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Neuropathy pain mechanisms involving gabapentinoids and the a2d-1 calcium channel subunit

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Abstract

The focus of perioperative pain management should be to attempt to minimise the nociceptive input and reduce the risk of transition to central sensitisation. Gabapentinoids are being increasingly used as adjuncts for management of peri- operative pain. Although gabapentinoids are classed as calcium channel blockers, their mechanisms of action are poorly understood. The analgesic effect in neuropathic pain is well evidenced but the role in postoperative pain is less certain. Medline and EMBASE database searches were conducted to identify studies relating to mechanisms of action and effects in experimental animal models of inflammatory and postoperative pain and human models of experimental pain. The effects of gabapentinoids may be attributed to depression of dorsal horn sensitivity through a multitude of mechanisms. They inhibit calcium mediated neurotransmitter release through effects on a2d-1 subunits. They inhibit forward traf- ficking of a2d-1 from the dorsal root ganglion, their recycling from endosomal compartments, thrombospondin mediated processes and stimulate glutamate uptake by excitatory amino acid transporters. Mechanisms not directly related to neurotransmitter release at dorsal horn include inhibition of descending serotonergic facilitation, stimulation of descending inhibition, anti-inflammatory actions, and influence on the affective component of pain. Gabapentinoids are effective analgesics in most animal models of inflammation and postoperative pain but effects in human models are variable.

Keywords: alpha 2-delta subunit 1 protein; gamma-aminobutyric acid; pregabalin

The gabapentinoids, pregabalin and gabapentin, have been the cornerstone of pharmacological management of neuropathic pain.[1] Despite the widespread use in neuropathic pain, the precise mechanism of action is uncertain. The effect of gaba- pentinoids in pain are assumed to be because of direct inhibi-
tion of voltage gated Ca^{2+} channels by binding to its a2d-1 subunit resulting in reduction of presynaptic Ca^{2+} influx and
subsequent release of excitatory neurotransmitters such as glutamate. This assumption is not correct as calcium cur- rents are not consistently reduced by acute application of gabapentinoids.[2] Despite this, most studies show that gaba- pentinoids inhibit release of neurotransmitters in neuronal tissues.[2] This review explores the possible mechanisms by which gabapentinoids inhibit neurotransmitter release despite the lack of acute effect on Ca^{2+} currents. This review has also sought to identify the analgesic mechanisms unrelated to the direct inhibition of neurotransmitter release at the dorsal horn. Although there is good evidence for the effect on neuro- pathic pain, the role in postoperative pain is less certain. Gabapentinoids are being increasingly used in the periopera- tive period s
evidence to support its use in postoperative pain is limited because of the poor quality of evidence from clinical trials.[4] This review sought to determine the effects of gabapenti- noids in animal models of postoperative and inflammatory pain and in human pain models. The

implications for clinical practice are discussed.

Methods

Medline and EMBASE database searches were conducted to identify studies relating to mechanisms of action and effects in experimental pain models (Appendix A). The reference lists of selected articles were explored for additional studies. Only manuscripts published in English were included. The level of evidence could not be graded as most studies were exploratory in nature. Various themes relating to mechanisms were identified and selected studies described.

Nociceptive pathways, voltage-gated calcium channels, and the a2d subunit

Nociceptors are pseudo-unipolar: the cell bodies are located in the dorsal root ganglion (DRG), a single process bifurcates into a central axon that project to second-order neurons and local interneurons in the dorsal horn of the spinal cord, and a pe- ripheral axon travels through the spinal nerve to the periph- ery.[5] After nerve injury or inflammation, the stimulation threshold of nociceptors is reduced. The sensitised nociceptors are activated by minimal stimulida process known as pe- ripheral sensitisation that causes primary hyperalgesia. The action potentials that are transmitted to the nociceptors are relayed to the spinal dorsal horn through the central axon and to the periphery through the peripheral axon. This causes membrane depolarisation, activation of voltage-gated calcium channels (VGCCs) and calcium influx, triggering release of glutamate as a major neurotransmitter along with neuro- modulators such as substance P, calcitonin gene-related pep- tide, and brain-derived neurotrophic factor. These are released both peripherally at the site of inflammation and in the dorsal horn, to produce an excitatory signal at the post synaptic tar- gets.[5] The effects on the dorsal horn neurons of the spinal cord are mediated by the postsynaptic glutamate receptorsda- amino-3-hydroxy-5-methyl-4-isoxazolepropionate, *N*-methyl- D-aspartate and kainite.[6] Many neurons also regulate neuro-transmitter release through expressing presynaptic glutamate receptors.[7] The excitatory interneurons in the dorsal horn are also glutamatergic. The enhanced release of glutamate in the dorsal horn of the spinal cord causes increased activation of

Voltage-gated calcium channels

Voltage-gated and ligand-gated channels that are permeable to inorganic ions such as sodium, potassium, chloride, and calcium are essential for electrical activity of excitable cells such as neurons.[9] Calcium differs from the other ions as it also serves as an important signalling entity. Influx of calcium ions through high-voltage activated (HVA) calcium channels trigger a wide range of responses including gene transcription, neurotransmitter release, neurite outgrowth and activation of calcium dependent enzymes.[9]

VGCCs are comprised of multiple subunits: a_1, b, g, and a_2d (Fig 1).[10] The a1subunit allows entry of calcium ions. It com- prises four homologous domains (IeIV), each of which contain six transmembrane helices (S1eS6). The extracellular a_2 sub- unit is attached to the d subunit via a disulfide linkage. The b subunit is entirely intracellular. VGCCs are classified into HVA (L-, P/Q-, N-, R-) and low-voltage activated (LVA; T-type) VGCCs.[11] Dorsal root ganglion cell bodies and presynaptic terminals that form synapses with dorsal horn neurons ex- press increased density of N-type VGCCs.[10] The L-type chan- nels are extensively found in both excitable and non-excitable tissues. Although less extensively studied, other subtypes may be involved in the pain pathway.[10]

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a2d subunit and pain

The auxiliary a_2d and b subunits have four isoforms and enhance the plasma membrane expression and function of HVA calcium channels but not LVA channels.[12] The a2d-1 isoform that medi- ates the effectsofgabapentinoids ispresent in the brain, skeletal, cardiac, and smooth muscle. a2d-2 and a2d-3 subunits are pre- sent in non-neuronal tissues in addition, whereas a2d-4 is expressed in retinal neurons and other non-neuronal tissues.[12]

The a2d-1 unit has widespread distribution in the mouse brain, especially in the cerebral cortex, hippocampus, and cerebellum.[12] Elevated concentration of a2d-1 subunit is clearly associated with augmented pain processing. DRG neurons show increased expression after peripheral nerve damage in animal models of neuropathic pain.[13e16] The peak expression of a_2d-1 occurs 7 days after injury and takes several months to decline, with a temporal relationship with the onset and resolution of evoked behaviours.[15] Deletion of a2d-1 gene in mice models of neuropathic pain is associated with marked behavioural deficit in mechanical and cold sensitivity.[17] Intrathecal antisense oligonucleotides (synthetic polymers that can alter synthesis of specific proteins) complementary to a region in the a2d-1 gene can reverse mechanical hypersen- sitivity in nerve ligation models.[14] The concentrations are elevated in the dorsal horn and mimic that of the DRG with decrease in concentrations and reversal of allodynia after

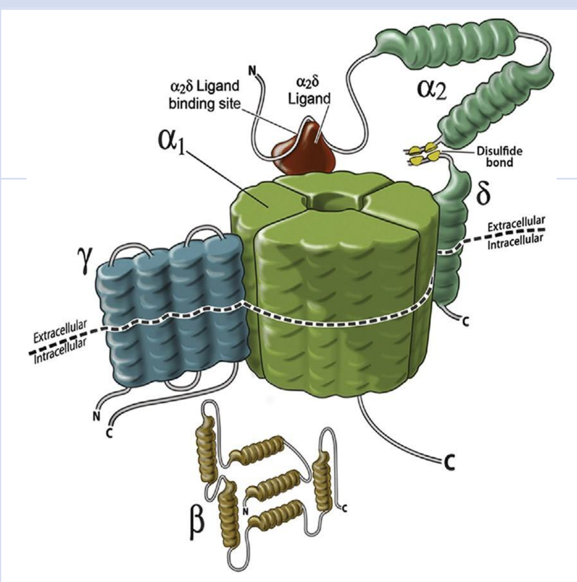

Fig 1. Voltage-gated calcium channels are composed of four subunits. The a1 subunit consists of four homologous domains, each containing six transmembrane segments. It is the pore-forming subunit. The b subunit is intracellular. The g subunit has four transmembrane segments. The d subunit has one transmembrane segment and is attached to the extracellular a2 subunit via a disulfide bond. Reprinted, with permission, from Elsevier.[10]

through system L-neutral amino acid transporters, pregabalin is rapidly and completely absorbed with peak plasma con- centrations within 1 h as opposed to 3 h with gabapentin.[27] Unlike gabapentin, absorption of pregabalin is not saturable, with a linear pharmacokinetic profile and less variable bioavailability.[27] Although peak plasma concentrations of gabapentinoids are achieved within 1e3 h, peak cerebrospinal fluid (CSF) concentrations may take significantly longer, with a median time of 8 h.[28] They do not influence spinal neuro- transmitter concentrations of glutamate, norepinephrine, substance P, and calcitonin gene-related peptide.[29] They are not metabolised by the liver and are excreted by the kidney with elimination half-lives of 6 h.[27]

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dorsal rhizotomy. This suggests that the elevated concentra- tion of presynaptic a2d-1 subunit in the dorsal horn is a result of transport by DRG neurons through their central axons.[14]

Increased concentrations of a_2d-1 can cause neuropathic pain even in the absence of nerve damage. Transgenic mice that overexpress a_2d-1 show symptoms of allodynia even when nerve damage is absent, which suggests that increased concentrations of a_2d-1 are sufficient to cause neuropathic pain.[18] The frequency of miniature excitatory postsynaptic currents in the dorsal horn is increased and is reversed by intrathecally delivered antagonists of glutamate receptors.[19] This suggests that a_2d mediates spinal sensitisation by increased presynaptic glutamate release that enhances the sensitivity of postsynaptic neurons in the dorsal horn. How- ever a2d is not always associated with neuropathic pain as the upregulation of a2d-1 is injury-specific with variable effects in animal models of neuropathic pain based on aetiology.[20]

Mechanism of action of gabapentinoids

Site of action

The actions of gabapentinoids are mainly at an intracellular site and require active uptake.[21] They were originally designed as g aminobutyric acid (GABA) analogues but do not have any effects on GABA receptors. Gabapentin binds to a_2d receptors with greater affinity to the a_2d-1 subtype.[22] Mutations of a_2d-1 or a_2d-2 block the neuronal actions of gabapentin by prevent- ing its binding, but not mutations in a_2d-3, indicating that the effects are mediated by a_2d subunits of VGCCs.[23] Several other sites of action have been described, such as NMDA receptors and sodium channels but the evidence is limited.[24e26] Although both drugs are absorbed by facilitated transport

Effect on DRG neurons

Gabapentinoids are considered to exert their effects by inhi- bition of calcium currents. There was modest inhibition of calcium currents in medium sized and isolectin B4 (IB4) negative DRG neurons (small sized neurons that express neuropeptides projecting to lamina I and II) after prolonged incubation.[30] The acute application of low concentrations of Mn^{2+}, which is a global VGCC blocker, was substantially more effective than gabapentinoids at inhibiting calcium currents in DRG neurons. If the classical mechanism is correct, Mn^{2+} should have been even more effective in suppressing synaptic transmission in the dorsal horn. However, the effectiveness was reversed in the dorsal horn. Gabapentinoids suppressed excitatory synaptic transmission in the substantia gelatinosa neurons, whereas Mn^{2+} had no effect. Clearly the moderate effect of gabapentinoids on calcium currents in DRG neurons alone could not explain its ability to reduce overall dorsal horn excitability. The reduction in neurotransmitter release because of gabapentin is therefore not entirely related to decreased calcium influx into presynaptic nerve terminals.

