additional clinical studies are warranted.

Gabapentin was designed as a structural analog of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Satzinger, 1994). Although gabapentin was originally modeled after the structure of GABA, it does not modulate GABA receptor function like conventional GABAergic drugs, and it is inactive at GABA receptors. This review outlines several potential mechanisms of pharmacological action of gabapentin. The various hypotheses are considered in approximate order of their discovery, and each is considered critically, with references to the published literature. Although a consensus has not yet been reached, it seems likely that several different sites of action may be necessary to account for all of the pharmacological actions of gabapentin.

2. Gabapentin and system L amino acid transporters

Gabapentin is an amino acid that exists at physiological pH as a zwitterion, and since it is doubly-charged, its native permeability to membrane barriers within the body is low. However, like several other amino acids, gabapentin is a substrate of the so-called system L transporter of gut (Stewart et al., 1993a) and of neurons and astrocytes (Su et al., 1995). This property allows gabapentin molecules to cross membrane barriers more easily. In addition to the facilitated transport across cell membranes, there is a smaller non-saturable component of transport (Stewart et al., 1993a; Su et al., 1995) that is likely due to passive diffusion. Due to differences in the influx and efflux rate of gabapentin via system L in cultured cells, it accumulates in cytosol to greater concentrations than in the bathing medium (Su et al., 1995). These transport properties of gabapentin probably account for the access of gabapentin to brain cytosol (Vollmer et al., 1986), where it is present at about ten-fold higher concentrations than in the brain extracellular space (Welty et al., 1993). The delayed anticonvulsant action of gabapentin in rats after a bolus intravenous dose (Welty et al., 1993) may be explained
Fig. 1. Schematic diagram of GABAergic inhibitory synapse in brain. In presynaptic ending, glutamate is synthesized primarily from glutamine (by glutaminase) and free GABA is synthesized from glutamate by the enzyme glutamic acid decarboxylase (GAD). GABA is degraded by the enzyme GABA-transaminase. The irreversible GABA-transaminase inhibitor, vigabatrin, is an anticonvulant. GABA is packaged into synaptic vesicles, where it is released in response to presynaptic calcium influx. GABA also can be released from the cytosol by reversal of the GABA transporter (GABA uptake). Post-synaptically, GABA activates GABA$_A$ receptors, which are modulated by anticonvulsant benzodiazepines and barbiturates. GABA$_A$ receptors are also activated by synaptic release of GABA or by the GABA$_B$ agonist, baclofen. GABA is taken up from the extracellular space by a specific transporter that is blocked by the anticonvulsant tiagabine. Nipecotic acid is both a blocker and substrate for the GABA transporter.

3. Gabapentin and function of GABA systems in brain

Numerous reports in the literature (Meldrum, 1985; Tunnicliff and Raess, 1991; Bowery, 1993; Macdonald and Olsen, 1994) indicate that $\gamma$-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in mammalian brain and that seizures occur if GABA synapses are impaired (Tunnicliff and Raess, 1991). A variety of GABA-enhancing drugs such as GABA$_A$ agonists, GABA$_A$ modulators (e.g. benzodiazepines), drugs converted metabolically to GABA, GABA uptake inhibitors (e.g. tiagabine (Gram et al., 1989)), and inhibitors of GABA degradation (e.g. vigabatrin (Meldrum, 1985)) prevent seizures in animal models or in clinical use (Suzdak and Jansen, 1995; Tunnicliff and Raess, 1991) (see Fig. 1). The similarity of chemical structures between GABA and gabapentin also suggests a functional relationship.

Gabapentin does not alter radioligand binding at GABA$_A$ or GABA$_B$ receptors at concentrations...
up to 100 μM (Taylor, 1995) nor does it alter [3H]GABA uptake into neuronal or glial cultures (Su et al., 1995). In addition, gabapentin does not alter neuronal responses to the application of GABA in electrophysiological experiments (Rock et al., 1993). These studies indicate that gabapentin does not interact directly with either GABA_A or GABA_B receptors, nor with high-affinity Na^+-dependent GABA transporters. Therefore, gabapentin’s actions are distinct from those of several drugs that directly modulate GABA_A receptor function (Macdonald and Olsen, 1994) such as benzodiazepines or barbiturates. Further, the actions of gabapentin are distinct from those of GABA uptake inhibitors (Suzdak and Jansen, 1995) and from drugs that alter GABA_B receptor function (Bowery, 1993).

