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REVIEW

P/Q-type calcium channel modulators

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P/Q-type calcium channels are high-voltage-gated calcium channels contributing to vesicle release at synaptic terminals. A number of neurological diseases have been attributed to malfunctioning of P/Q channels, including ataxia, migraine and Alzheimer's disease. To date, only two specific P/Q-type blockers are known: both are peptides deriving from the spider venom of *Agelenopsis aperta*, ω-agatoxins. Other peptidic calcium channel blockers with activity at P/Q channels are available, albeit with less selectivity. A number of low molecular weight compounds modulate P/Q-type currents with different characteristics, and some exhibit a peculiar bidirectional pattern of modulation. Interestingly, there are a number of therapeutics in clinical use, which also show P/Q channel activity. Because selectivity as well as the exact mode of action is different between all P/Q-type channel modulators, the interpretation of clinical and experimental data is complicated and needs a comprehensive understanding of their target profile. The situation is further complicated by the fact that information on potency varies vastly in the literature, which may be the result of different experimental systems, conditions or the splice variants of the P/Q channel. This review attempts to provide a comprehensive overview of the compounds available that affect the P/Q-type channel and should help with the interpretation of results of *in vitro* experiments and animal models. It also aims to explain some clinical observations by implementing current knowledge about P/Q channel modulation of therapeutically used non-selective drugs. Chances and challenges of the development of P/Q channel-selective molecules are discussed.

Abbreviations

Aβ, amyloid-β; AD, Alzheimer's disease; CDK, cycline-dependent kinase; LMW, low molecular weight; VGCC, voltage-gated calcium channel

Introduction

The P/Q-type calcium channel (also referred to as $Ca_v2.1$) is a presynaptic high-voltage-gated calcium channel, which couples neuronal excitation to secretion of neurotransmitter (Ishikawa *et al.*, 2005). The ion-conducting pore is formed by four domains of the α_{1A} subunit, whereas accessory subunits (β, α2δ) modulate channel kinetics and the level of expression. P-type currents were first identified in Purkinje neurons of the cerebellum (Llinás *et al.*, 1989) and are distinguished from Q-type currents identified in cerebellar granule neurons (Randall and Tsien, 1995). Both are characterized by their sensitivity to the venom of *Agelenopsis aperta*, ω-agatoxin IVA (Mintz *et al.*, 1992a), and are generated by ion channels encoded by the CACNA1A gene. A number of splice variants may explain different phenotypic characteristics of P- and Q-type channels (Bourinet *et al.*, 1999). For convenience and because distinction between these channel subtypes is not always clear, we refer throughout this review to P/Q-type channels. Expression of P/Q-type channels often overlaps with its close analogue, the N-type calcium channel. Yet, the P/Q-type channel is preferably expressed in neurons of the CNS (Bourinet *et al.*, 1999), making it an interesting target for therapeutics addressing neurological disorders.

A number of conditions have been related to P/Q-type channels, some linked by human mutations occurring in familiar inherited diseases (Kisilevsky and Zamponi, 2008). Familiar hemiphlegic migraine is an example of a disorder with altered P/Q-type activity. Here, different mutations in the CACNA1A gene lead to altered calcium influx, possibly