Gabapentinoids have anti-allodynic rather than anaesthetic effect as not all DRG neurons are sensitive to gabapentio- noids.[30] They preferentially affect medium sized neurons associated with Ad fibres that are often associated with noci- ceptive transmission and small IB4e neurons, with a complete lack of effect on the large and IB4+ neurons. Medium sized neurons and IB4e neurons project to excitatory neurons in the substantia gelatinosa, whereas large neurons associated with Ab fibres and IB4+ neurons project to inhibitory neurons.[30]

Spontaneous firing of injured sensory neurons mediated by voltage-gated persistent sodium currents is implicated in the generation of neuropathic pain. Gabapentin decreased the amplitude of resonance and abolished the subthreshold membrane potential oscillations of A-type DRG neurons, in a chronic compressed dorsal root ganglion model.[25]

Effect on forward trafficking

The effects could be related to the forward trafficking of a_2d to neurotransmitter release sites. a2d and b subunits could in- fluence the HVA channel current density by increasing the expression of the pore forming a1 subunits. Gabapentinoids applied chronically could reduce calcium influx by suppressing the forward trafficking of a_2d and calcium channels to the plasma membrane.[13] This could explain the significant effects on dorsal horn excitability in spite of the moderate effects on DRG neurons. The prolonged duration required for gabapenti- noid action *in vitro* can then be explained as a consequence of the time required to transport recently synthesised pore-

forming units to the terminals in the dorsal horn.[31] However, it is clear that decreased expression of HVA calcium channels at nerve terminals may not be relevant as their blockade by Mn^{2+} has very little effect on neurotransmitter release.[30,31]

Protein trafficking *in vivo* can be studied by obstructing trafficking by nerve section or ligation. The proteins that accumulate at the site of obstruction can then be studied. a_2d-1 was found to accumulate proximal to the spinal nerve ligation (SNL) site but was not exclusively presynaptic. It was found in both the central and peripheral terminals, and this increased over the days after ligation, suggesting that they are trans- ported to these terminals from their site of production in DRG cell bodies. Increased a2d-1 concentrations in the dorsal horn are important for development of neuropathic pain. Chronic treatment of these spinal nerve ligated animals with pre- gabalin had a significant antiallodynic effect.[32] It reduced the accumulation of a_2d-1 at the SNL site, in ascending DRG axons of the fasciculus gracilis and reduced the elevated concen- trations of a_2d-1 in the presynaptic terminals of DRG neurons in the spinal cord dorsal horn. However, it had no effect on the up-regulation of a_2d-1 in DRG neurons. This indicates that the *in vivo* antiallodynic effects of chronic gabapentinoids are a result of inhibition of anterograde trafficking of a_2d-1, thereby inhibiting neurotransmitter release.[32]

The reduction in forward trafficking of a_2d with gabapentin may be a consequence of inhibition of its Rab11-dependent recycling.[33] Rab11 is involved in the regulation of recycling of endocytosed proteins.[34] By inhibiting the recycling of a2d in neurons, gabapentin might reduce the expression of plasma membrane calcium channels at presynaptic terminals. How- ever, intrathecal administration of pregabalin had analgesic effects but did not inhibit the accumulation of a_2d-1 at primary afferent terminals after peripheral nerve injury in rats.[35] This lack of a_2d-1 accumulation may be a result of reduced drug concentrations at effect sites with intrathecal administration as compared with systemic administration.[35] The transport of a1:a2d-1-subunit complexes to the cell surface is b-subunit dependent and can be influenced by gabapentinoid action on the b4a subtype.[36]

Effect on neurotransmitter release sites

a2d increases the density of HVA calcium channels at release sites and promotes increased exocytosis. This enhanced effect is seen at decreased Ca^{2+} influx, indicating that elevated con- centrations of a2d allow synapses to make more efficient use of Ca^{2+} entry to drive neurotransmitter release.[37] Pregabalin ac- tions were attenuated in knockout mice lacking the protein syntaxin 1A, a component of the synaptic vesicle release ma- chinery, indicating that syntaxin 1A is required for pregabalin to exert its full presynaptic inhibitory effects.[38] The inhibitory ef- fects can therefore be explained by the interruption of the ability of a2d to facilitate interaction of HVA calcium channels with neurotransmitter release sites. The a2d-mediated impaired synaptic transmission is attenuated by gabapentinoids.[39]

Effect on thrombospondin

Astrocytes are involved in many neuronal mechanisms, including the formation of new synapses.[40] Astrocyte-derived thrombospondins are involved in presynaptic plasticity through their actions on a_2d-1, by binding to their von Wille- brand factor domain.[40] Gabapentin can inhibit the formation of excitatory synapses by blocking the binding ofthrombospondin to a_2d-1.[41] It is unlikely, however, that the slow process of synaptogenesis contributes to the rapid effects of gabapentinoids. In contrast, gabapentin reversed neuro-pathic pain after intrathecal injection of thrombospondin-4 (TSP4) with return of withdrawal threshold to control con- centrations within 24 h.[42] The rapid effects may be a result of interference with processes dependent on the interaction of TSP4 and calcium channels that may be key even before long- term changes such as synaptogenesis.[42] Increased TSP4 con-tributes to hypersensitivity by reduced expression of HVA and enhancing LVA in DRG neurons.[43]

Effect on descending serotonergic facilitation, descending inhibition and cortical mechanisms

Some of the analgesic effects are mediated through modula- tion of descending pathways. Increased descending seroto- nergic facilitation on spinal 5HT3 receptors is associated with the development of pain.[44e46] Antinociceptive effects of gabapentinoids were blocked by prior administration of sero- tonin receptor blockers and by selective ablation of superficial dorsal horn neurons expressing the neurokinin-1 receptor for substance P.[47e49] These neurons project to descending brain- stem serotonergic pathways that increase spinal excitability. Activation of spinal 5-HT3 receptors in normal animals allowed gabapentin to inhibit neuronal responses where pre- viously it was ineffective. Gabapentin induces glutamate release from astrocytes in the locus coeruleus that is the principal site of noradrenaline synthesis.[50] The analgesic ef-fect of gabapentin in neuropathic pain in a rat SNL model was reduced because of downregulation of astroglial glutamate-1 transporter in the locus coeruleus that reduced spinal norad- renergic inhibition but was reversed by oral valproate that is an inhibitor of histone deacetylase.[51]

Gabapentinoid effects on the affective component of pain can explain some of the analgesic effects. Positron emission tomography imaging indicated that the analgesic effect is mediated by suppressing medial prefrontal cortex, a brain area involved in the affective response to pain, with extensive connections to the limbic system.[52] The supraspinal mecha- nisms that modulate the affective-motivational qualities of pain require engagement of cortical endogenous opioid cir-cuits that activate mesolimbic reward system involved in motivational aspects of pain behaviour.[53]

Effect on glutamate transport

Glutamate released by excitatory stimulation can accumulate extracellularly and cause excitotoxicity. Its concentrations are regulated by rapid removal by excitatory amino acid trans-porters (EAATs).[54] The glial cells take up glutamate that is metabolised to glutamine by glutamine synthase and is transported back to the neurons to replenish glutamate at the presynaptic membrane.[54] The EAAT1 and EAAT2 subtypes are more widely distributed in the neuronal tissue and are responsible for most of the glutamate uptake, the reduced expression of which contributes to the development of neuropathic pain.[55] All subtypes are expressed in the dorsal horn post synaptic membranes.[56] EAAT3, however, is less abundant as compared with the other subtypes and may act by influencing glutamate metabolism rather than neurotrans-mission.[56,57] Pregabalin increased activity of EAAT3 in EAAT3- expressing oocytes in a dose-dependent manner, indicating that it may work by enhancing its trafficking to the plasma

Table 1 Continued

Source	Experiment	Result
	Chronic incubation with 100 mM or 1 mM gabapentin for 48 h before assessment of the plasma membrane expression level of a2d-2 BBS	
Effect at neurotransmitter release sites		
Hoppa and colleagues[37] Ca^{2+} signals but Y	Hippocampal neurons Effect of a2d concentrations on the probability synapses overexpressing a2d that a vesicle in the readily-releasable pool controls. [In action potential will undergo fusion with a single action calcium influx suggests potential (n≥7) subunits results in	Single APs resulted in robust by 40% in compared with stimulus in spite of Y stimulus and calcium influx that overexpression of a2d a tighter spatial relationship between sites of Ca^{2+} entry and exocytosis
Matsuzawa and colleagues[38] Amplitude of electrically evoked eEPSC in	Effects of pregabalin 100 mM on excitatory synaptic transmission in superficial dorsal as compared to knockout mice horn in syntaxin 1A knockout (n=12) and wild-type mice (n=11)	Y wild type mice
Zhou and Lou[39] frequency in superficial dorsal horn	SNL model, a2d1 overexpressing transgenic mice transgenic mice Effect of gabapentin (10e100 mg) on a2d1 gabapentin. No effect on mediated presynaptic neurotransmission in No effect on mIPSC in either superficial dorsal horn SNL model: mEPSC (SNL, n=58; sham, n=30); mIPSC (SNL, n=17; sham, n=17) Transgenic model: mEPSC (transgenic, n=20; wild type, n=22), mIPSC (transgenic, n=19; wild type, n=18)	[mEPSC neurons after SNL and in normalised by amplitude. model
Effect on thrombospondin		
Park and colleagues[42]	Gabapentin effect on intrathecal TSP4-induced effect of C_{av}a2d1 with gabapentin neuropathic pain (n=8) or saline (n=6); effect pain on TSP4 mediated mEPSC frequency or[control levels with control (n≥7 each) gabapentin, reduced to the Intrathecal gabapentin (300 mg) effect on PWT in after 24 h TSP4 induced neuropathic pain Y to the control Gabapentin (50 mg) effect on elevated mEPSC the control group frequency significantly	Blocking the reversed TSP4 mediated PWT to near the intrathecal pretreatment level Elevated mEPSC frequency level, but its basal level in was not affected

Pan and colleagues[43] Effect of gabapentin (25 mM) pretreatment on TSP4-
induced reduction of HVA I_{Ca} and the effects of
thrombospondin-4 (TSP4) on elevation of LVA I_{Ca}
were eliminated by
HVA and LVA voltage-gated calcium channels
gabapentin. It also blocked
effects of TSP4 on HVA I_{Ca} (vehicle, n=9; gabapentin, n=7;
TSP4, the intracellular Ca^{2+}
transient
n=7; gabapentin + TSP4,
n=7) and LVA I_{Ca} (vehicle,
n=8; gabapentin, n=7;
TSP4, n=7; gabapentin +
TSP4, n=7)
Calcium transients: HVA
(vehicle, n=68; gabapentin,
n=46; TSP4, n=66;
gabapentin + TSP4, n=34),
LVA (vehicle, n=40:
gabapentin, n=53; TSP4,
n=53; gabapentin + TSP4,
n=39)

Effects on descending serotonergic facilitation, descending inhibition and cortical mechanisms

