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# Drugs of the Future: Review

# Cellular and molecular action of the putative GABA-mimetic, gabapentin

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**Abstract.** Gabapentin was originally designed as an anticonvulsant γ-aminobutyric acid (GABA) mimetic capable of crossing the blood-brain barrier. In the present review we show that although gabapentin is not a GABA mimetic, it has great utility as an add-on therapy for epilepsy and as a first-line treatment for neuropathic pain. We summarise the studies that have been performed which demonstrate that gabapentin appears to interact with a novel binding site expressed at high density within the central nervous system (CNS), namely the $\alpha 2\delta$ voltage-dependent calcium channel subunit. The review continues by examining the effects of gabapentin on calcium channel function and neurotransmitter release before, in the latter part of the review, summarising the more recently discovered actions of gabapentin in relation to intracellular signalling.

**Key words.** Gabapentin; voltage-gated calcium channel; epilepsy; pain; mitogen-activated protein kinase.

## Introduction

Gabapentin (Neurontin) was originally designed as an anticonvulsant γ-aminobutyric acid (GABA) mimetic capable of crossing the blood-brain barrier. Although its anticonvulsant effects within the central nervous system (CNS) have been well documented both preclinically and clinically, these effects do not appear to be mediated through interaction with GABA receptors. Gabapentin is currently licensed worldwide as an add-on therapy for patients with partial seizures resistant to conventional therapies and in a number of countries for neuropathic pain. Gabapentin is also widely used for many other off-licence indications such as anxiety and sleep disorders due to its apparent lack of toxicity.

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The wide range of therapeutic indications for gabapentin and its remarkable safety profile have stimulated a large amount of effort in the pursuit of its mechanism of action, which continues to be the subject of much speculation. In the present review we summarise the main findings on gabapentin, from pharmacological, clinical, molecular and physiological perspectives.

## Preclinical and clinical pharmacology of gabapentin

### Effect of gabapentin in animal models

#### Seizures

Gabapentin (see fig. 1 for structure) has been shown to be effective in a number of animal seizure models, elicited by both physical (e.g. electroshock or audiogenic) and

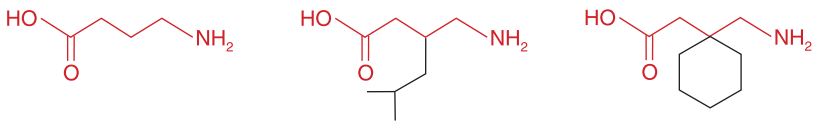


Figure 1. Chemical structures showing the similarities and differences between GABA ($\gamma$-aminobutyric acid), gabapentin and pregabalin [(SR)-3-isobutyl GABA]. The regions in red indicate the homology of gabapentin and pregabalin to GABA.

chemical (e.g. pentylenetetrazol, thiosemicarbamide, isoniazid, bicuculline, picrotoxin or 3-mercaptopropionate [1–4]) means. From the profile shown in animal models it can be expected that gabapentin would work clinically against complex partial and secondary generalised seizures, being especially effective for partial and tonic-clonic seizures. However, although gabapentin is effective in several genetic models of seizures, it fails to prevent spike/wave events in the electroencephalogram (EEG) of rats with genetic absence seizures, suggesting that it would not be active in absence seizures [2].

As we shall see, although the anticonvulsant effect of gabapentin is directly related to its activity at $\alpha_2\delta$ sites, it has also been shown that the glycine/*N*-methyl-D-aspartic acid (NMDA) receptor agonist D-serine reverses the anticonvulsant actions of gabapentin [5]. suggesting the potential involvement of other sites of action.

### Anxiety

Gabapentin has also been shown to have anxiolytic-like effects in a variety of animal models in several species. Gabapentin is active in the rat conflict test, the mouse light/dark box, the rat elevated plus maze and the marmoset human threat test [6]. The magnitude of gabapentin effect in these tests is similar to that of the benzodiazepines [7], whilst its wide spectrum of action suggests that it may be superior to other non-benzodiazepine anxiolytic compounds.