In vivo results suggest an interaction of gabapentin with GABA systems. Gabapentin treatment causes decreased firing rates in rat substantia nigra pars reticulata GABA neurons (Bloms-Funke and Löschler, 1996). Spontaneous firing rates are reduced substantially by other GABAergic drugs such as benzodiazepines and muscimol. In animals, tonic extensor convulsions caused by the GABA synthesis inhibitor thiosemicarbazide are blocked more potently than seizures from several other convulsant agents (Bartoszyk et al., 1986; Taylor, 1995). Thiosemicarbazide is an inhibitor of pyridoxyl 5'-phosphate (Tapia and Salazar, 1991), an enzymatic cofactor needed for GABA synthesis by the cellular enzyme glutamic acid decarboxylase (GAD).

Thiosemicarbazide acts to trap pyridoxyl 5'-phosphate by combining with the free carbonyl group to form an inert complex. Clonic convulsions caused by antagonism of GABA receptors are prevented by gabapentin less potently than convulsions from inhibition of GABA synthesis. Gabapentin does not prevent clonic convulsions from antagonism of spinal glycine receptors. These results suggest that gabapentin may selectively counteract GAD inhibitors. The results also suggest that gabapentin does not interact directly with GABA receptors or with glycine inhibitory synapses of spinal cord.

Gabapentin is a mixed-type inhibitor of GABA-transaminase at high concentrations, but this action is probably not relevant for anticonvulsant activity (Goldlust et al., 1995). Gabapentin in vitro increases the activity of partially-purified glutamic acid decarboxylase (GAD), suggesting that gabapentin treatment might increase the synthesis of GABA from glutamate in brain tissues (Taylor et al., 1992). GAD exists in two isoforms with different molecular and functional properties. GAD_{67} has a larger molecular weight, and exists mostly in neuronal cell bodies; GAD_{65} is localized more in nerve endings and synaptosomes (Erlander and Tobin, 1991; Erlander et al., 1991; Martin et al., 1991). The in vitro activity of GAD_{65} is increased sevenfold in saturating concentrations of pyridoxal 5'-phosphate, while GAD_{67} is only modulated 2-fold (Erlander and Tobin, 1991). Gabapentin may modulate GAD activity in vivo. Gabapentin treatment (25 mg/kg IP) increases brain concentrations of GABA in rats pretreated with aminooxyacetic acid (AOAA) (Löschler et al., 1991). AOAA inhibits GABA transaminase and prevents GABA degradation. Therefore, changes in brain GABA concentrations after AOAA treatment suggest that gabapentin enhances the GABA synthesis rate by 50–100% in several brain regions in vivo. A recent study (Leach et al., 1997) with mice given single or twice-daily doses of gabapentin showed some significant changes in brain glutamate and glutamine concentrations and also changes in GABA-T activity, suggesting alterations in brain amino acid metabolism. In vivo NMR spectroscopy indicates that brain GABA concentrations are elevated in human patients taking gabapentin, and that elevation of GABA is related to seizure control (Petroff et al., 1996; Mattson et al., 1997). Recent results with NMR spectroscopy of rat brain tissues indicates that gabapentin treatment elevates brain GABA concentration and also decreases brain glutamate concentration (Petroff et al., 1997).

Gabapentin increases GABA release from rat striatal brain slices in vitro (Götz et al., 1993), and this action is prevented by the GABA_A antagonist, bicuculline. Although the mechanism of the bicuculline-sensitive effect is not clear, GABA can be released from neuronal tissues by either Ca^{2+}-dependent or Ca^{2+}-independent mechanisms, the
latter depending upon reversal of the GABA uptake carriers (Szerb, 1982; Pin and Bockaert, 1989). Gabapentin increases electrophysiological responses caused by the non-vesicular release of GABA in rat optic nerves in a sucrose-gap apparatus (Kocsis and Honmou, 1994). Very similar increases in GABA responses were seen upon inward current in voltage-clamped pyramidal neurons in rat hippocampal slices in vitro (Honmou et al., 1995a,b, see Fig. 2. In both experiments, GABA release was triggered by nipecotic acid, a substrate and competitive inhibitor of GABA transport that is not active at GABA receptors. Application of nipecotic acid disrupts the normal equilibrium of the GABA transporter, and causes the release of cytosolic GABA by hetero-exchange (Szerb, 1982; Kocsis and Honmou, 1994). The responses are due to activation of GABA_A receptors, and are blocked by bicuculline.

In addition, in vivo, gabapentin treatment decreases paired-pulse inhibition in the rat dentate gyrus (Xiong and Stringer, 1997). At first glance, decreased paired-pulse inhibition suggests a decrease in GABA function, but similar results were obtained in another study with vigabatrin (Sayin et al., 1997) (a known inhibitor of GABA degradation). The vigabatrin results were partially reversed by co-application of a GABA_B blocker, suggesting that higher extracellular GABA concentrations activate presynaptic GABA_B receptors (Sayin et al., 1997).