Rahman and colleagues[47] Osteoarthritis induced by
intra-articular Ondansetron: Y evoked
responses to innocuous injection of MIA into the knee joint;
spinal stimuli in MIA only, Y
response to noxious ondansetron (n=8, sham=8) or
pregabalin stimuli in both groups
(n=9, sham=8) on evoked responses of dorsal Pregabalin: Y
responses to noxious stimuli in
horn neurones to electrical, mechanical and the MIA-
treated group only; efficacy lost in thermal stimuli;
pregabalin alone or with the presence of
ondansetron
ondansetron (n=7)
Spinal ondansetron (10e100 mg/50 ml) or
systemic pregabalin (0.3e10
mg kg^{-1}); pregabalin (10 mg
kg^{-1}) in pretreated MIA rats

Suzuki and colleagues[48] SNL model; gabapentin (10, 30, 100 mg kg^{-1} s.c.) SP-SAP
Y tactile and cold hypersensitivity and
after SAP and SP-SAP to target neurokinin-1 abnormal
neuronal coding (including expressing neurons that drive
serotonergic spontaneous activity,
expansion of receptive facilitation field size) seen after SNL
Effect of ablation of neurokinin-1 neurons with No
neuronal response with gabapentin in SAP: hind paw
withdrawal frequency to absence of injury
mechanical/cooling stimuli: SNL (SAP, n=17; Y Punctuate,
heat and brush evoked neuronal SP-SAP, n=23) sham (SAP,
n=13; SP-SAP, n=9) responses with gabapentin in
SAP but not SP-
Effect of SAP on neuronal plasticity: SNL (SAP, SAP even in SNL
n=50e57; SP-SAP, n=41e44) sham (SAP, n=24 Gabapentin
effect lost with ondansetron pre-
e33; SP-SAP, n=21e32) treatment; spinal 5HT3
activation allowed

Continued

Table 1 Continued

Source	Experiment	Result
	Effect of spinal 5HT3 block on gabapentin inhibit responses in uninjured efficacy: intrathecal ondansetron 10 mg before gabapentin 100 mg kg^{-1} Spinal 5HT3 activation: spinal neurons of naı¨ve, neuropathic SP-SAP, intrathecal activator 2-methyl 5HT 0.1 mg before gabapentin 100 mg kg^{-1}	gabapentin to animals
Suto and colleagues[50]	Effect on glutamate concentrations in LC with concentrations in microdialysates saline (normal, n=10; SNL, n=8) or gabapentin saline, with a 50 mg kg^{-1} i.v. (normal, n=11; SNL, n=12) gabapentin 60 min after Gabapentin effect on increased glutamate after [Glutamate in both normal and knockdown GLT-1 (n=22) rats with local perfusion Depletion of noradrenaline with intra-LC GLT-1 abolished gabapentin- injection of saporin 0.25 mg/rat (n=17) extracellular glutamate Effect on extracellular glutamate concentrations in spinal cord, vehicle or noradrenergic neurons did not gabapentin 10 mM perfused into the spinal increase in dorsal horn for 90 min (n=9 in each group) LC	[Glutamate from the LC compared with peak effect of injection. SNL Knockdown of induced increase in in the LC Depletion of alter gabapentin-induced extracellular glutamate in the In contrast to its effect in the LC, glutamate concentrations from the spinal cord reduced within 30 min compared with vehicle and was maintained for at least 90 min
Kimura and colleagues[51]	Effects of SNL on antihypersensitivity effects of effect size (>30 g pressure) efficacy gabapentin or saline (n=9 in each), single (>20 g) and injection of intraperitoneal saline or (>10 g) effect sizes, efficacy lost at 6e10 gabapentin (100 mg kg^{-1}) at 0, 1, 2, 4, 6, 8, and 10 weeks after SNL surgery weeks but not 10 Effects of intrathecal a2-adrenoceptor adrenoceptor antagonist idazoxan (30 mg) or saline injected that SNL time- 30 min after gabapentin injection (100 mg gabapentin related to kg^{-1}) in rats at 2, 6, and 10 weeks after SNL inhibition (n=7 or 8) weeks post SNL, Effect of glutamate transporter GLT-1 selective with GLT-1 small small interfering RNA or control on effect on Oral valproate treatment [paw withdrawal with gabapentin 100 mg kg^{-1} thresholds compared (n=8) the Effects of increasing GLT-1 expression in the LC	For a large lost around 5 weeks; for middle small weeks in half SNL rats Y Gabapentin efficacy at 2e6 weeks with intrathecal a2- antagonist idazoxan -suggests dependently Y effect of descending noradrenergic Y Expression of GLT-1 in LC at 6 Y gabapentin efficacy interfering RNA; mechanical withdrawal with the control and restored

antihypersensitivity effect of
gabapentin, by histone deacetylase inhibition on which was
abolished by idazoxan gabapentin's efficacy >6 weeks after
nerve
injury (n=9)
Sodium valproate (histone
deacetylase inhibitor) by a
feeding tube (200 mg kg$^{—1}$
daily) for 14 days beginning 6
weeks after SNL
surgery

Lin and colleagues[52] SNI model, brain glucose metabolic rate at the Y Glucose
metabolism in the media prefrontal effective dose of gabapentin in
SNI rats, (SNI, cortex, anterior cingulate
cortex, thalamus, n=12; sham, n=10) and cerebellar
vermis but [in the bilateral
18 F-fluorodeoxyglucose-positron emission upper lip regions of
primary somatosensory
tomography before and after SNI and after cortex
and ipsilateral AG gabapentin

Bannister and colleagues[53] Effect of i.v. gabapentin 50 mg
kg$^{—1}$ on tactile I.V. gabapentin Y allodynia with
peak effect in allodynia (sham, n=7; SNL, n=9), CPP (sham,
20 min, significant preference
seen for the n=10; SNL, n=17), and NAc DA release (sham,
chamber paired with gabapentin-
indicates n=11; SNL, n=12) gabapentin is not rewarding in
a normal
Effect of 200 mg intrathecal gabapentin on pain state, and that its
rewarding quality in SNL
thresholds (saline, n=9; gabapentin, n=10), rats is probably a
result of relief of ongoing CPP (sham, n=10; SNL, n=12), NAc DA
release aversiveness associated with
pain, [DA only (saline, n=7; gabapentin, n=8) in SNL
Effect of block with irreversible m-opioid Intrathecal gabapentin Y
pain thresholds, [DA
receptor antagonist b-FNA into the rACC in and preference for
gabapentin chamber in SNL on antiallodynia (saline, n=4; b-FNA,
n=6) SNL but not sham
CPP (saline, n=7; b-FNA, n=12) and NAc DA Pretreatment with
saline or b-FNA into the release (saline, n=8; b-FNA, n=9)
rACC did not influence
gabapentin efficacy;
Effect of gabapentin in rACC on allodynia (n=5 robust CPP and
[DA with saline but no saline or gabapentin each), CPP (sham,
n=10; change in CPP or DA after
b-FNA
SNL, n=8) or NAc DA concentrations (saline,
pretreatmentdindicates
endogenous opioid
n=8; gabapentin, n=5) signalling in the rACC is
required for rewarding actions
but not antiallodynic effect
Gabapentin injected in rACC
produced CPP, [NAc DA
release selectively in SNL rats but
did not reverse tactile allodynia

Continued

Table 1 Continued

Source	Experiment	Result
Effect on glutamate transport		
Ryu and colleagues[58]	Voltage patch clamp of oocytes, effect of Exposing oocytes injected with EAAT3 mRNA to pregabalin on EAAT3 activity (n=20e25 in serial concentration of pregabalin increased each group) and Michaelis constant (Km) and their responses to glutamate concentration maximum velocity (Vmax) values of EAAT3 dependently transport kinetics for glutamate (n=25 in each Pregabalin [Vmax without changing the Km group) Treatment with PMA or pregabalin [Effect of PKC activator (n=17e25 in each group: transporter currents but no additive effect control, PMA, pregabalin, pregabalin + PMA) PKC inhibitors Y pregabalin- enhanced EAAT3 Effects of PKC inhibitors on EAAT3 activity activity, treatment with PI3K Ybasal EAAT3 (n=10e19 in each group: control, PKC activity inhibitor, pregabalin, PKC inhibitor + Pregabalin [EAAT3 activity and PKC and PI3K pregabalin) were found to contribute to this effect Role of PI3K on the regulation of EAAT3 activity by pregabalin (n=15e19 in each group)	

membrane of neurons and glial cells.[58] However, gabapentin had an opposite effect with decreased EAAT3 activity in a similar model.[59] This discrepancy may be related to the duration of exposure to the drug. Oocytes were exposed to pregabalin for 72 h as opposed to 3 min to gabapentin.

Time course of action of gabapentinoids

The actions of gabapentinoids in neuropathic pain take several hours to develop *in vitro*, but develop rapidly *in vivo*.[31,60] A likely explanation is that the *in vitro* studies are often done in uninjured animals whereas the *in vivo* studies are done on nerve-injured animals where a$_2$d-1 has been already upregu- lated.[31] The effects are probably pronounced with increased expression of a$_2$d-1 subunit with a higher rate of turnover of channel complexes that may be more vulnerable to gaba- pentinoids.[31] The rapid effects *in vivo* may be a result of rapid intracellular neuronal uptake that is absent *in vitro*. Substantia gelatinosa neurons obtained *ex vivo* after acute administration of intraperitoneal gabapentin *in vivo* in chronic constriction injury model were less excitable.[61] The rapid effects may be a result of effects on descending serotonergic and noradrenergic pathways and cortical mechanisms that might be unrelated to increased a$_2$d-1 expression). Selected studies relating to anal- gesic mechanisms are described in Table 1 and Fig. 2 illus- trates a summary of these mechanisms.

Effect on inflammation and postoperative pain models

Elevated proinflammatory cytokines are intimately associated with the development of neuropathic pain as part of the neuroinflammatory response and are inhibited by gabapentinoids.[62e66] Neuropathic and inflammatory pain differ in their aetiology but have common underlying mech- anisms such as elevated tumour necrosis factor, Nav1.7, Nav1.8, glutamate, increased glial activation, and glutamate receptor function.[67] Animal studies of inflammatory pain induce inflammation after injection of a wide range of irritants and evaluate reflexive behavioural responses to thermal, me- chanical, or electrical stimuli. Gabapentinoids are effective antinociceptives in most animal models of inflammatory pain (Table 2).[68e83] However, some studies show limited effects.[84e87] Gabapentinoids reduce inflammatory media- tors[69,73,78] and suppress dorsal horn activity[79,83] but contrast- ing effects have been shown.[84] There is some evidence to support pre-emptive treatment.[72,73,85] Gabapentin was effec- tive in suppressing single motor unit response but not wind- up, indicating a peripheral mechanism of action.[86] Mechani- cal and thermal responses were attenuated but reduction in activity was not improved acutely as compared with non- steroidal anti-inflammatory drugs (NSAIDs).[87] Only chronic gabapentin had an effect on ambulatory-evoked pain.[76]

The neurophysiology of incisional pain, however, is different from inflammatory and neuropathic pain with unique sensiti- sation processes.[88,89] In the plantar incision model, a 1 cm lon- gitudinal incision is performed through the glabrous skin, fascia, and plantar muscle of the rat hind paw. Short-lasting non-evoked guarding and longer lasting evoked pain-related behaviour are seen and used as surrogates for pain at rest and evoked pain (lasting several days to weeks) after surgery, respectively. Mechanical and heat butnot coldhyperalgesiaand anxiety behaviours develop after injury. The skin and muscle retraction injury model was developed to investigate prolonged pain after surgical incision. Gabapentinoids are effective in most animal models of postoperative pain (Table 2). They demonstrate analgesic effects with synergistic effects in com- bination with opioids and NSAIDs.[90e97] They did not influence activity in a knee arthroplasty model but improved weight bearing when combined with opioids in an incision model.[92,98]