### Pain

The major therapeutic value of gabapentin has proven to be its use as an analgesic. Gabapentin does not block physiological pain [8, 9] but is effective against hypersensitivity induced by tissue damage or neuropathy. Its action seems to be centrally mediated, as seen from the selective blockade of the late phase of formalin test [6] and from its effect blocking the maintenance of carrageenan-induced sensitisation of dorsal horn neurones [10]. Furthermore, administration of gabapentin into the paw has no effect [11], but the compound is active in a number of animal models of pain when administered intrathecally [12–14].

Although gabapentin is very effective in inflammatory and surgical models of pain [15], the real breakthrough for the compound is its effect in models of neuropathic pain. It is effective in the chronic constriction injury (CCI), Chung and streptozocin models of neuropathic pain, blocking not only mechanical and thermal hyperalgesia, but also allodynia [8, 16] and abnormal neuronal responses [17]. In contrast to morphine and amitriptyline, which are only effective against static allodynia, gabapentin also blocks dynamic allodynia [18], thus demonstrating a superior antiallodynic profile to current therapies. This wide range of action in animal models of pain makes gabapentin a favourite treatment for chronic pain syndromes (see below for clinical data).

## Clinical efficacy of gabapentin

### Epilepsy

Gabapentin is licensed as an add-on therapy for the treatment of partial seizures, with and without generalisation. Its clinical efficacy as an anticonvulsant is proven and has been extensively reviewed [19, 20]. In general it is well tolerated. One main advantage of the compound is its lack of drug-drug interactions, due to its lack of binding to plasma proteins and lack of liver metabolism (for review see [21, 22]).

### Neuropathic pain

Among the number of nonepileptic uses for gabapentin, the largest is for the treatment of neuropathic pain. Described as one of the greatest challenges in pain management, neuropathic pain has traditionally been managed with antidepressants or other anticonvulsants, which have important adverse effects that limit their use (for review see [23]). In humans, gabapentin exhibits clinically effective antihyperalgesic activity against a wide range 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 to 2400 mg/day were effective in treating diabetic neuropathy [24], postherpetic neuralgia [25], trigeminal neuralgia, migraine and pain associated with cancer and multiple sclerosis ([26–28; see also [29–31]). Gabapentin is currently widely prescribed for patients with neuropathic pain, due not only to its demonstrated efficacy but also to its lack of significant adverse side effects (for review see [32]). However, cost issues and limited experience presently restrict the use of gabapentin as a first-line option [30].

### Neurologic and psychiatric indications

Together with the therapeutic use of gabapentin in epilepsy and neuropathic pain, a wide range of neurologic and psychiatric indications have recently emerged for gabapentin, including movement disorders, migraine prophylaxis and cocaine dependence [29]. Gabapentin has been reported to be effective as therapy for bipolar disorders (for review see [33]) and social phobia [34]. Gabapentin has recently been described to improve sleep [35], suggesting yet another potential therapeutic use for this versatile compound.

## Cellular and molecular aspects of gabapentin action

### Gabapentin effects on GABA mechanisms

As a drug, gabapentin was originally designed as a structural analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA; see fig. 1 for structure). Nevertheless, initial studies suggested that gabapentin did not bind to either $GABA_A$ or $GABA_B$ receptors [36, 37], nor was it converted metabolically into GABA [38]. In vitro and at high concentration, gabapentin is a mixed-type inhibitor of GABA-transaminase [39] and increases the activity of partially purified glutamic acid decarboxylase [40]. Since in vivo nuclear magnetic resonance (NMR) spectroscopic studies have shown that GABA concentrations are elevated in human patients taking gabapentin, and that this elevation of GABA is related to seizure control [41, 42], it is possible that these actions are clinically significant. However, this possibility requires further investigation.