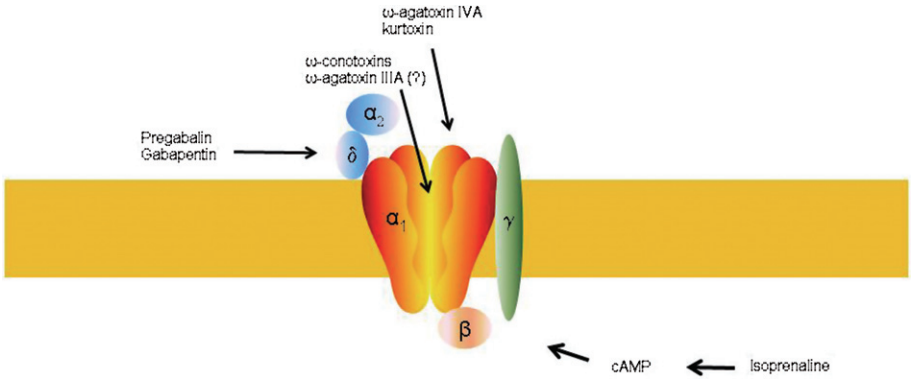

Figure 1

Topology of the P/Q-type channel with potential binding sites of channel modulators. ω-Agatoxin IVA and kurtoxin bind to the outer mouth of the pore forming subunit (linker of the S3–S4 domain), ω-agatoxin IIIA probably to a pore site. ω-Conotoxins bind to the pore region. Pregabalin and gabapentin have been suggested to interact with the α2δ subunit. Isoprenaline probably enhances P/Q currents via second messenger cascades.

causing cortical spreading depression, which is thought to underlie migraine aura (Plomp *et al.*, 2001; van den Maagdenberg *et al.*, 2004). In contrast, decreased P/Q channel activity may lead to absence epilepsy and ataxia (Ophoff *et al.*, 1998). It has recently been shown that amyloid-β (Aβ) oligomers directly increase the recombinant P/Q-type calcium current, and it has been suggested that such modulation can lead to excitotoxic neurodegeneration in Alzheimer's disease (AD; Mezler *et al.*, 2012a). For most of these conditions, there are few or no medications on the market.

In spite of this high, unmet medical need, no specific low molecular weight blockers are known with scaffolds that could serve as structures for lead optimization. This might be due to the fact that the P/Q-type channel is highly homologous to the N-type channel, and that high-throughput assay technology may not successfully deliver specific compounds for lead optimization. Furthermore, development of P/Q-type blockers may be hampered by the fact that peptide tool compounds do not pass the blood–brain barrier, thus do not allow appropriate proof-of-concept studies in animals.

A closer look at the available compound collection may open avenues for drug development, especially when compounds with different biophysical properties are examined for P/Q modulation and may provide clues for a structure–activity relationship. Some proof-of-concept may also come through the interpretation of clinical studies with less specific calcium channel blockers, when taking into account their P/Q-channel activity.

Currently available compounds that carry P/Q-type channel activity can be divided in several groups: (i) Peptidic ion channel blockers deriving from venom of different invertebrate animal species. This group is also host to a subgroup of compounds with two peptides of high selectivity for the P/Q-type channel: the ω-agatoxins. (ii) Low molecular weight compounds that show some efficacy for the P/Q-type channel, but which are not used as therapeutics. (iii) Therapeutics that also affect the P/Q-type channel. Some of those are traditionally named 'calcium antagonists' and thought to target the L-type calcium channel. It also comprises some anti-epileptics with P/Q channel activity as well as volatile anaesthetics.

P/Q channels as drug target

Voltage-gated calcium channels (VGCC) are protein complexes that mediate calcium influx in response to membrane depolarization. High threshold VGCC (L-type, P/Q-type, N-type and R-type) are activated by strong depolarization, whereas low threshold calcium channels (T-type) open in response to mild depolarization steps. The topology of the P/Q-type channel is illustrated in Figure 1 (for review, see Pietrobon, 2002). The characteristic of the P/Q-type channel is mainly determined by the α_{1A} subunit, which contains the conducting channel and the voltage sensor. The auxiliary subunits β and α2δ (and sometimes the γ-subunit) occur in most VGCC and influence trafficking or have a regulatory function (Dolphin, 2009). The pore consists of four homologous domains (I–IV), each of which is composed of six transmembrane segments (S1–S6). S4 is thought to be the voltage sensor. The P/Q-type calcium channel is located at axon terminals as well as somatodendritic compartments of central and peripheral neurons, with some preference for the CNS. At presynaptic sites, opening of the channel mediates synaptic vesicle release via an increase in the local calcium concentration. The pore forming subunit of the P/Q-type calcium channel is encoded by the CACNA1A gene, and multiple splice variants exist that are differentially distributed in the CNS.