Effects in human experimental pain models

Various models that explore the effect of gabapentinoids in human experimental pain have been described (Table 3). The Ultraviolet B (UVB) model is widely used for assessing efficacy of anti-inflammatory drugs.[115] Hyperalgesia is evoked by exposing an area of skin to an individualised dose of UVB. Gabapentinoids did not have any effect on heat pain percep- tion and secondary hyperalgesia in this model of inflamma- tion.[99,100] The burn model is considered a model of inflammatory pain but both central and peripheral mecha- nisms are involved.[116] Gabapentin did not have any effect on heat pain detection threshold, pain during burn and secondary hyperalgesia.[101] However, it reduced area of secondary hyperalgesia after brief thermal stimulation of thigh.[102] The capsaicin model is used as a surrogate model of changes in neuropathic pain resulting in sensitisation.[117] Gabapentin had limited effects on measures of hyperalgesia in this mod- el.[103e105] Capsaicin mediates hyperalgesia through effects on

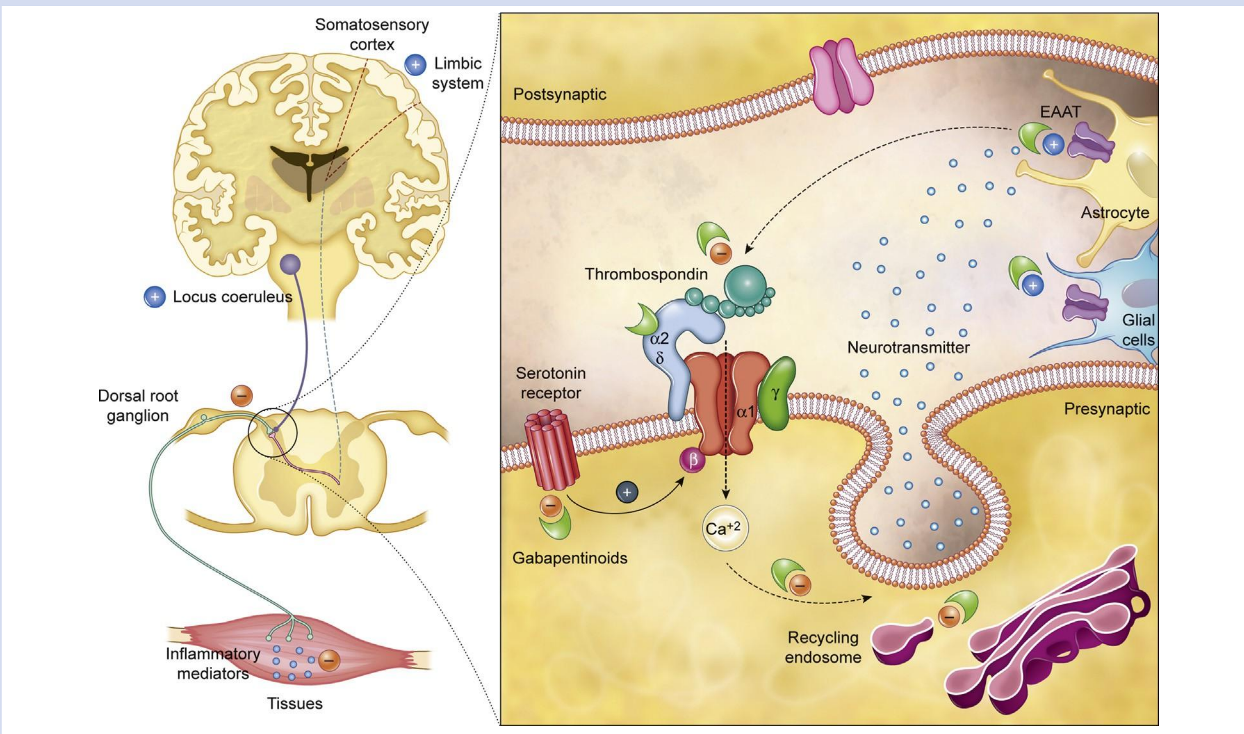

Fig 2. Gabapentinoids inhibit calcium mediated neurotransmitter release through effects on a2d-1 subunits. They inhibit forward traf- ficking of a2d-1 from the dorsal root ganglion, their recycling from endosomal compartments, thrombospondin mediated processes and stimulate glutamate uptake by excitatory amino acid transporters (EAAT). Mechanisms not directly related to neurotransmitter release at dorsal horn include inhibition of descending serotonergic facilitation, stimulation of descending inhibition, anti-inflammatory actions and influence on the affective component of pain.

Table 2 Effects of gabapentinoids in animal pain models. CFA, complete Freund's adjuvant; FGF, fibroblast growth factor; PWT, paw withdrawal threshold; TNF, tumour necrosis factor; IL, interleukin; PKC, protein kinase C; ERK, extracellular signal regulated kinase; PWL, paw withdrawal latency; EPSC, excitatory postsynaptic currents; NS, nociceptive specific

Source	Experimental model	Result
Animal models of inflammation		
Sun and colleagues[68]	CFA induced arthritis (n=30 divided into control, treated and untreated group) Intraperitoneal gabapentin 50 mg kg^{-1} daily for 8 days starting 7 days after induction, FGF antagonist FD173074 2 mg kg^{-1} i.v. every 2 days	Y Expression of FGF2 and FGF receptor 1 in dorsal root ganglia with gabapentin but [in control [PWT with gabapentin but Y in control
Anfuso and colleagues[69]	Lipopolysaccharide stimulated rabbit corneal cells, endotoxin-induced uveitis in rabbits Gabapentin (10 mg ml^{-1}) or control, inflammation induced in with lipopolysaccharide (1 mg ml^{-1}) for 24 h	Y Expression of inflammatory mediators (TNF-a, IL-1b, phosphorylated protein expression, PGE2 and COX-2 in stimulated rabbit corneal cells), Y clinical signs and biomarkers of inflammation with topical treatment, at 7 and 24 h
Park and colleagues[70]	Glucose-6-phosphate isomerase (n=43) and collagen type II antibody model-induced arthritis models with controls (n=4) Intraperitoneal gabapentin (100 mg kg^{-1}) or ketorolac (15 mg kg^{-1}) using a conditioned place preference paradigm	Collagen type II antibody (n=16) model- gabapentin produced a preference in the early phase and a trend in the late phase, ketorolac ineffective Glucose-6-phosphate isomerase model- both gabapentin and ketorolac produced a preference for the drug-paired compartment in the early phase. Gabapentin, but not ketorolac, resulted in a place preference during late phase

Continued

Table 2 Continued

Source	Experimental model	Result
Zhang and Pain behaviour with gabapentin at 30 colleagues[71] 30 min before intracolonic min; Y pain	Visceral inflammatory pain model (formalin induced colitis), injection ERK inhibitors Behavioural effects (n=9 in six groups), electrophysiological recordings (n=6 in seven groups), PKC translocation and wide dynamic range neurons ERK1/2 phosphorylation (n=6 in six groups for each) after	Y gabapentin 100 mg kge1 min, no difference at 60 behaviour with PKC and at 30 min Y Firing in with gabapentin in 90 min injection, Y at 30 min with inhibitors Y Translocation of PKC and ERK 1/2 phosphorylation with gabapentin, no difference at 120 min
Hummig and grooming induced by capsaicin colleagues[72]	Orofacial capsaicin and formalin tests for pretreatment. (n=8 e10), Inflammatory irritant carrageenan into the upper lip pretreatment. No (n=8e10), constriction of the infraorbital nerve (n=7e8), (first 3 min) of facial cancer model Y second Pregabalin 10 and 30 mg kg^{-1} 1 h before, control group (saline (12e30 min after injection) at + pregabalin vehicle) and vehicle of the group; morphine Heat hyperalgesia 2.5 mg kg^{-1} as positive control in formalin group carrageenan in lip, nerve	Facial abolished by response to first phase the formalin response, but phase both doses. Y induced by injury and facial cancer
Dias and oedema with gabapentin and colleagues[73] saline, indomethacin 10 mg kg^{-1}, pretreatment in	Carrageenan induced paw oedema: six groups (n=5 each); 0.1, 0.5, 1 mg kg^{-1} gabapentin 1 h before carrageenan and dextran induced Paw oedema induced by dextran, serotonin, histamine, leucocyte counts in bradykinin, PGE2, 48/80 (n=5 each for control, levels of intraperitoneal saline, indomethacin, gabapentin 1 mg activity in the kg^{-1}) and TNF-a Carrageenan induced peritonitis- pretreatment with concentrations in the peritoneal gabapentin 1 mg kg^{-1}, indomethacin or saline 1 h before [concentrations of (n=5 each) malondialdehyde	Y Paw control, intraperitoneal indomethacin carrageenan oedema over 4 h, Y peritonitis model, myeloperoxidase plantar tissue, IL-1b exudates, glutathione and Y into the peritoneal fluid
Yang and Activation of spinal microglia, spinal colleagues[74]	CFA induced monoarthritis in rats; sham, control, gabapentin voltage-gated calcium channel a2d-1 Intraperitoneal gabapentin 100 mg kg^{-1} once daily for 4 days CX3CL1 with the first injection 60 min before intra-articular CFA	Y groups (n=6 each) subunits by Day 4, concentrations and

thermal Thermal hyperalgesia and spinal microglia sham, arthritis,
hyperalgesia from
Day 2e6
gabapentin (n=6 each); a2d-1
concentrations (naive n=5, arthritis n=4,
gabapentin n=3); CXCL3 concentrations
(naive n=5, arthritis n=4, gabapentin n=3)

Abdel-Salam and Carrageenan induced paw oedema, intraplantar capsaicin, 12.5
mg kg^{-1} produced analgesia. 100 mg Sleem[75] intraperitoneal acetic
acid, gastric lesions caused by kg^{-1} produced maximal
increase in
indomethacin or ethanol in rats (n=6 in each group, 2e4 hot plate
latency of 68% 1 h after groups and control) administration. [Current
threshold in
Gabapentin (12.5, 25, 50,100, 200 mg kg^{-1}) tail electrical stimulation
with 25, 50 or 100 mg kg^{-1}. Y duration of paw licking
after intraplantar capsaicin.
No antinociceptive action
in a mouse acetic-acid
induced writhing assay. Y
Paw oedema. Y
Indomethacin induced
gastric mucosal lesions
with 12.5e50 mg kg^{-1} but
higher doses increased
gastric acid secretion

Vonsy and Unilateral knee OA using monosodium iodoacetate in rats Y
Mechanical and thermal sensitivity colleagues[76] Twice daily morphine (3
mg kg^{-1} s.c.) or gabapentin (30 mg and ambulatory-evoked
pain after
kg^{-1} s.c.) or vehicle administered for 5 days; von Frey 1, 6, 8 both acute
and chronic morphine
g acetone drop (four groups each), latency to fall, whilst only chronic
gabapentin had an ambulatory-evoked pain score (two groups each);
n=7 for effect
each group

Zhang and CFA induced monoarthritis in rats Dose dependent [in PWL
with both colleagues[77] Intraperitoneal injection of
dexmedetomidine (2.5, 5, 10, and agents. Y Thermal
hyperalgesia for 60
20 mg kg^{-1}) or gabapentin (25, 50, 100, and 200 mg kg^{-1}) min with
dexmedetomidine +
Monoarthritis rats divided into 15 groups in blind
randomised
gabape
ntin fashion: drugs alone or in combination or normal
saline,
behaviour testing 15e150 min after injection (n=6e10 each
group)