More recent studies based on the structural similarity between baclofen ($GABA_B$ agonist) and gabapentin, together with the overlapping CNS distribution of gabapentin binding with $GABA_B$ receptors [43], have led Ng et al. (2001) to propose that gabapentin is a $GABA_B$ receptor agonist. Using the *Xenopus laevis* oocyte expression system, gabapentin has been shown to selectively activate $GABA_B$ gb1a-gb2 heterodimers coupled to Kir 3.1/3.2 inwardly rectifying potassium channels in a manner that can be blocked by $GABA_B$ antagonists. Similarly, the same authors have demonstrated that gabapentin activates potassium currents in CA1 pyramidal neurones and inhibits voltage-dependent calcium channels in a mouse pituitary cell line via interaction with $GABA_B$ receptors [44]. However, recently published data suggest that gabapentin action is not mediated via $GABA_B$ receptors [45], since gabapentin displays no effect upon $GABA_{B(1a,2)}$ or $GABA_{B(1b,2)}$ heterodimers at concentrations up to 1 mM when expressed in *Xenopus laevis* oocytes or mammalian cells. Clearly, these data cast a shadow on the existence of any gabapentin-$GABA_B$ receptor interaction. Similarly, Lanneau et al. [46] were not able to reproduce the results of Ng et al. [46a] using similar tissue preparations. Also, Martin et al. [47] reported that gabapentin does not interact with $GABA_A$ or $GABA_B$ receptors in cultured dorsal root ganglion (DRG) cells.

Patel et al. [48] have compared the effects of $GABA_B$ agonists and gabapentin on mechanical hyperalgesia in rat models of neuropathic (partial sciatic ligation) and inflammatory (Freund's complete adjuvant) pain. In both models, whilst the effects of $GABA_B$ agonists were blocked by a selective $GABA_B$ antagonist, the effects of gabapentin were unaffected by this antagonist. Together with original binding data establishing the very low affinity of gabapentin for the $GABA_B$ receptor [37], these results also seem to suggest that the gabapentin effects shown in these two models are not mediated by the $GABA_B$ receptor, or at least the mechanism of action of gabapentin is quite different from that of other $GABA_B$ agonists.

Another possibility that has recently gained interest is that gabapentin may act to modulate GABA transporter function. The GAT1 GABA transporter is a plasma membrane protein involved in regulating synaptic levels of GABA. Whitworth et al. [49] have recently demonstrated that 2-h preincubation of hippocampal cultures with gabapentin or the more potent gabapentin mimetic (*SR*)-3-isobutyl GABA (pregabalin; see fig. 1 for structure) caused a two-fold increase in subsequent GABA uptake, which was concentration and time dependent. This effect appears to arise from a redistribution of GAT1 protein from intracellular locations to the plasma membrane. On the other hand, Eckstein et al. [50] have shown gabapentin to inhibit the uptake of GABA. Recently, evidence was also given of a low-affinity inhibitory effect of gabapentin on the L-type amino acid transporter (LAT1; [51]).

Clearly, these areas of gabapentin research are particularly confused and in need of further study. Recently, a $GABA_B$ null mutant mouse (lacking $GABA_{B1}$ subunit) has been reported [52] and shown to exhibit spontaneous seizures, hyperalgesia, hyperlocomotor activity and memory impairment. This mouse should provide a unique opportunity to evaluate the relevance of $GABA_B$ receptors in mediating gabapentin effects.

### Gabapentin binding sites

The lack of definitive evidence to support an interaction between gabapentin and GABAergic pathways has led researchers to consider other possibilities to account for the therapeutic effects of this compound. Initial radioligand-binding analysis revealed that gabapentin did not interact with a wide variety of commonly studied drug, neurotransmitter and ion-channel binding sites [53]. However, [$^3$H]gabapentin was shown to bind with high affinity to a single population of binding sites present in homogenised brain membranes from a variety of mammalian species