Measurements of GABA release have been confirmed in studies of [3H]GABA release from brain slices (Fichter et al., 1996). Rat striatal brain slices were preincubated with either [3H]GABA or [3H]glutamine to differentially label two GABA pools. GABA release was measured by scintillation counting (for pre-loaded [3H]GABA) or by cation-exchange chromatography followed by scintillation counting (for [3H]GABA synthesized from [3H]glutamine). [3H]GABA release after preloading with [3H]glutamine was caused by application of nipecotic acid (1 mM) and was enhanced approximately 50% by gabapentin pretreatment. In contrast, [3H]GABA release after preloading with [3H]GABA was not altered by gabapentin. These results are consistent with the idea that gabapentin increases synthesis of GABA via GAD. Furthermore, since results were obtained in the presence of the GABA-transaminase inhibitor AOAA, the action of gabapentin is not likely to be caused by GABA-T inhibition. The electrophysiological and biochemical results with gabapentin on GABA systems (Kocsis and Honmou, 1994; Honmou et al., 1995a,b; Fichter et al., 1996) are consistent with increased GAD activity, since hippocampal tissues (Tapia and Salazar, 1991), optic nerves (Ochi et al., 1993) and striatal tissues each contain significant amounts of GAD.

Although nipecotic acid normally is not present in brain, it is a convenient probe for the GABA uptake transporter. [3H]Nicotinic acid (During et al., 1995) or [3H] derivatives of other selective GABA uptake inhibitors (Suzdak et al., 1994) have been used to study the localization and function of GABA transporters. The stoichiometry of GABA transporters has been described (Keynan and Kanner, 1988; Liron et al., 1988)

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**Fig. 2.** Gabapentin enhances the nipecotic acid-induced release of GABA in rat hippocampal slices in vitro. A whole-cell voltage clamp recording was made from a single CA1 pyramidal neuron, and nipecotic acid (10 mM) was applied for 2.0 s by a pressure pulse to a nearby micropipette. The nipecotate caused non-vesicular GABA to be released from neighboring cellular elements, resulting in a bicuculline-sensitive inward current that was measured at intervals of 5 min. The application of gabapentin (100 μM, triangles) at time zero caused a significant increase in inward current in comparison to control experiments with no drug present (circles); N = 5 for control experiments, N = 7 for gabapentin experiments. Reproduced from Honmou et al. (1995a) with permission.
and changes in the cellular microenvironment would cause reversal of the normal inward flux of GABA via transporters. Either cytosolic Na\(^+\) loads, cellular depolarization, or particularly activation of glutamate receptors (which causes both depolarization and Na\(^+\) loading) cause net efflux of GABA by reversed transport (Pin and Bockaert, 1989). Glutamate release and cellular depolarization occur during seizures, and a recent report (During et al., 1995) suggests that in human epileptic brain tissues, non-vesicular GABA release is reduced while calcium-dependent GABA release occurs normally. Therefore, gabapentin may compensate for a pathological reduction in transport-mediated GABA release in epileptic brain tissues (Kocsis and Mattson, 1996). This hypothesis remains to be tested critically, but experiments with gabapentin using microdialysis to measure synaptic and non-vesicular GABA release might help address this idea.

4. Receptor binding studies with \[^3\text{H}\]gabapentin

Gabapentin does not affect ligand binding at a wide variety of commonly-studied drug and neurotransmitter binding sites and voltage-activated ion channels including GABA, glutamate, and glycine receptors of several types (Taylor, 1995). However, gabapentin itself has been used to define a novel binding site in brain tissues.

Tritiated gabapentin binds with high affinity to a single population of sites in rat (\(K_D\) 38 nM), mouse (\(K_D\) 14 nM) and pig (\(K_D\) 17 nM) synaptic plasma membranes prepared from cerebral cortex (Suman et al., 1993; Thurlow et al., 1993). The maximum binding capacity of \[^3\text{H}\]gabapentin reported for rat brain membranes is 4.6 pmol/mg (Suman et al., 1993), and similar values have been obtained for mouse and pig tissue (Thurlow et al., 1993). Mapping of \[^3\text{H}\]gabapentin binding sites in rat brain has been achieved by autoradiographic
A variety of compounds have been tested for inhibition of specific \[^{3}H\]gabapentin binding to brain membranes. Inactive compounds (IC\(_{50}\) > 1 mM) included those that interact with GABA receptors (kojic amine, muscimol, bicuculline, isonicotic acid), NMDA receptors (glutamate, glycine, D-serine, N-methyl-D-aspartic acid, 7-chlorokynurenic acid and trans-crotonic acid) and the GABA transporter (nipecotic acid and THPO (4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol)) (Suman et al., 1993). The low affinities of GABA, baclofen and (+)-MK-801 for the \[^{3}H\]gabapentin binding site suggest that \[^{3}H\]gabapentin does not interact with either GABA or NMDA receptors (Suman et al., 1993). Mg\(^{2+}\) and the polyamines, spermine and spermidine, which are known allosteric modulators of NMDA receptors (Foster and Fagg, 1987), inhibited \[^{3}H\]gabapentin binding in a non-competitive manner with IC\(_{50}\) values of 27, 12 and 15 \(\mu\)M, respectively. Maximal inhibition with Mg\(^{2+}\) or polyamines was only about 60%. A combination of 3 mM MgCl\(_{2}\) and 3 mM spermine did not inhibit \[^{3}H\]gabapentin binding to a greater extent than either compound alone, suggesting the possibility of a common site of action. A range of anticonvulsant drugs including diazepam, carbamazepine, phenobarbital, phenytoin, pentobarbital, sodium valproate, ethosuximide and \(\beta\)-hydroxy-GABA were all inactive (IC\(_{50}\) > 1 mM) supporting the idea that \[^{3}H\]gabapentin labels a novel pharmacological site.