Fehrenbacher and CFA induced inflammation in rats. Inflammation induced
Release of immunoreactive peptides colleagues[78]
neuropeptide release with pregabalin, gabapentin (n=7 from
non-inflamed animals was not
each) and untreated (n=14); effect on protein kinase C (PKC) altered by
either drug. Yenhanced
activator induced neuropeptide release (n=3 each for release of
peptides after inflammation pregabalin, gabapentin, untreated, PKC
activator) with both drugs. Y Release
of
10 mM gabapentin or pregabalin on inflammation induced
immunoreactive
neuropeptides in

Continued

Table 2 Continued

Source	Experimental model	Result
	neuropeptide release; pretreatment with 10 or 100 mM tissues pre-treated with PKC pregabalin for assessing activator induced neuropeptide release	spinal effect of PKC activator
Liu and colleagues[79]	recordings from substantia evoked gelatinosa neurons from adult rat spinal cord slices in monosynaptic carrageenan induced inflammation 25%. No reduction in evoked Gabapentin (5e20 mM for 5 min) or control, effect on monosynaptic EPSCs monosynaptic and polysynaptic EPSC in normal (n=6 and 3) normal rats. Gabapentin failed to and inflammation (n=10 and 5); effect on N-methyl-D- aspartate induced aspartate induced currents	Whole-cell voltage-clamp Y Dorsal root Ad fibre polysynaptic, but not EPSC by polysynaptic or in block N-methyl-d- slow excitatory currents.
Hurley and colleagues[80]	Carrageenan induced inflammation in rats (n=up to 17) naproxen and pregabalin (3.0e300.0 mg kg^{-1}), naproxen (0.1e30.0 mg kg^{-1}), pregabalin (3.0e30.0 mg kg^{-1}) or a mixture of additively to reverse gabapentin and naproxen (0.0001e300.0 mg kg^{-1} total dose) associated with or pregabalin and naproxen (0.1e30.0 mg kg^{-1} total dose); 17 groups for drugs alone (8e17 each group); 12 groups gabapentin + naproxen (8e16 each group); nine groups pregabalin + naproxen (7e17 each group)	Gabapentin + Vehicle, gabapentin + naproxen can interact synergistically or thermal hyperalgesia peripheral inflammation.
Patel and colleagues[81]	CFA induced inflammation in rats, gabapentin s.c. 30, 100, 250 mg kg^{-1} or vehicle (n=6 in each group)	[PWT, 40% reversal of hyperalgesia with 250 mg kg^{-1}
Lu and colleagues[82]	induced arthritis in rats response to Intrathecal gabapentin or control (n=6 each) 1.5 h before posture not induction in pretreatment; three groups for post- secondary hyperalgesia to treatment: control, gabapentin in cord, gabapentin s.c. (n=6 effect on knee in each group)	Carrageenan and kaolin Pretreatment: at 4 h PWL radiant heat and the changed, Y radiant heat, no circumference Post-treatment: at 1.5 h PWL back to baseline with spinal gabapentin, improved secondary hyperalgesia and pain behaviour, no effect on knee circumference; s.c. gabapentin did not affect PWL and pain behaviour
Stanfa and colleagues[83]	Carrageenan induced inflammation in rats response of recordings, effect of gabapentin (10, [post- 30 and 100 mg kg^{-1} s.c.) in normal (n=5e6) and after [Ad fibre response with 100 carrageenan (n=5) mg opposite effects	Gabapentin [C-fibre evoked Single unit extracellular the dorsal horn neurones, discharge, kg^{-1}, none to Ab;

after carrageenan
Camara and Sciatic nerve constriction, carrageenan induced paw oedema Y Heat-
induced hyperalgesia on the 5th colleagues[84] in rats day
with 60 and 120 mg kg$^{—1}$. [Nerve Spontaneous behaviour (n=at least 10), effect on TNF-a,
and MPO, TNF-a, and IL-1b concentrations
IL-1b and IL-10 (n=at least 5 in each group) with 60 mg kg$^{—1}$, Y anti-
inflammatory Oral gabapentin 30, 60, and 120 mg kg$^{—1}$, 60 min before
cytokine IL-10 nerve
concentrations
chronic constriction of the sciatic nerve (CCSN) and for 5 with 120 mg kg$^{—1}$
days postinjury, saline as control [Carrageenan-induced paw oedema

and peritoneal
macrophage migration
with 60 and 120 mg kg$^{—1}$
You and Intact and spinalised rats: repetitive electrical stimulation ofY C-fibre
mediated spinal NS neurons'
colleagues[85] single DRG late responses but effects
on

97 NS neurons in the deep dorsal horn area of the spinal cord
nociception not observed
until 30 min from 79 intact and 18 spinalised rats after administration.
No inhibitory
Pregabalin 20, 40, 80 mg kg$^{—1}$ i.v., untreated control, saline- effect on A-d
fibre mediated early
early and late response, after-discharges and windup; 80 responses.
Inhibitory effects absent in mg/kg comparing intact and spinalised
animals spinalised animals,
suggesting mainly
s.c. bee venom induced inflammation- mechanical/heat central
effects involving supraspinal stimulation after pregabalin 80 mg/kg:
(untreated n=8, centres via descending
inhibitory saline n=8, pre-treatment n=9, post-treatment n=9)
controls. Markedly Y s.c.
bee venom

elicited spontaneous
neuronal responses with
pre-treatment but not post-
treatment with pregabalin
(80 mg kg$^{—1}$), and noxious
mechanical/heat stimuli
evoked hyperactivities of
spinal NS neurons,
suggesting role for pre-
emptive analgesia
Curros-Criado and Carrageenan induced monoarthritis (n=6), sciatic nerve Gabapentin
effective in arthritic and Herrero[86] ligation mononeuropathy
(n=9), normal (n=6) neuropathic rats but not
normal rats;
Gabapentin 7e224 mg kg$^{—1}$, effect on single motor unit windup was
dose dependently
response to noxious mechanical stimulation reduced in neuropathic but
not normal
and arthritic rats

Continued

Table 2 Continued

Source	Experimental model	Result
Matson and colleagues[87]	Bilateral inflammation of the knee joints by CFA in rats Multiple doses of ibuprofen, rofecoxib, celecoxib, piroxicam, and dexamethasone and gabapentin 300 mg kg^{-1}, and amitriptyline 30 mg kg^{-1} (five groups including control n=8 per group) (3 h pretreatment)	Reduction in activity was dose-dependently reversed by ibuprofen, rofecoxib, celecoxib, piroxicam, and dexamethasone, whereas gabapentin and amitriptyline were ineffective
Animal models of postoperative pain		
Papathanasiou and colleagues[90]	Rat plantar incision Morphine (1, 3 and 7 mg kg^{-1}), gabapentin (10, 30 and 100 mg kg^{-1}) or their combination (nine combinations in total)	Dose dependent synergistic effects with morphine + gabapentin
Narai and colleagues[91]	Rat plantar incision Intrathecal gabapentin (4, 40, 400 mg), diclofenac or saline (n=6 in each group); 30 min before incision	Only 400 mg gabapentin [PWT from 2 h up to 7 days. Gabapentin + diclofenac more effective than individual drugs two combinations with
McKeon and colleagues[92]	Rat plantar incision Intraperitoneal tramadol 10 mg kg^{-1} + s.c. gabapentin 80 mg kg^{-1}, and saline, 30 min before incision (n=6 per group); tramadol repeated every 12 h for 60 h and gabapentin every 24 h for 48 h; tests after 1 day 2 and 4.	Saline or tramadol group: hyperalgesia on Days 1e4 and 1e3 after surgery, respectively. Tramadol + gabapentin- reduced thermal hyperalgesia on Days 2 and 4. Only the combination did not show reduction in weight bearing
Flatters[93]	Skin/muscle incision and retraction in rats Intraperitoneal gabapentin 100 mg kg^{-1} or saline (n=9 each), testing after 9e13 days	100 mg kg^{-1} almost completely reversed mechanical hypersensitivity after 1 h
Hayashida and colleagues[94]	Rat plantar incision Oral (vehicle, 30, 100, 300 mg kg^{-1}) or intracerebroventricular gabapentin (vehicle, 10, 30, 100 mg; n=6 or 7 in each group), tests after 24 h; cerebrospinal fluid norepinephrine after preoperative gabapentin 1200 mg	[PWT for 1e4 h with oral and 15e60 min with intraventricular, effects reversed with a2-adrenergic receptor antagonist idazoxan and G protein-coupled inwardly rectifying potassium channel antagonist tertiapin-Q, [norepinephrine concentration in cerebrospinal fluid
Whiteside and colleagues[95]	Rat plantar incision Intraperitoneal gabapentin (10, 30, 300 mg kg^{-1}) compared	[Pressure PWT, maximum reversal of mechanical

hyperalgesia 64% with100
with different doses of morphine, celecoxib, indomethacin, mg
kg^{-1}, no effect at 5 h; maximum etoricoxib, naproxen (n=8e10
each group) reversal of tactile
allodynia by 19% as

measured by von
Frey withdrawal
threshold, no
effect at 1 and 5 h

Cheng and Rat plantar incision [PWT in dose
dependent fashion colleagues[96] Intrathecal
gabapentin 10, 30, and 100 mg, control (n=6e12 in
each group), 2 h after incision

Field and Rat plantar incision 330 mg kg^{-1}
[thermal PWL with 30 mg colleagues[97] Gabapentin 3e30 mg kg^{-1} s.c., 1 h
before incision, control kg^{-1} effective for 24
h; [PWT with 10 (n=8e12 in each group) and 30 mg kg^{-1} lasting for 25 and 49
h

Buvanendran andKnee surgery model in rats Pregabalin 15 mg did
not improve
colleagues[98] Knee surgery/drug, knee surgery/vehicle, sham skin incision/
spontaneous
activity postsurgery vehicle (n=8 in each group), evaluated
after 24 h

transient receptor potential channel subfamily V member 1 (TRPV1). The heat-capsaicin model combines heat exposure with capsaicin to potentiate the effects, as TRPV1 receptors are also activated by heat. Gabapentin reduced secondary hyper- algesia and had modulatory effect on functional magnetic resonance imaging brain responses to nociceptive inputs in this model.[102,106] The electrical hyperalgesia model is consid- ered representative of central sensitisation as repetitive elec- trical stimulation of skin and muscles can induce temporal summation. Contrasting effects were seen on the threshold of pain summation to this repetitive stimulation.[99,107] Area of allodynia was reduced.[108,109] Contrasting effects were also seen after sural nerve stimulation.[108,110] The continuous electrical stimulation model induces both central sensitisation and continuous C-fibre mediated pain. Gabapentin increased the current strength required to induce pain and reduced area of secondary hyperalgesia but did not influence pain detection threshold in area of hyperalgesia.[111] Gabapentinoids had

modest effects on the pain tolerance threshold to single elec- trical stimulus.[99,111] Chemical stimulation of muscle by i.m. injection of hypertonic saline mimics musculoskeletal pain resulting in deep and diffuse pain as a result of activation of C- fibres.[117] Contrasting effects were seen with gabapentin.[107,111] Gabapentin reduced pain intensity in the cold pressor test model but only in combination with morphine unlike pre- gabalin that was effective alone.[99,112] Conditioned pain mod- ulation, which is a measure of endogenous pain inhibitory pathways, was not influenced.[99,113] Pregabalin enhanced both opioid analgesia and respiratory

Discussion

Gabapentinoids certainly act on a2d receptors and attenuate the enhanced dorsal horn excitability. It is, however, evident that various effects not directly related to neurotransmitter release at pre-synaptic terminals also contribute to analgesia.