Several neurological disorders are caused by mutations in the CACNA1A gene (for review, see Pietrobon, 2010; Rajakulendran *et al.*, 2012). Familiar hemiplegic migraine 1 (FHM1) is a rare, but severe autosomal-dominant subtype of migraine with aura characterized by typical migraine symptoms like unilateral headaches and nausea, but also presents other neurological symptoms such as motor weakness and hemiparesis. Nearly all mutations described in the literature lead to amino acid changes in the α_{1A} subunit, causing a gain-of-function of

the P/Q-type calcium channel. A knock-in mouse carrying a FHM1 mutation showed an increased P/Q-type current and a higher susceptibility to cortical spreading depression (van den Maagdenberg *et al.*, 2004; Tottene *et al.*, 2009). The latter is thought to be the pathophysiological correlate of migraine aura. These studies suggest that cortical hyperexcitability may be an underlying cause for the vulnerability of migraine. A key role of cortical spreading depression in migraine pathogenesis has also been derived from human imaging studies (Hadjikhani *et al.*, 2001). Drugs inhibiting cortical spreading depression may thus be candidates for the prophylaxis of migraine. P/Q channel blockade in the CNS will lower neurotransmission and can thus decrease cortical excitability. Several studies have shown that selective P/Q-type channel blockade can prevent spreading depression (Kunkler and Kraig, 2004; Tottene *et al.*, 2011). Taken together, these data encourage the development of P/Q-type channel blockers as a therapeutic strategy for migraine prophylactic treatment.

Mutations in the P/Q-type calcium channel may also lead to a higher susceptibility for epilepsy. Mice with spontaneous mutations in the CACNA1A gene like *tottering* or *learner* show patterns of generalized seizures (Fletcher *et al.*, 1996). Mutations in the P/Q-type channel have also been linked to epilepsy in humans (reviewed by Khosravani and Zamponi, 2006), although a robust causal relationship has not been demonstrated. Several types of ataxia have been linked to P/Q-type channel mutations: In episodic ataxia type 2, an acetozolamide-responsive type of generalized ataxia, two mutations have been identified that cause a shift in the open reading frame and result in a truncated α_{1A} subunit. Spinocerebellar ataxia type 6 is a progressive form of ataxia caused by an expansion of the polyglutamate repeat in the C-terminus of the α_{1A} subunit (Zhuchenko *et al.*, 1997). As some of these mutations lead to gain-of-function and others to a lack-of-function of the P/Q-type channel, a single P/Q channel modulator may not be sufficient to treat all P/Q-related disorders.

The P/Q-type calcium channel has also been suggested to contribute to the pathology of AD. It is now an accepted view that Aβ oligomers cause cognitive decline by altering synaptic function in patients with AD. Early studies using non-specific Aβ peptides reported that application of Aβ to neurons causes an increase in calcium currents (He *et al.*, 2002; Rovira *et al.*, 2002). I.c.v. injection of Aβ peptides caused disturbance of synaptic plasticity in rats, which was reversed by calcium antagonists (Freir *et al.*, 2003). Several publications then showed that application of Aβ peptides to neurons increased N and P/Q-type calcium currents (MacManus *et al.*, 2000; Ramsden *et al.*, 2002). We recently tested the effect of Aβ oligomers on recombinantly expressed P/Q-type calcium currents in *Xenopus* oocytes and showed that the α_{1A} subunit of the channel was specifically modulated, leading to an increased calcium influx. It was speculated that this increase might cause excitotoxicity and lead to synaptic decline in AD (Mezler *et al.*, 2012a). The view that Aβ protein interacts with presynaptic calcium channels in AD patients was supported by the observation that Aβ oligomers co-localize with axon terminals in AD brains (Kokubo *et al.*, 2005; Noguchi *et al.*, 2009). It has also been shown that endogenous Aβ increases the frequency of EPSCs (Abramov *et al.*, 2009), indicating an up-regulation of presynaptic function by amyloid protein.

P/Q-type channels have also been discussed as a drug target for pain (Yakash, 2006; Lewis *et al.*, 2012). Although P/Q-type channels contribute to neurotransmission at nociceptive synapses (Heinke *et al.*, 2004), and efficacy in pain models has been reported (Nebe *et al.*, 1997), N-type channels are likely to be the preferred target for this therapeutic area (for review, see Lewis *et al.*, 2012).