Compounds from two chemical series have been identified as potent inhibitors of \[^{3}H\]gabapentin binding. Several 3-substituted analogues of GABA, most notably (SR)-3-isobutyl-GABA (IC\(_{50}\) = 80 nM), were found to be active (Suman et al., 1993). The S(+-)-enantiomer of 3-isobutyl-GABA was 10-fold more potent than the R(−)-enantiomer (Taylor et al., 1993). The rank order of potency of gabapentin and the two enantiomers of 3-isobutyl GABA were the same both in the \[^{3}H\]gabapentin binding assay and in animal seizure models (Taylor et al., 1993). This observation suggests that the binding of gabapentin to the \[^{3}H\]gabapentin binding site may be important to the antiepileptic activity of the drug. Potent and stereospecific displacement of \[^{3}H\]gabapentin binding was also apparent with several large neutral amino acids (Thurlow et al., 1993). For each amino acid tested, the L-enantiomer was 100–1000-fold more potent than the corresponding D-enantiomer. These findings together with the observation that \[^{3}H\]gabapentin is transported across membranes by a system L transporter (Stewart et al., 1993a,b; Su et al., 1995) led to the hypothesis that \[^{3}H\]gabapentin may label the recognition site of a neuronal transporter similar to system L (Thurlow et al., 1993).

4.1. Purification and identification of the \[^{3}H\]Gabapentin binding protein

To clarify the molecular nature of the \[^{3}H\]gabapentin binding protein (GBP), it was purified from pig brain. Preliminary biochemical studies suggested that the GBP was a membrane-associated protein. Brain membranes were solubilized with a non-ionic detergent, Tween 20, and the GBP was purified to near-homogeneity by sequential chromatography over six matrices (Gee et al., 1996). Electrophoretic analysis revealed the purified GBP to have a subunit molecular weight of approximately 130 000. The partial N-terminal sequence of the purified protein was identical to that reported for the \(\alpha_2\delta\) subunit of the L-type voltage-dependent Ca\(^{2+}\) channel from skeletal muscle (Hamilton et al., 1989). Binding of
[\textsuperscript{3}H]gabapentin to COS-7 cells transiently expressing rabbit skeletal muscle \(z_\delta\) cDNA and to partially purified muscle Ca\(^{2+}\) channel subunits confirmed that the GBP and the \(z_\delta\) Ca\(^{2+}\) channel subunit are the same protein (Gee et al., 1996). Gabapentin is the first ligand described that interacts with the \(z_\delta\) subunit of a Ca\(^{2+}\) channel.