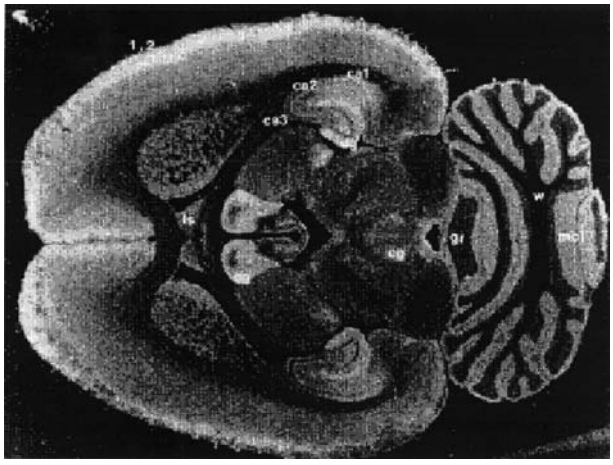

Figure 2. Autoradiograph showing the localization of [$^3$H]-gabapentin binding sites in a horizontal section through the rat brain. Highest levels of binding were found in layers I and II of the frontal, parietal, entorhinal and occipital cortex, whereas binding in the white matter was almost nonexistent. The hippocampus, dentate gyrus and cerebellum also displayed noticeable levels of binding. In the hippocampus, binding was dense except in the pyramidal cell layer. In the cerebellum, the molecular layer showed highest density of binding. Reprinted from [55] with permission from Elsevier Science.

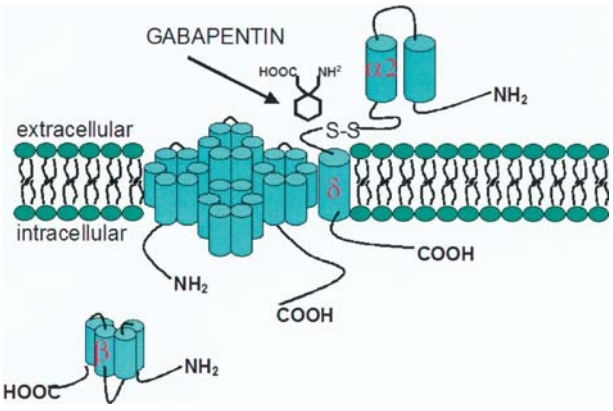


Figure 3. Schematic representation of the voltage-dependent calcium channel complex. The primary structures and transmembrane organisation of the subunits are illustrated. Cylinders represent probable $\alpha$-helical segments, and bold lines represent the polypeptide chains of each subunit. As shown, gabapentin is believed to interact with the $\alpha_2\delta$ subunit. Modified from [60] with permission from Taylor and Francis Group.

($K_d$ in rat 38 nM; [37, 54]). The ability of a large variety of neuroactive chemicals to displace [$^3$H]gabapentin from these binding sites has been examined. Of the wide range of agents tested, the vast majority were inactive, supporting the idea that gabapentin interacts with a unique pharmacological site [38]. However, potent and stereoselective displacement of [$^3$H]gabapentin was achieved with large neutral amino acids [54] and several 3-substituted analogues of GABA, most notably pregabalin [37]. Subsequent autoradiographical studies with [$^3$H]gabapentin demonstrated that these binding sites are heterogeneously expressed throughout the brain and are probably located on neurones rather than glia ([55], see fig. 2).

The [$^3$H]gabapentin binding protein was subsequently purified from pig brain and shown to be the $\alpha_2\delta$ subunit of the voltage-dependent calcium channel complex [56]. Voltage-gated calcium channels are multisubunit complexes found not only in the CNS but also peripheral tissues such as skeletal muscle and heart. These channels consist of a voltage-sensing $\alpha_1$ pore-forming subunit that conducts current and modulating accessory subunits, including $\alpha_2\delta$, $\beta$ and $\gamma$ (in muscles; for review see [57]; fig. 3). For each subunit, multiple genes have been identified, each of which can exhibit multiple splice variants, providing the potential for enormous molecular heterogeneity.