Table 3 Continued

Source	Experimental model	Result
Chizh and colleagues[109]	Double-blind, two-period, placebo-controlled study using incomplete block design (n=32) Electrical hyperalgesia; pregabalin (titrated to 300 mg) or aprepitant (titrated to 320 mg), or placebo over 6 days, sensitisation was assessed over 3 h; at 2 h, either parecoxib (40 mg) or saline i.v.	Y Areas of punctate mechanical hyperalgesia and dynamic touch allodynia with pregabalin, no response to aprepitant Y Area of allodynia with pregabalin + aprepitant but no effect on area of hyperalgesia
Enggard and colleagues[110]	Double-blind, randomised, placebo-controlled cross-over study (n=18) Pain summation model; gabapentin 600 mg three times per day over 24 h against placebo, tests electrical sural nerve stimulation and cold pressor test before and 24 h after administration	[Threshold of pain summation to repetitive stimulation and PTT to single electrical sural nerve stimulation; no effect on PDT to single electrical sural nerve stimulation
Segerdahl[111]	Double-blind, placebo controlled, three-session cross-over study (n=16) Hypertonic saline induced muscle pain, electrical stimulation; gabapentin 0, 1200, 1800 and 2600 mg (pre-treatment, titrated over four doses) or placebo; VAS, area of local and referred pain, PDT to pinprick in area of hyperalgesia	Y Sensitivity to electrical induction of skin pain by 14% by pre-treatment with 1800 mg, Y area of secondary hyperalgesia but mechanical pain thresholds were unaffected. No effect on ongoing pain. Pain induced by i.m. infusion of hypertonic saline was not affected by gabapentin Dizziness and fatigue more common with increasing doses
Eckhardt and colleagues[112]	Double-blind, randomised, placebo-controlled four-way cross-over design (n=12) Cold pressor test; oral morphine 60 mg slow release or gabapentin 600 mg	Area under curve of pain tolerance from 0e6 h for gabapentin similar to placebo. However, gabapentin enhanced the analgesic effects of morphine
Olesen and colleagues[113]	Placebo-controlled study (n=64) Painful chronic pancreatitis; pain thresholds to pressure and electric tetanic stimulation and CPM after pregabalin 150e300 mg twice daily for 3 weeks	Electrical pain detection ratio but not CPM was predictive for pregabalin effect
Myhre and colleagues[114]	Double-blind, randomised, placebo- controlled, cross-over study (n=12) Cold pressor test; Pregabalin 150 mg/placebo twice orally, Remifentanil/placebo at effect-site target-controlled infusion: 0.6, 1.2, and 2.4 ng/ml; VAS score, spirometry, colour eword interference and rapid information processing	Pregabalin + remifentanil had additive analgesic effects but adversely affected cognition, pregabalin potentiated remifentanil induced respiratory depression

The absence of effects in spinalised animals supports the role of supraspinal mechanisms.[85] Elevated concentration of CSF norepinephrine after preoperative gabapentin indicates that some effects are mediated by descending noradrenergic inhi- bition.[94] However, there was no effect on conditioned pain modulation that is a measure of inhibitory influences.[99,113] The interaction of gabapentinoids with serotonin pathways is complex with permissive conditions for their effects dependent on the up-regulation of serotonergic facilitatory systems.[47,48] The role of serotonin in pain has not been fully elucidated with both facilitatory and inhibitory influences on nociception depending on the presence of injury.[118] This may explain the state dependent actions of gabapentinoids that have effects only in the presence of injury.[86,101] The influence on affective component of pain is independent of analgesic effects.[53] Pregabalin has well known anxiolytic effects that may have positive effects on postoperative rehabilita- tion.[119,120] The discrepancy between the acute effects *in vivo* as opposed to the prolonged duration of exposure required *in vitro* can be explained conceptually on the greater sensitivity of elevated levels of a2d1 in nerve injury models to gaba- pentinoid action, rapid uptake by *in vivo* models, anti- inflammatory actions and by modulation of descending in- fluences and the affective component of pain.

There are no studies looking at the role of a2d in inflam- mation and incisional pain models. Elevated levels of a2d-1 may be specific to neuropathic pain. Gabapentinoids seem to be effective antinociceptives in most animal models of in- flammatory pain but prolonged administration may be required to achieve improved ambulation.[76] This explains the lack of improvement in activity in a model of complete Freund's adjuvant-induced arthritis.[87] Gabapentin was effec- tive in all animal models of postoperative pain with synergistic effects with opioids. However, the difference in neurobiology of nociceptive systems between species limits the extrapola- tion of findings from animal studies to humans.[121,122] The few studies that have explored the effects in human models of inflammatory pain do not show convincing evidence of ben- efit.[99e101] It is difficult to explain the contrasting effects on pain induced by chemical stimulation of muscles and repeti- tive electrical stimulation. The lack of effect on secondary hyperalgesia induced by continuous electrical stimulation may be a result of the higher strength of current required in the premedicated group.[111] The lack of effect of gabapentin as compared with pregabalin in the cold pressor model may be a result of differences in pain assessment methods.[114]

There are no studies looking at effects in human models of postoperative pain. However, the effects on animal models suggest that gabapentinoids could contribute to multimodal analgesia. Several meta-analyses have shown a modest reduction in the use of opioids after surgery.[123e127] However, the quality of the trials is moderate to very low.[4] There is con- flicting evidence with regards to the timing (preoperative or postoperative) and the optimal dose.[3] Although peak CSF con- centrations are achieved at a median time of 8 h, the relevance is uncertain, as pregabalin in clinical doses does not influence spinal neurotransmitter concentrations.[29] The optimal dose has not been defined as the few studies that have attempted to address this question, are limited by their sample size. Higher preoperative doses and continued use in the postoperative period may provide better analgesia.[3,128] This makes intuitive sense as a_2d-1 concentrations are raised for several days after injury.[20] Only chronic use was associated with improved ambulation in a knee inflammation model.[76] This suggests that continued use is necessary for any potential benefit.

This approach might, however, increase the risk of adverse effects such as dizziness and increased sedation.[4] Gabapenti- noids have synergistic analgesic effects with opioids but also enhance opioid-induced respiratory depression.[114] Significant number of healthy subjects in experimental studies experienced adverse effects, particularly with increasing dosage.[99,103,104,108,109] Gabapentinoids are often utilised in enhanced recovery pathways, particularly for hip and knee replacement surgery. These procedures are often performed in the elderly, who may be more vulnerable to the potential side effects ofgabapentinoids.[129] Itthereforemakes intuitive sense to tailor the use of gabapentinoids to the clinical situation. Peri-operative use is appropriate in patients having ‘pro-nociceptive surgery’, such as spine surgery that may be associated with nerve damage.[130] Patients on high dose opioids who are tolerant to their effects could benefit from even the modest effects of gabapentinoids. They might be helpful in situations where even marginal additional improvements in analgesia could potentially influence outcome (e.g. multiple rib fractures resulting in impaired ventilation as a result of poor pain control).[131]

The evidence for the role of gabapentinoids in prevention of chronic pain is limited. They have modest analgesic effects in chronic neuropathic pain with numbers needed to treat of 7.7 (6.5e9.4) for pregabalin and 7.2 (5.9e9.2) for gabapentin.[132] A Cochrane review suggested that gabapentinoids do not have a preventative role.[83] Recent systematic reviews have differing conclusions with regards to effectiveness in preventing persistent pain.[126,133] The lack of evidence may be because of inadequate sample size and poor trial designs.[134] It is not surprising, however, that a clear benefit in terms of prevention of chronic pain has not been found as, although a2d-1 is required for rapid development of hypersensitivity, its absence does not prevent sensitisation.[17] This suggests that there are other mechanisms involved that contribute to the development of hypersensitivity. It is possible that these subunits are no longer upregulated in chronic pain, resulting in lack of efficacy.[135] The poor efficacy in chronic pain might be a result of differential upregulation of splice variants with reduced affinity to gabapentin.[136] Downregulation of gluta-
mate transporter-1 can reduce long-term efficacy, but addition of valproate can restore efficacy.[51]

Translational studies studying the effects of analgesics in human models of postoperative pain are required to bridge the gap between human clinical studies and animal models. A human surrogate model of postoperative pain has been described.[137] There is no information regarding persistence of a2d upregulation in both animal and human studies. Future studies could focus on persistence of a2d and any possible correlation between the efficacy of gabapentinoids and concentrations of a2d and the presence of variants. This could allow targeted treatments with gabapentinoids for neuropathic pain and reduce the risk of exposing patients who might otherwise not benefit, to adverse effects. The
potential of valproate to restore the efficacy of gabapenti- noids needs to be explored. Well designed, large-scale trials are required to evaluate the effects on development of persistent pain.

Conclusion

Blockade of calcium channels is not the only mechanism by which gabapentinoids exert their effects. Their actual process is more accurately described as the reduction of presynaptic excitatory input to dorsal horn neurons via interactions with the increased a2d-1 subunits that occur after damage. In addition to promoting glutamate absorption by EAATs, they impede thrombospondin-mediated activities, recycling of a2d-1 from endosomal compartments, and forward trafficking from the dorsal root ganglia. Inhibition of descending serotonergic facilitation, stimulation of descending inhibition, anti-inflammatory activities, and impact on the emotional component of pain are mechanisms that are not directly connected to neurotransmitter release at dorsal horn. Effects in human models of inflammation and surgical pain are inconsistent, whereas gabapentinoids are efficient analgesics in most animal models. The potential for negative consequences is heightened when the dose is raised. Preemptively initiating and maintaining gabapentinoids throughout the perioperative period is recommended when the risk-benefit analysis supports their use.

References

One study that looked at the evidence for pharmaceutical therapy of neuropathic pain was conducted by Finnerup NB, Sindrup SH, and Jensen TS. Discomfort, 2010; 150: 573–581

Second, Uchitel OD, Di Guilmi MN, Urbano FJ, and Gonzalez- Inchauspe C. Acute regulation of synaptic transmission and calcium currents by gabapentinoids. Journal of Channels, 2010; 4: 490e6

Thirdly, perioperative gabapentinoids: agent selection, dosage, timing, and impact on persistent postoperative pain (Schmidt PC, Ruchelli G, Mackey SC, Carroll IR). Journal of Anesthesiology 2013; 119: 1215–1221.

The authors of the manuscript are Fabritius ML, Strøm C, Koyuncu S, and others. Pregabalin for the treatment of acute pain: a comprehensive review including meta-analyses and trial sequence analyses of its benefits and harms. Anesthesiology in Britain 2017; 119: 775–791.
Dubin AE, Patapoutian A. Nociceptors: pain pathway sensors. The citation is from the Journal of Clinical Investigation in 2010 (volume 120, pages 3760-82).
Glutamate receptors and the sensation of pain. 6. Bleakman D, Alt A, Nisenbaum ES. In 2006, the article was published in Semin Cell Dev Biol and ran from pages 592 to 604.
Zhuo M. is number seven. Transmission and regulation of pain via glutamate-kainate receptors. As editor, Zhuo M. Chemical agony. Published by Springer in 2007. From pages 97 to 106
What constitutes the transition from acute to chronic pain? 8. Voscopoulos C, Lema M. The reference is from the British Journal of Anaesthesia, 2010; 105: i69–e85.
Section 9. Simmons BA, Zamponi GW. Structure, function, and dysfunction of neuronal voltage-gated calcium channels. Research in Neurology, Volume 82, Issues 24–45, 2014.
Ten. Dean L. Calcium channels that are gated by voltage and discomfort. Publication date: 2010; volume: 14, pages 42–47.
Eleventh, Dolphin AC. A brief history of voltage-gated calcium channels. Published in the British Journal of Pharmacology in 2006, volume 147, pages S56–e62.