4.2. Structure of voltage-dependent calcium channels

Ca\(^{2+}\) channels are multi-subunit complexes found not only in the brain but also in peripheral tissues such as skeletal muscle, heart and lung (Catterall, 1995). The distribution of \([\textsuperscript{3}H]gabapentin binding sites in rat peripheral tissues is consistent with an interaction of gabapentin at Ca\(^{2+}\) channels (Gee et al., 1996). Ca\(^{2+}\) channels consist of at least three subunits (for review, see Dolphin (1995)): \(\alpha_1\) (the pore-forming subunit), \(\alpha_\delta\) and \(\beta\). Skeletal muscle Ca\(^{2+}\) channels also have a \(\gamma\) subunit. High-threshold Ca\(^{2+}\) channels are classified as L-, N-, P-, or R-types on the basis of their sensitivity to various organic ligands and several neurotoxins. Apart from gabapentin, all Ca\(^{2+}\) channel ligands appear to bind to \(\alpha_1\) subunits. Molecular cloning studies have revealed at least six genes encoding \(\alpha_1\) subunits, and a number of splice variants have also been identified (for review, see Hofmann et al. (1994)). The \(\beta\) subunit is a hydrophilic protein that interacts with the \(\alpha_1\) subunit (Pragnall et al., 1994). Four genes encode \(\beta\) subunits and splice variants for three of them have been described (Perez-Reyes and Schneider, 1994). A single gene encodes the \(z_\delta\) subunit (DeJongh et al., 1990) and a number of splice variants have been found (Perez-Reyes and Schneider, 1994). The \(z_\delta\) subunit is synthesized as a pre-protein that undergoes extensive post-translational modifications. The membrane targeting signal sequence is removed and a second proteolytic cleavage event generates a small C-terminal fragment (\(\delta\)) that remains attached to the larger fragment (\(z_\delta\)) by a disulfide bridge. The protein is also heavily glycosylated (Sharp and Campbell, 1989; Jay et al., 1991). The membrane topology of the \(z_\delta\) subunit is unclear, thus the \([\textsuperscript{3}H]gabapentin site could be intracellularly or extracellularly disposed (see Fig. 4). Hydrophilicity plots suggest that the \(z_\delta\) polypeptide contains two transmembrane domains (Ellis et al., 1988) but biochemical data support a model where the \(z_\delta\) polypeptide is wholly extracellular (Jay et al., 1991; Brickley et al., 1995). The presence of a single transmembrane anchor in the \(\delta\) polypeptide is widely accepted.

4.3. Roles of the subunits in calcium channel function

Only the N-type channel from rabbit brain (Witcher et al., 1993) and the L-type Ca\(^{2+}\) channel from skeletal muscle (Leung et al., 1988; Jay et al., 1991) have been purified along with their associated auxiliary subunits. Thus, the particular subunit combinations that occur in vivo for other channels are unclear. This should be borne in mind when considering the results of ‘mix and match’ heterologous expression studies with cloned Ca\(^{2+}\) channel subunits. Most of these studies have utilized the Xenopus laevis oocyte expression system. Functional channels require only the \(\alpha_1\) subunit (Mori et al., 1991). However, significant changes to the electrophysiology of the channel are effected by co-expression with the auxiliary subunits. A peak barium current (\(I_{\text{Ba}}\)) of 31 nA was recorded for the N-type \(\alpha_{1\text{NB}}\) subunit alone, whereas expression with the skeletal muscle \(z_\delta\) or skeletal muscle \(\beta_1\) yielded a peak \(I_{\text{Ba}}\) of 89 and 664 nA, respectively. Significant co-operativity of the auxiliary subunits was demonstrated by co-expression of all three subunits (\(z_\delta\), \(\beta_1\)) which yielded an \(I_{\text{Ba}}\) of > 6 \(\mu\)A (Mori et al., 1991). Several other groups also report a co-operative effect of \(z_\delta\) and \(\beta\) on \(\alpha_1\) (Singer et al., 1991; Williams et al., 1992). Shistik et al. (1995) have dissected the electrophysiological and biochemical mechanisms of modulating Ca\(^{2+}\) channel current characteristics by auxiliary subunits. Whole-cell calcium channel currents of \(\alpha_{1C}\) (cardiac) were increased 8–10 fold by either \(z_\delta\) or \(\beta_2\) alone but 100-fold with both subunits. They found that the increase in the current by \(z_\delta\) resulted both from a three-fold increase in the amount of \(\alpha_{1C}\) present in the membrane and from modulation of the gating characteristics of \(\alpha_{1C}\). On the other hand,
Fig. 4. Schematic diagram of the structure of the Ca$^{2+}$ channel $\alpha_1$, $\alpha_2$ and $\beta$ subunits. Taken with permission from Gurnett et al. (1996). The cell plasma membrane is shown as a lipid bilayer with intracellular and extracellular media labeled. Small branched structures indicate extracellular sites of glycosylation (sugar polymers attached to protein). 'S–S' denotes sites of putative cysteine linkages. The main voltage-sensing and ion-conducting subunit (central part of $\alpha_1$) is shown in its postulated conformation spanning the lipid bilayer. The gabapentin binding protein ($\alpha_2\beta$) is shown in its presumed conformation anchored in the lipid bilayer and associating with the $\alpha_1$ subunit, but with most amino acids exposed to the extracellular fluid. Recent experiments with site-directed deletions of $\alpha_2\beta$ Gurnett et al. (1996) indicate that the extracellular region of this protein enhances Ca$^{2+}$ current amplitude and the intramembrane portion interacts directly with the $\alpha_1$ subunit (arrows). It is not yet known what part of the $\alpha_2\beta$ protein gabapentin binds to.