The $\alpha_2\delta$ family consists of three genes. $\alpha_2\delta$-1 was first identified from skeletal muscle [58] and is now known to exist as five tissue-specific splice variants [59]. $\alpha_2\delta$ proteins are synthesised as preproteins that undergo extensive posttranslational modification. The membrane targeting signal is proteolytically removed, whilst further cleavage generates a small C-terminal fragment ($\delta$) that remains attached to the larger ($\alpha_2$) fragment by a disulphide bridge. It is generally believed that the $\delta$ subunit forms a single transmembrane-spanning segment which anchors the wholly extracellular $\alpha_2$ subunit to the calcium channel complex (for review see [60]), fig. 3). Gabapentin binding appears to be dependent on the presence of both $\alpha$ and $\delta$ subunits, since neither individual subunit appears capable of binding the drug when expressed alone [61]. Mutational analysis of $\alpha_2\delta$-1 has led to the identification of regions 206–222, 516–537 and 583–603 within the $\alpha_2$ subunit that are essential for gabapentin binding with an arginine residue at position 217 being critical for this interaction [61].

Since these findings, the complexity of the field has increased with the discovery of two novel $\alpha_2\delta$ genes, $\alpha_2\delta$-2 and $\alpha_2\delta$-3 by [62]. Each subunit is widely expressed throughout the body in a tissue-specific manner [63, 64]. Interestingly, $\alpha_2\delta$-2 but not $\alpha_2\delta$-3 has been shown to bind gabapentin with a similar affinity to that shown by $\alpha_2\delta$-1 [65]. Analysis of the gene structure of these proteins has revealed the presence of arginine at position 217 in the $\alpha_2\delta$-2 gene and its absence in the $\alpha_2\delta$-3 gene, further underlining the importance of this residue in gabapentin binding. The physiological importance of $\alpha_2\delta$-2 has also been further underlined by the discovery of the first natural $\alpha_2\delta$ mutation in the mouse mutant ducky, which exhibits a form of absence epilepsy underlined by a reduced calcium current in cerebellar Purkinje neurones [66].

Further evidence supporting the therapeutic relevance of the $\alpha_2\delta$ binding site in gabapentin action has been provided by Taylor et al. [67] who demonstrated that the differing $\alpha_2\delta$ binding affinities of stereoselective analogues

of gabapentin correlate well with their structure-activity relationships in animal models of epilepsy. This has recently shown also to be the case in animal models of pain [68].

### Gabapentin effects on ion channels

Heterologous expression studies have shown that $\alpha_2\delta$ does indeed functionally interact and modulate a range of calcium channel $\alpha_1$ subunits (reviewed in [69, 70]). As the only ligand identified to date that interacts with the $\alpha_2\delta$ subunit, gabapentin may therefore provide a unique, alternative candidate for mediating voltage-dependent calcium influx.

In 1998, Stefani et al. [71] were the first group to demonstrate that gabapentin inhibited voltage-dependent calcium channel currents recorded from cortical neurons; however, the gabapentin-mediated reduction in current varied between different cell types. For example, electrophysiological recordings have failed to detect any gabapentin-mediated change in the calcium channel currents recorded from hippocampal neurones taken from patients with temporal lobe epilepsy [72]. Nevertheless, gabapentin has been shown to prevent increased duration of seizure discharge in the rat hippocampus in a similar manner to the L-type voltage-dependent calcium channel blocker, nimodipine [73].

Recent work using both electrophysiological and calcium-imaging techniques has served to verify and extend these findings to certain other specific cell types, including DRG neurones [47, 74, 75], but not other cell types such as cardiac myocytes [76].

These studies have shown that the sensitivity of calcium currents to gabapentin is greatly influenced by the physiological state of the cells and the calcium channel subunits subsequently expressed. For example, the relative amount of $\alpha_2\delta$ messenger RNA (mRNA) present in cells appears to be an important determinant of gabapentin sensitivity [47, 76], presumably as a result of increased incorporation of specific subunits in the expressed calcium channel complexes. Gabapentin sensitivity also appears to be influenced by the activity of protein kinases [47], which is also known to be affected by underlying pathophysiology (e.g. [77]).