Twelve, Dolphin AC. The voltage-gated calcium channel a2d subunits. 2013; 1828: 1541e9 Biochim BiophysActa Biomembr.
Chaucer CS, Nieto-Rostro M, Rahman W, et al., 13. Presgabalin, an a2d ligand, inhibits the enhanced trafficking of the calcium channel subunit a2d-1 to pre-synapsic terminals in neuropathic pain. Research in Neuroscience 2009; 29: 4076–88
Li CY, Song YH, Higuera ES, and Luo ZD. Upregulation of the spinal dorsal horn calcium channel a2d-1 subunit is associated with tactile allodynia caused by peripheral nerve damage. The Journal of Neuroscience, 2004; 24: 8494e9
15.Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME. Upregulation of dorsal root ganglion a2d calcium channel subunit and its cor- relation with allodynia in spinal nerve-injured rats. Journal of Neuroscience 2001; 21: 1868–1875
After a partial sciatic nerve injury, the expression of the calcium channel a2d-1 subunit is enhanced in dorsal root ganglion neurons, according to a study by Newton RA, Bingham S, Case PC, Sanger GJ, and Lawson SN. Experimental Neurology 2001; 95: 1e8
The a2d-1 gene was studied by Patel, Bauer, Nieto-Rostro, and colleagues (17). Additionally,
The function of somatosensory neurons is impacted by deletion, which also delays the onset of mechanical hypersensitivity in response to injury to peripheral nerves. Journal of Neuroscience 2013; 33: 16412–26
18—Li CY, Zhang XL, Matthews EA, and others. Spinal hyperexcitability facilitates pain modulation via the a2d1 subunit of calcium channels. Analgesia 2006; 125: 20–34
Nguyen, Deng, Matthews, and colleagues (1986). The spinal sensitization and behavioral hypersensitivity mediated by the calcium channel a2d-1 protein are contributed to by enhanced presynaptic glutamate release in the deep dorsal horn. Chemical Pain, 2009, 5, 6.
20.Luo ZD, Calcutt NA, Higuera ES, et al. The antiallodynic effects of gabapentin are correlated with the up-regulation of injury type specific calcium channel a2d-1 subunit in rat neuropathic pain models. The Journal of Pharmaceutical Research, 2002, vol. 303, pages 1199–205.
Transport of gabapentin, a g-amino acid medication, via system l a-amino acid transporters: a comparative investigation in astrocytes, synaptosomes, and CHO cells (Su TZ, Lunney E, Campbell G, Oxender DL, 2021). The Journal of Neurochemistry, 1995, 64, 2125–231.
Calcium channel alpha(2)delta subunits: structure and gabapentin binding. Mol Pharmacol 2001; 59: 1243e8. 22. Marais E, Klugbauer N, Hofmann F.
23.Hendrich J, Van Minh AT, Heblich F, et al. The pharmaco-logical inhibitor gabapentin blocks the trafficking of calcium channels. The publication is titled "Proc Natl Acad Sci U S A 2008; 105: 3628e33."
24-Hara K, Sata T. Gabapentin inhibits the activity of xenopus oocyte-expressed N-methyl-d-aspartate receptors. The authors published their findings in the 2007 edition of Acta Anaesthesiol Scand (51: 122e8).
Injured type-A dorsal root ganglion neurons have their persistent sodium current selectively reduced by gabapentin (25. Yang RH, Wang WT, Chen JY, Xie RG, Hu SJ). Discomfort 2009; 143: 48–55
Cheng JK, Chen CC, Yang JR, and Chiou LC. Who is intrathecal gabapentin's antiallodynic action target: Ca2+ channels, KATP channels, or N-methyl-d-aspartic acid receptors?
Anesthaea and Analgesia 2006; 102: 182–187.1
A study comparing the pharmacokinetics and pharmacodynamics of gabapentin with pregabalin was conducted by Blockbrader, Wesche, Miller, Chapel, Janiczek, and Burger in 27. The clinical pharmacology journal published the article in 2010 with the DOI: 49: 661-10.
Can 300 mg of pregabalin approach acute

levels of antihyperalgesics in the brain and spinal cord?
Submitted to the Journal of the Royal Society for the Study of Pain in 2010; volume 35, pages 535–538.
29.Buvanendran A, Kroin JS, Della Valle CJ, Moric M, Tuman KJ. Changes in cerebrospinal fluid neurotransmitters over

the perioperative phase in patients having total knee replacement: a randomized experiment]. Anesthesiology and Analgesia 2012; 114: 434–441.
Biggs JE, Ganesan N, Boakye PA, and colleagues on page 30. Dorsal root ganglia and substantia gelatinosa long-term effects of gabapentin and pregabalin. The Journal of Neurophysiology, 2014, 112, 2398–412,
Myths, paradoxes, and acute effects of the anti-allodynic gabapentinoids (Alles SR, Smith PA, 31). Psychological Research 2016; 23: 40–55
32.Bauer CS, Rahman W, Tran-Van-Minh A, Lujan R, Dickenson AH, Dolphin AC. Pregabalin, an anti-allodynic a2d ligand, blocks the a2d-1 subunit of the calcium channel from reaching presynaptic terminals in living organisms. "Biochem Soc Trans" from 2010 with the reference number 38, pages 525–528.
Inhibition of Rab11-dependent recycling of the calcium channel subunit a2d-2 is achieved by the a2d ligand gabapentin, according to research by Tran-Van-Minh A and Dolphin AC (33). Neuroscience 2010; 30: 12856–1888
Takahashi, Kubo, Waguri, et al., 34 is the reference. Rab11 controls recycling vesicle exocytosis at the plasma membrane. Journal of Cell Science 2012; 125: 4049–e57
The 35th study by Yang et al. found that pregabalin, a common analgesic, did not alleviate the chronic pain that rats experienced after peripheral nerve damage. Analgesia 2014; 155: 356–66
Thirdly, alternative Ca2+ splicing (Mich PM, Horne WA)
The selectivity for gabapentin inhibition of Cav2.1 trafficking is conferred by the channel beta4 subunit. Chemistry and Molecular Biology, 2008, 74, 904–e12.
“Hoppa MB, Lana B, Margas W, Dolphin AC, Ryan TA” is the 37th author. a2d
Expression determines the number and release probability of presynaptic calcium channels. The published article is "Nature 2012; 486: 122e5".
Number 38: Matsuzawa, Fujiwara, Nemoto, et al. Additional characterisation of presynaptic mechanisms: the inhibitory effects of pregabalin on excitatory transmission in the superficial dorsal horn of the spinal cord of mice. Neuroscience Letters 2014, 558, 186–189.
Enhanced calcium channel alpha-2-delta-1 subunit protein sensitivity of spinal neurons: an electrophysiological study (Zhou C, Luo ZD, 39). The European Journal of Pain, 2014, 18, 649–658.e
In vitro development of hippocampal presynaptic plasticity is mediated by astrocyte-derived thrombospondins (Crawford DC, Jiang X, Taylor A, Mennerick S. 40). Journal of Neuroscience 2012; 32: 13100-15
Erolu G, Allen NJ, Susman MW, and others. The excitatory CNS synaptogenesis is regulated by the gabapentin receptor a2d-1, a neuronal thrombospondin receptor. Citation: Cell 2009; 139: 380–882.
42.The authors of the study are Park J, Yu YP, and Zhou C-Y. Central pathways mediating thrombospondin-4-induced pain states. Science 2016; 291: 13335–e48
No. 43: Pan, Guo, Wu, et al. In sensory neurons after nerve damage, thrombospondin-4 subtly controls voltage-gated Ca2+ channel subtypes. Analgesia 2016; 157:
Twenty-68 est.
According to a study by Suzuki, Rahman, Hunt, and Dickenson (44), deep dorsal horn neurons show improved descending facilitatory regulation of mechanically evoked responses after peripheral nerve damage. Neuroscience 2004; 1019: 68–76

Using a rat model of cancer-induced bone pain, the authors Donovan-Rodriguez, Urch, and Dickenson found evidence for descending serotonergic facilitation. Science Letters in 2006, 393: 237–242.
Asante CO, Dickenson AH. Rats' spinal rapamycin-sensitive pathways are engaged by ascending serotonergic facilitation via spinal 5-HT3 receptors. 46. Brain Research Letters 2010; 484: 108–12
In a rat model of osteoarthritis pain, Rahman W, Bauer CS, Bannister K, Vonsy JL, Dolphin AC, and Dickenson AH investigated the antinociceptive effects of pregabalin and the descending serotonergic facilitation. The Journal of Manual Pain, 2009, 5, 1–17
The spinal-supraspinal serotonergic pathways that control neuropathic pain and its alleviation with gabapentin (48. Suzuki R, Rahman W, Rygh LJ, Webber M, Hunt SP, Dickenson AH). Analgesia 2005; 117: 292–303
The descending noradrenergic system is activated to treat neuropathic pain in the mouse by pregabalin, S- (+)-3-isobutylgaba, according to 49. Takeuchi Y, Takasu K, Ono H, Tanabe M.
A model for partial sciatic nerve ligation. Clinical pharmacology
47, no. 53, 842–853.
According to research conducted by Suto, Severino, Eisenach, and Hayashida (2020), gabapentin raises the amount of glutamate outside of cells in the locus coeruleus via processes that are reliant on astroglial glutamate transporters. "Neurophysiology" (2014, 81: 95–100).
The involvement of glutamate transporter-1 in the locus coeruleus: how gabapentin loses its effectiveness following nerve damage in rats over time (51). Physical pain, 2016; 157: 2024–2032
In rats suffering from neuropathic pain, gabapentin suppresses glucose metabolism in the medial prefrontal cortex and reverses central hypersensitivity (52.Lin HC, Huang YH, Chao THH, Lin WY, Sun WZ, Yen CT). Metabolic Pain, 2014, 10, 63.
Bannister K, Qu C, Navratilova E, and colleagues were 53. Gamapentin alleviates persistent experimental neuropathic pain

via a variety of mechanisms. The published article is Pain 2017; 158: 2386–95.
Centelles, J.J. 55. The brain's glutamate transporters are involved in the regulation of excitatory and excitotoxic glutamate. Scientific Reports 2016; 2
Glutamate transporter malfunction linked to nerve injury-induced discomfort in mice (55). Authors: Napier IA, Mohammadi SA, Christie MJ. Publication date: 2012, volume 107, pages 649–657 of the Journal of Neurophysiology.
The location of sodium-dependent excitatory amino acid transporters in the rat spinal cord was studied by Queen SA, Kesslak JP, and Bridges RJ in 55. Back pain journal, 2007; 30: 263–271.
Holmseth S, Huang YH, Dehnes Y, and colleagues (57). The density of glutamate transporters produced by neurons in the central nervous system of mammals, namely EAAC1 (EAAT3). Proceedings of the National Academy of Sciences, 2012; 32: 6000–e13.
Glutamate transporter type 3 activity as affected by pre-gabalin (58. Ryu JH, Lee PB, Kim JH, Do SH, Kim CS). The British Journal of Anaesthesia 2012; 109: 234–239
According to a study conducted by Gil YS, Kim JH, Kim CH, Han JI, Zuo Z, and Baik HJ, gabapentin blocks the action of the rat excitatory glutamate transporter 3 that is expressed in Xenopus oocytes. Journal of European Pharmacology 2015; 762: 112e7.
60.Baba H, Petrenko AB, Fujiwara N. An intracellular calcium-imaging research in adult rat spinal cord slices showed that the excitation of dorsal horn neurons was unaffected by clinically relevant concentrations of pregabalin, whether the pain was normal or neuropathic. Research in the field of brain science, 2016; 1648: 445 E58
61.Alles SRA, Bandet MV, Eppler K, et al. How gabapentin works quickly to alleviate pain in the dorsal horn and main