$\beta_2$ alone affected only the gating characteristics. Few electrophysiological studies on the effects of gabapentin on Ca$^{2+}$ channels have been reported and further work in this area is required.

However, several observations are consistent with the idea that gabapentin modulates Ca$^{2+}$ channels, particularly if channels are modulated in a subtle manner. It is possible that inhibition of monoamine neurotransmitter release (Reimann, 1983; Schlicker et al., 1985; Dooley et al., 1996) is caused by an interaction of gabapentin with Ca$^{2+}$ channels. Monoamine release is never inhibited by more than 50% by gabapentin (Reimann, 1983; Schlicker et al., 1985; Dooley et al., 1996). Re-
cently, voltage-clamp records of high-threshold Ca\(^{2+}\) currents from rat neocortical pyramidal neurons show that gabapentin (10–100 \(\mu\)M) reversibly reduces the nitrendipine-sensitive component of current by about 35% (Stefani et al., 1997). However, another study with acutely-isolated human dentate granule neurons showed no effect of gabapentin on total cellular Ca\(^{2+}\) currents (Schumacher et al., 1997). These findings suggest that gabapentin may have subtle actions on Ca\(^{2+}\) channels that are only apparent in certain sub-populations or experimental protocols.

5. Gabapentin and other neurotransmitters

Gabapentin has actions on monoamine neurotransmitter release in vitro. Gabapentin causes significant decreases (10–15% blockade) in the electrically- or 20–50 mM K\(^+\)-evoked release of noradrenaline, dopamine and serotonin from brain slices (Reimann, 1983; Schlicker et al., 1985; Dooley et al., 1996). The inhibitory action of gabapentin on striatal dopamine release is clearly different from that of the GABA\(_B\) agonist baclofen (Reimann, 1983). Reduced monoamine release may relate either to an action on Ca\(^{2+}\) channels (see section above) or to changes in monoamine metabolism. Recent results indicate that pretreatment of rats with gabapentin significantly reduces the augmented noradrenaline and dopamine turnover caused by systemic administration of 3,4-diaminopyridine (Pugsley et al., 1997). These results indirectly suggest that gabapentin may alter the function of Ca\(^{2+}\) channels involved with monoamine release (see section above). Although changes in monoamine function induced by gabapentin might not relate directly to anticonvulsant effects, alterations in monoamine neurotransmission might underlie behavioral effects (anxiolytic-like action or analgesia).

6. Gabapentin and voltage-sensitive Na\(^+\) channels

Gabapentin does not alter voltage-clamped sodium currents in the manner of phenytoin, carbamazepine or lamotrigine (Taylor, 1993), nor did it alter sustained firing of action potentials in cultured neurons (Rock et al., 1993). However, with longer incubation periods in vitro, it decreases sustained firing of Na\(^+\)-dependent action potentials (Wamill and McLean, 1994). In addition, a recent report suggests that gabapentin has other electrophysiological actions that may account for reduced excitability (Kawasaki et al., 1995). It is not yet clear whether these in vitro findings are relevant for anticonvulsant or other pharmacological actions of gabapentin in vivo.

7. Gabapentin and serotonin concentration in whole blood

Gabapentin given to healthy human volunteers increases the concentration of serotonin in whole blood (Rao et al., 1988). These authors speculate that increased serotonin might be due to changes in serotonin metabolism or uptake in platelets. However, this has not been studied in vitro. They also speculate that changes in serotonin could explain changes that were observed in sleep patterns (increased duration of stages 3 and 4 without changes in total sleep time, REM sleep time or REM latency).

8. Gabapentin and neuroprotection (glutamate metabolism)

Although seizures induced by glutamate agonists are not prevented by gabapentin, high doses significantly delay such seizures, suggesting that gabapentin might interact with glutamatergic synapses (Bartoszyk et al., 1986; Taylor, 1995). Studies with gabapentin in an in vitro model meant to mimic some aspects of motor neuron disease (amyotrophic lateral sclerosis or ALS) indicate that neuronal cell death is prevented by gabapentin treatment (Rothstein and Kuncl, 1995). The model utilizes the application of a selective inhibitor of glutamate uptake, and neuronal death is reduced by selective antagonists of the AMPA type of glutamate receptor. Therefore, it is reasonable to postulate that protective effects of gabapentin might arise from changes in gluta-
mate metabolism or release. Additionally, studies with a transgenic mouse model of ALS (Gurney et al., 1994) indicate that chronic treatment with high dosages of gabapentin significantly delays paralysis and death in transgenic mice (Gurney et al., 1996).