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 side effects. In addition, gabapentin is not effective in all patients or cell preparations [30, 78], a finding which may reflect a biovariability in the pharmacokinetic properties or target binding interactions of gabapentin. The recent identification of multiple $\alpha_2\delta$ subtypes which differentially bind gabapentin and exhibit a tissue- [62, 63] and pathophysiology-dependent [79–81] distribution provides the potential for subunit-specific and localised interactions between gabapentin and calcium channels.

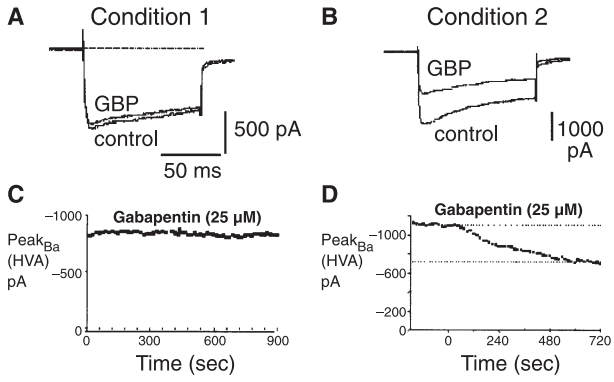


Figure 4. High voltage activated (HVA) calcium currents recorded from dorsal root ganglion (DRG) neurones under two different culture conditions, illustrating the differential effect of gabapentin (GBP) under each condition. Representative calcium currents recorded using barium as the charge carrier from DRG neurons cultured in media 1 [panel *A*, condition 1: serum and 10 ng/ml nerve growth factor (NGF-2.5s)] were significantly less sensitive to gabapentin compared with the calcium currents recorded from DRG neurons cultured in media 2 (panel *B*, condition 2: serum-free and 100 ng/ml NGF-7s). Calcium currents were obtained under voltage clamp conditions by a step depolarisation from −80 mV to 0 mV for 100 ms. In panels *C* and *D* the time course of gabapentin effect on the peak barium current (PeakBa) is plotted with respect to time. Reprinted from [47] with permission from Elsevier Science.

Recent recordings taken from dorsal horn neurons in spinal cord slices from adult hyperalgesic rats or neonatal control slices have confirmed a presynaptic site of action for gabapentin [82, 83]. Postsynaptic affects have also been demonstrated in which gabapentin variably modulates either NMDA- or $\alpha$-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-mediated synaptic transmission in freshly dissociated rat DRGs, control slices and in vivo [78, 83, 84].

### Gabapentin and neurotransmitter release

Several independent studies have now shown that given the right conditions, gabapentin can produce a reduction in calcium influx in presynaptic nerve terminals and inhibit the release of excitatory amino acids in a regionally selective manner [85, 86]. Recent data also describe an inhibitory effect of gabapentin on $K^+$-stimulated [$^3$H]-noradrenaline release from human neocortex [87]. Other early reports mentioning an inhibitory effect of gabapentin on dopamine release in the rabbit striatum [88] and of 5-hydroxytryptamine levels in whole blood of healthy young men [89] remain anecdotal and have not been substantiated by further studies. In general, the

gabapentin effects mentioned in these studies are consistent but not very pronounced.

Recent evidence also suggests that the effects of gabapentin can be stimulus-dependent in certain systems. For example, in rat caudal trigeminal slices, gabapentin has no effect on the $K^+$-evoked release of [$^3$H] glutamate but is able to inhibit the facilitatory effects of substance P or calcitonin gene-related peptide on glutamate release [90]. Gabapentin also inhibits the enhancement of $K^+$-evoked [$^3$H] glutamate release by activators of protein kinase C (PKC) or adenylate cyclase in these slices [91], suggesting that it may act to modulate calcium influx following phosphorylation.