correlating behavioral and physiological data in the somatosensory cortex. The article was published in the journal Neuropharmacology in 2017 and can be found on pages 576 to 590.
Leung L. and Cahill CM. TNF-a and neuropathic pain: a review
look at. Scientific Reports on Neuroinflammation, 2010; 7: 27
A connection between neuroinflammation and excitotoxicity: tumor necrosis factor alpha (63.Olmos G, Llado' J.). Mass media 2014; 2014. no. 861231
Kremer, Yalcin, Nexon, and colleagues (64). In neuropathic pain, pregabalin has an antiallodynic effect that is not reliant on the opioid system. Report from Mol Pain in 2016; 12. The number 1744806916633477
Bao YH, Zhou QH, Chen R, and colleagues—65. In rats, gabapentin activates the interleukin-10-heme oxygenase-1 signaling pathway, amplifying the anti-nociceptive effects of morphine in neuropathic pain. Journal of Molecular Neuroscience 2014; 54: 137–146
Lee BS, Jun IG, Kim SH, and Park JY. 66. In a rat model of neuropathic pain, intrathecal gabapentin enhances interleukin-10 expression and suppresses pro-inflammatory cytokine. Article cited in the Korean Journal of Medical Sciences, 2013; 28: 308–14.
The pathophysiology of inflammatory pain and neuropathic pain: a quick comparison (Xu Q, Yaksh TL, 2017). Current Opinion in Anesthesiology 2011; 24: 400–700
Research by Sun, Yang, Wang, et al. In a rat model of arthritis, gabapentin controls the production of FGF2 and FGFR1 in the dorsal root ganglia by means of microRNA-15a. Journal of Orthopaedic Science 2017; 22: 1112e9
A group of researchers included Anfuso, Olivieri, Fidilio, and others (69). Studies in vitro and in vivo show that gabapentin attenuates ocular inflammation. The article "Front Pharmacol 2017; 8: 173" was published in 2017. T
Researchers Park, Sandor, McQueen, and colleagues did 70. How gabapentin and ketorolac influence inflammation caused by antibodies in terms of allodynia and conditioned site preference. Journal of Pain 2016; 20: 917–925
Chapter 71: Zhang Y, Guo Z, Li M, et al. Modulation of protein kinase C-ERK1/2 signaling in the spinal cord of rats subjected to visceral inflammatory pain caused by formalin: the role of gabapentin. 2010; published in PLoS One. No. 1,141,142
In rat models of orofacial pain, 72. Hummig W, Kopruszinski C, Chichorro J. found that pregabalin increased both the acute inflammatory pain and the chronic pain linked to nerve damage and cancer. The article is titled "Journal of Oral and Facial Pain and Headache 2014; 24: 320e9."
Dias JM, Brito TV, Aguiar Magalha~es D, and others.
The synthetic version of gamma amino butyric acid, gabapentin, alleviates oxidative stress and systemic acute inflammation in rats. Injury 2014; 37: 1826–36
J-L Yang, B. Xu, S.-S. Li, and colleagues... In rats with monoarthritis, gabapentin inhibits the activation of spinal microglia and decreases CX3CL1 signaling. Molecular Brain, 2012, 5, 18.
The analgesic, anti-inflammatory, and gastrointestinal effects of gabapentin were studied by Abdel-Salam OM and Sleem AA. Research on Drugs, 2009, 3, 18–26
In a rat model of osteoarthritis pain, 76. Vonsy JL, Ghandehari J, Dickenson AH. Differential analgesic effects of gabapentin and morphine on behavioral assessments of pain and disability. Western Journal of Pain 2009; 13: 786–893.
The antihyperalgesic impact of systemic dexmedetomidine and gabapentin in a rat model of monoarthritis was studied by Zhang WS, Xu H, Xu B, Sun S, Deng XM, and Zhang YQ. Scientific Reports, 2009, 1264: 57–76
Research conducted by Fehrenbacher JC, Taylor CP, and Vasko MR found that the release of substance P and CGRP from rat spinal tissues can only be reduced by pregabalin and gabapentin after inflammation or protein kinase C activation. Article published in Pain in 2003, volume 105, pages 133–141.

The impact of gabapentin on excitatory postsynaptic currents elicited by A delta fiber in rat spinal cord substantia gelatinosa neurons is shown by inflammation (79. Liu Z, Xu R, Yang K.). Journal of Chinese Medicine (English Edition) 2003; 116: 883–883.
The anti-hyperalgesic effects of gabapentin and pregabalin may be amplified when taken with naproxen, according to research 80 by Hurley RW, Chatterjea D, Rose Feng M, Taylor CP, and Hammond DL. The article is published in the journal Anesthesiology in 2002 and spans pages 1263–1273.
Paper 81 by Patel S, Naeem S, Kesingland A, and others. Mechanical hyperalgesia in rat models of inflammatory and neuropathic pain: effects of gabapentin and GABAB agonists. "Pain" (2001, 90: 217–266)
Lu Y, Westlund KN. Gabapentin reduces pain-related behaviors in a rat model of acute arthritis. 82. Published in 1999 in the Journal of Pharmaceutical Research, volume 290, pages 214-119.
Stanfa LC, Singh L, Williams RG, and Dickenson AH found that gaba-pentin significantly decreased C-fiber evoked responses during inflammation, whereas it had no impact on normal rats. Brain Research, 1997, 8, 587–590
Casa CC, Ramos HF, da Silva AP, and colleagues (84). Gapitentin orally
therapy intensifies the inflammatory reactions in the nerves and peripheral tissues of Wistar rats during nerve constriction experiments. The Citation: Neuroscience Letters 2013; 556: 93e8.
You HJ, Lei J, and Arendt-Nielsen L. found that pregabalin inhibited nociception in both intact and spinalized rats via peripheral C fibers, but not A-delta fibers. The article is published in the journal Neuroscience in 2009 and spans pages 1845–1853.
The kind of sensitization-induced hyperalgesia is connected to the antinociceptive action of systemic gabapentin (86.Curros-Criado MM, Herrero JF). Publication date: 2007; volume: 4; pages 1–8
A new paradigm to examine the impact of analgesics in rats: inflammation-induced decrease of spontaneous activity by adjuvant (Matson DJ, Broom DC, Carson SR, Baldassari J, Kehne J, Cortright DN). Research in Pharmaceutical Sciences 2007; 320: 194–201
Brennan, T.J. (88). What causes discomfort after surgery? Pain
publication year 2011; volume 152: pages S33–e40
Postoperative pain: a review of causes, mechanisms, and treatments (89. Pogatzki-Zahn EM, Segelcke D, Schug SA). 2017—Pain Report 2. The e588
90.Papathanasiou T, Juul RV, Heegaard A-M, Kreilgaard M, Lund TM. A rat model of postoperative pain shows dose-dependent synergistic benefits when morphine and gabapentin are administered together. Published in 2016 in the European Journal of Pharmaceutical Science, volume 82, pages 97–105....
The antihyperalgesic effects of diclofenac sodium are enhanced by gabapentin in a rat postoperative pain model, according to 91. Narai Y, Imamachi N, and Saito Y. The article "Anesthraethol Analg 2012; 115: 189e93" provides further information.
92. McCleon GP, Long CT, Pacharinsak C, et al. In an incisional pain model in rats (Rattus norvegicus), the analgesic effects of tramadol, tramadol-gabapentin, and buprenorphine were studied. Journal of the American Society for Laboratory Animal Science, 2011; 50: 192–197.
Smashing JL (93). The impact of analgesic guidelines on the persistence of pain after surgery caused by skin/muscle incision and retraction (SMIR). Brain Research Letters 2010; 477: 43–47
Hayashida K-I, DeGoes S, Curry R, and Eisenach JC. 94. Gaba-pentin increases spinal noradrenergic activity in rats and humans and lowers hypersensitivity after surgery. Journal of Anesthesiology 2007; 106: 557–562.
A group of researchers including Whiteside, Harrison, Boulet, and others made 95 findings. Pharmacological characterisation of a rat model of incisional pain. Br J Pharmacol 2004; 141: 85e91
96.
Cheng JK, Pan HL, Eisenach JC. Antiallodynic effect of intrathecal gabapentin and its interaction with clonidine in a rat model of postoperative pain. Anesthesiology 2000; 92: 1126e31
97.Field MJ, Holloman EF, McCleary S, Hughes J, Singh L. Evaluation of gabapentin and S-(+)-3-isobutylgaba in a rat model of postoperative pain. J Pharmacol Exp Ther
1997, volume 282, pages 1242–1246.
98.Buvanendran A, Kroin JS, Kari MR, Tuman KJ. A new knee surgery model in rats to evaluate functional mea- sures of postoperative pain. Anesth Analg 2008; 107: 300e8
99.Okkerse P, van Amerongen G, de Kam ML, et al. The use of a battery of pain models to detect analgesic properties of compounds: a two-part four-way crossover study. Br J Clin Pharmacol 2017; 83: 976e90
100.Gustorff B, Hoechtl K, Sycha T, Felouzis E, Lehr S, Kress HG. The effects of remifentanil and gabapentin on hyperalgesia in a new extended inflammatory skin pain model in healthy volunteers. Anesth Analg 2004; 98: 401e7
101.Werner MU, Perkins FM, Holte K, Pedersen JL, Kehlet H. Effects of gabapentin in acute inflammatory pain in humans. Reg Anesth Pain Med 2001; 26: 322e8
102.Dirks J, Petersen KL, Rowbotham MC, Dahl JB. Gaba- pentin suppresses cutaneous hyperalgesia following heat-capsaicin sensitization. Anesthesiology 2002; 97: 102e7
103.Gottrup H, Juhl G, Kristensen AD, et al. Chronic oral gabapentin reduces elements of central sensitization in human

experimental hyperalgesia. Anesthesiology 2004; 101: 1400e8
104.Wallace MS, Schulteis G. Effect of chronic oral gaba- pentin on capsaicin-induced pain and hyperalgesia. Clin J Pain 2008; 24: 544e9
105.Wang H, Bolognese J, Calder N, et al. Effect of morphine and pregabalin compared with diphenhydramine hy- drochloride and placebo on hyperalgesia and allodynia induced by intradermal capsaicin in healthy male sub- jects. J Pain 2008; 9: 1088e95
106.Iannetti GD, Zambreanu L, Wise RG, et al. Pharmacolog- ical modulation of pain-related brain activity during normal and central sensitization states in humans. Proc Natl Acad Sci U S A 2005; 102: 18195e200
107.Arendt-Nielsen L, Frokjar J, Staahl C, et al. Effects of gabapentin on experimental somatic pain and temporal summation. Reg Anesth Pain Med 2007; 32: 382e8
108.Boyle Y, Fernando D, Kurz H, Miller SR, Zucchetto M, Storey J. The effect of a combination of gabapentin and donepezil in an experimental pain model in healthy volunteers: results of a randomized controlled trial. Pain 2014; 155: 2510e6
109.Chizh BA, Go€hring M, Tro€ster A, Quartey GK, Schmelz M,
Koppert W. Effects of oral pregabalin and aprepitant on pain and central sensitization in the electrical hyper- algesia model in human volunteers. Br J Anaesth 2007; 98: 246e54
110.Enggaard TP, Mikkelsen SS, Zwisler ST, Klitgaard NA, Sindrup SH. The effects of gabapentin in human exper- imental pain models. Scand J Pain 2010; 1: 143e8
111.Segerdahl M. Multiple dose gabapentin attenuates cuta- neous pain and central sensitisation but not muscle pain in healthy volunteers. Pain 2006; 125: 158e64