A recent paper (Welty et al., 1995) details two hypotheses for the neuroprotective action of gabapentin. One pathway for glutamate synthesis in brain tissues arises from transamination of α-ketoglutarate by branched-chain amino acids (leucine, isoleucine, valine) by the enzyme branched-chain amino acid aminotransferase (BCAA-T). Gabapentin is a competitive inhibitor of BCAA-T with inhibition constants ($K_i = 0.6–1.2$ mM) equivalent to the substrate affinity for endogenous branched-chain amino acids ($K_m = 0.4–1.2$ mM) (Goldlust et al., 1995). Recent in vitro studies show that this action of gabapentin is selective for the isoform of BCAA-T found in brain cytosol (neuronal form), with little effect on the mitochondrial form of BCAA-T (astroglial form) (Hutson et al., 1995). Since the therapeutic range of concentrations for gabapentin (10–100 μM) is similar to the endogenous concentrations of branched-chain amino acids, gabapentin may significantly inhibit the action of BCAA-T in vivo, and this may result in decreased cytosolic concentrations of glutamate that would reduce glutamate-dependent cell death. Decreased synthesis of glutamate from labeled L-leucine in the presence of gabapentin has been demonstrated in vitro, although total tissue content of glutamate was not altered (Kapetanovic et al., 1995). Decreases in whole-brain glutamate of 20% after gabapentin treatment have been demonstrated by NMR spectroscopy of rat brain extracts (Petroff et al., 1997). A decrease in the synthesis of glutamate from L-leucine after administration of gabapentin in vivo remains to be demonstrated.

In addition, gabapentin in vitro stimulates the activity of the catabolic enzyme glutamate dehydrogenase (GDH) at high concentrations (Goldlust et al., 1995). It is possible that gabapentin administration would enhance GDH activity in vivo, as has been proposed for the treatment of ALS with high-dose administration of endogenous branched-chain amino acids (Plaitakis, 1990). This hypothesis for the action of gabapentin by enhanced degradation of glutamate also remains to be tested in vivo.

Gabapentin in vitro decreases the tissue content of glutamine (a metabolic precursor of glutamate and GABA) in isolated hippocampal tissue (Kapetanovic et al., 1995). Although the metabolic pathway involved with this effect has not been described, changes in glutamine metabolism or transport might be relevant for decreased glutamate synthesis.

### 9. Effects in animal models of anxiety

Gabapentin was investigated in several animal models that predict utility for the treatment of anxiety. These include the marmoset human threat test, the rat elevated X-maze and the rat conflict test (Singh et al., 1996). In all of these models, gabapentin produced anxiolytic-like effects with minimum effective doses ranging from 3 to 30 mg/kg (Singh et al., 1996). In all three models, gabapentin produced activity with similar efficacy to that of benzodiazepines. Most non-benzodiazepine drugs produce much weaker activity than gabapentin in the rat elevated X-maze and the marmoset human threat test. For example, gabapentin’s activity is more pronounced than that of buspirone or other experimental compounds such as cholecystokinin CCK$_R$ antagonists (Singh et al., 1991) or serotonin 5-HT$_3$ receptor antagonists (Broekkamp et al., 1989). A recent study of the antidepressant, phenylzine (Paslawski et al., 1996) suggests that elevation of whole brain GABA concentration (see above) could be related to antianxiety effects.

### 10. Effects in animal models of pain

Gabapentin administration to rats or mice does not alter acute responses to thermal or chemical stimulation. However, delayed pain responses in several animal models are reduced. Formalin and carrageenan are two chemical irritants that are widely used in studies of tonic pain and hyperalgesia from peripheral inflammation. The subcuta-
neous administration of formalin into the plantar surface of the rodent paw produces a biphasic nocifensive behavioral response. The early phase consists of intense licking and biting of the injected paw and lasts up to 10 min but a second late phase of licking and biting occurs from 10 to 60 min after injection (Dubuisson and Dennis, 1977). The late phase is a state of facilitated pain response (hyperalgesia) associated with inflammation. This behavioral response has been shown to correlate with a biphasic increase in the activity of C-fiber primary afferent neurons after formalin injection (McCall et al., 1996). Carrageenan elicits little or no immediate pain response (Wheeler-Aceto et al., 1990), but causes hyperalgesic responses to thermal or mechanical stimuli with a maximum 3–4 h after injection into the footpad (Hargreaves et al., 1988). The formalin and carrageenan behavioral tests involve sensitization of sensory neurons of the spinal dorsal horn in response to injury or intense artificial activation of C-fiber afferents (Woolf and Wall, 1986). Gabapentin (30–300 mg/kg) selectively blocks the tonic phase of formalin nociception without changing the peripheral swelling caused by carrageenan, but gabapentin reduces the mechanical and thermal hyperalgesia from carrageenan (Singh et al., 1996). Therefore, gabapentin may act within the spinal cord or brain to reduce sensitization of dorsal horn sensory neurons.