**Gabapentin effects on signal transduction**

Recent studies have shown that the onset of pain is associated with marked changes in the phosphorylation state of spinal cord neurones [77, 92]. A key enzyme cascade which appears crucial in the modulation of this process and therefore to the induction of pain is the mitogen-activated protein (MAP) kinase pathway. For example Ji et al. [77] have demonstrated that intense electrical C-fiber stimulation increases the number of phosphoERK (extracellular signal-related kinase)-positive neurons in lamina I and II of the ipsilateral dorsal horn. This increase in activity can be blocked by the NMDA receptor antagonist MK-801 or pretreatment with gabapentin. At present it is unclear whether the effects of gabapentin on this pathway contribute to the analgesic effects of this drug or whether the reduction of phosphoERK immunoreactivity is a consequence of the analgesia produced by gabapentin acting on some other system.

In relation to this, Greenberg et al. have recently reported that activation of L-type voltage-activated calcium channels could lead to activation of transcription factors such as cyclic AMP response element (CREB) and myogenic enhancer factor 2 (MEF-2) [93]. Furthermore, it was shown that binding of calcium-calmodulin complex to the carboxyl terminus of calcium channel leads to activation of the RAS/MAP kinase pathway, which conveys local calcium signals to the nucleus. It is conceivable that the high-affinity binding of gabapentin to $\alpha_2\delta$ can modulate the interaction between the calcium channel and calmodulin and hence cause a downstream effect on the MAP kinase signalling pathway. This is a particularly provocative hypothesis that is likely to draw considerable attention in the future.

**Conclusions and future directions in gabapentin research**

A large and continuously growing body of preclinical and clinical data exists which indicates that gabapentin is effective in an extraordinarily diverse range of conditions and at the same time exerts only few untoward side effects. As the list of clinical indications grows, the confusion surrounding the drug's mechanism of action also increases. It remains difficult to understand how a chemical with such robust and important therapeutic activity in vivo can fail to elicit equally robust activity in vitro. There is little agreement among investigators regarding the exact molecular target of gabapentin. It may be that there is no one individual target and that gabapentin has a small modulatory effect on a large number of systems.

In the current paper we attempted to review the findings which have led to our present understanding of gabapentin action from the initial observations that this compound was not a simple GABA mimetic, to the discovery of a unique gabapentin binding site and the more recent linkage between gabapentin and complex intracellular signalling pathways.

Over the years that we have worked with this compound, both collectively and individually, we have developed the idea that gabapentin may be an ideal drug that acts selectively on pathophysiological systems. This would explain the difficulty that exists in finding robust effects of gabapentin in vitro, where true pathophysiological events are difficult to replicate.

Indeed, more recent gabapentin literature appears to support the hypothesis that cells need to be subjected to certain conditions in order to display gabapentin sensitivity. Clearly then, a key area of future research will be to understand the mechanisms that underlie pathology and the effects these mechanisms have upon putative gabapentin targets. An obvious first step in understanding gabapentin action process will be to thoroughly delineate the role of the $\alpha 2\delta$ subunits in gabapentin-sensitive disease models. Here, it is likely that $\alpha 2\delta$ subunit knockout animals will have an important role. Key experiments will include an examination of the phenotype of various knockouts, their response to the pathology and the ability of gabapentin to treat these pathologies.

Recent studies have provided evidence that gabapentin may not necessarily act via the $\alpha 2\delta$ binding site, and future work should also be performed to determine whether this is truly the case. Once again, the use of the available knockout models will be important to fully explore these hypotheses.

In summary, there remain many questions which need to be answered before we can confidently delineate the mechanisms by which gabapentin achieves its therapeutic effects. Solution of these difficult and intriguing questions will surely continue to offer a greater insight into the disease process as well as hopefully providing opportunities for newer and more effective drugs.

*Acknowledgements.* We have tried throughout this review to include all relevant literature. However, since the gabapentin-associated literature is growing at an ever increasing pace, we apologise in advance for any omissions that we may have made. We would also like to acknowledge the help and support that has been offered from fellow scientists working on this project. In particular we would like to acknowledge the endeavours of former colleagues at the Parke-Davis pharmaceutical company who over the years took gabapentin from being an obscure chemical entity to a drug that has benefited patients throughout the world.

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