Gabapentin reduces pain responses from neuropathy produced by chronic constriction of the sciatic nerve (Hunter et al., 1997; Xiao and Bennett, 1997) or by ligation of spinal nerves at the L5 and L6 levels (Hunter et al., 1997; Hwang and Yaksh, 1997). These results in various animal models show that gabapentin reduces mechanical hyperalgesia (pin prick response), mechanical allodynia (Von Frey nylon monofilament), thermal hyperalgesia (from radiant heat) and thermal allodynia (from cold water). The intrathecal administration of gabapentin blocked thermal and mechanical hyperalgesia (Hwang and Yaksh, 1997; Xiao and Bennett, 1997), suggesting that gabapentin may work from a spinal site of action. Thermal hyperalgesia is mediated primarily by C-fiber sensory afferents, which produce their actions in the spinal cord mainly via NMDA-type glutamate receptors on dorsal horn neurons of the spinal cord. It remains to be seen whether gabapentin alters NMDA responses in the spinal cord, but NMDA antagonists have similar actions to gabapentin in the formalin test.

The antihyperalgesic action of gabapentin does not depend on activation of opiate receptors, and is not altered by the opiate antagonist, naloxone (Field et al., 1997b). Unlike morphine, gabapentin does not reduce gut motility in rats. Sedation and ataxia are caused by gabapentin only at doses ten times higher than those preventing pain responses. Repeated administration of gabapentin does not result in tolerance to antihyperalgesia. Morphine tolerance does not cross-generalize to gabapentin (Field et al., 1997b). Therefore, gabapentin’s analgesic actions are distinct from those of opiate analgesics.

Results of an open-label clinical study in patients with reflex sympathetic dystrophy (RSD) suggest that gabapentin may reduce neuropathic pain (Mellick et al., 1995). RSD is characterized by burning pain, allodynia, hyperpathia, vasomotor and sudomotor disturbances, edema, and trophic changes to bone, skin and soft tissues. Satisfactory (scored good to excellent) pain relief was obtained in all eight patients given 300 or 600 mg gabapentin daily. Gabapentin treatment corrected skin temperature and color, and reduced allodynia, hyperalgesia and hyperpathia in most patients. These results suggest that a placebo-controlled clinical study of gabapentin for RSD is warranted. In addition, results of a double-blinded clinical study of gabapentin for pain from diabetic neuropathy showed a significant reduction of pain scores in comparison to placebo (Backonja et al., 1997).

In summary, gabapentin is active in animal models that require sensitization of pain responses but is not active in transient models of pain. Therefore, it may not reduce immediate pain from injury, but it appears to reduce abnormal hypersensitivity (allodynia and hyperalgesia) induced by inflammatory responses or nerve injury. When considered with gabapentin’s relative lack of undesirable side effects, gabapentin may eventually be shown to improve treatment for several chronic pain syndromes; three additional placebo-
controlled clinical studies for various pain syndromes are presently underway.

11. Electrophysiology studies of glutamate responses

Rock et al. (1993) used electrophysiological recordings from single cultured neurons from rat cortex, mouse spinal cord and rat sensory neurons to evaluate potential actions of gabapentin on neurotransmitter responses and voltage-dependent ion channels. Gabapentin (concentrations up to 500 μM) had no effects on repetitive sodium action potentials nor on isolated calcium currents (whole-cell currents included components of both high N/L- and low threshold T-type currents). Inhibitory responses produced by the iontophoretic application of GABA or glycine were not altered by gabapentin. The only effects of gabapentin observed were on NMDA responses in cortical neurons. Gabapentin (100 μM) enhanced the sustained portion of NMDA responses in seven out of 18 neurons in the absence of exogenous glycine. No effects of gabapentin were seen when experiments were repeated in the presence of excess glycine, nor were any effects of gabapentin reported on single NMDA channels recorded from outside-out membrane patches taken from cortical neurons, either in the absence or presence of glycine. No effects of gabapentin were seen with currents induced by the excitatory amino acids, kainate and quisqualate.

12. Conclusions

Although many studies have attempted to establish the cellular and molecular targets of the actions of gabapentin, a clear consensus still has not been obtained. It is quite likely that several different cellular actions account for various aspects of gabapentin pharmacology. At present several laboratories have demonstrated that gabapentin treatment alters the metabolism or concentrations of glutamate, glutamine or GABA in brain tissues. Several laboratories also have demonstrated that gabapentin (and several endogenous amino acids) interact with an auxiliary subunit of voltage-gated Ca\(^{2+}\) channels. Additional studies are needed to establish which of the various potential mechanisms account for activity of gabapentin in anticonvulsant, antinociceptive, anxiolytic and neuroprotective models.

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