The P/Q-type channel is widely expressed in the CNS. Its general expression in all brain areas – especially in the cerebellum – may be a challenge for drug development, as P/Q blockade in the cerebellum may cause gait and movement disturbances. Indeed, P/Q channel knock-out mice exhibit symptoms of ataxia and dystonia (Jun *et al.*, 1999; Fletcher *et al.*, 2001). Addressing a particular splice variant expressed in the brain region of interest could be a sophisticated approach for drug development to bypass the effects of the cerebellum. Bourinet *et al.* (1999) identified a number of splice variants with different pharmacological properties. A larger group of splice variants was later identified and exemplifies the diversity of P/Q channel variation (Soong *et al.*, 2002). The challenge would be the identification of a compound with sufficient selectivity for a given splice variant. So far, there is no detailed expression map of the various isoforms available that would support a particular splice variant as drug target. Variant $\alpha_{1A\text{-}b}$ shows preferential expression in hippocampal areas (Bourinet *et al.*, 1999) and could be an interesting target for development of compounds against AD. Yet, its full pattern of CNS distribution is not known. A second approach, which is increasingly implemented in drug discovery, is the development of state-dependent therapeutics. These molecules are designed to preferably bind to the inactivated state of the channel and thus are thought to target channels at overactive synapses (and thus only under pathological conditions), while sparing normal synapses. A compound with high level of state-dependency may be favourable, particularly for the indications migraine and epilepsy, where the pathophysiology involves prolonged depolarization of the membrane over seconds or minutes. Other conditions like pain may benefit from use-dependent compounds, which do not block the channel at normal firing patterns, but instead bind to the channel during high-frequency firing. In the pharmaceutical development of ion channel blockers it is now state-of-the-art to strive for a high level of state- or use-dependence in order to increase the therapeutic window. We recently described a high-throughput screening assay with a subsequent electrophysiological secondary screening, which was designed to identify state-dependent P/Q-type channel blockers (Mezler *et al.*, 2012b). Whether these approaches will actually reduce the number and intensity of adverse effects in humans has yet to be shown in clinical trials.

In contrast, some compounds bind to the open state of the channel and thereby delay its deactivation. These drugs lead to a facilitation of calcium influx and may be beneficial in certain types of ataxia, where calcium entry through the P/Q channel is diminished.

Here, we have attempted to give an overview on the available compounds with P/Q channel modulating activity. Only two peptide toxins are selective for P/Q-type channels, the majority of the compounds described are non-selective and often more potent for other targets.

Table 1

Reported peptide blockers with P/Q-type channel activity (in alphabetical order)

Compound	P/Q channel activity	Activity on other channels	Reference	Species
Calcicludine	Complete block at 10 nM (native P-type current) to slight block at 100 nM (recombinant P/Q channel)	Block of N and L-type channels at 25–250 nM	Schweitz *et al.*, 1994; Stotz *et al.*, 2000	*Dendroaspis angusticeps*
DW13.3	IC_{50} = 4.3 nM	Blocks N-type channels with an IC_{50} of 14.4 nM, L-type channels with 26.8 nM and R-type channels 96.4 nM	Sutton *et al.*, 1998	*Filistata hibernalis*
Kurtoxin	50% inhibition of initial current amplitude (K_D = 14 nM), but facilitation of steady-state current	Block of N, L and T-type currents (K_D 456, 72 and 49 nM respectively)	Sidach and Mintz, 2002	*Parabuthus transvaalicus*
Phonetoxin IIA	>70% block at 10 nM	Full block of N-type currents at 3.5 nM, 20% block of R-type currents (17 nM)	Dos Santos *et al.*, 2002	*Phonoetrica nigriventer*
PnTx3-6	IC_{50} = 263 nM	IC_{50} for N-type channel 136 nM, R-type channel 607 nM and L-type channel 122 nM	Vieira *et al.*, 2005	*Phonoetrica nigriventer*
SNX482	partical block at 300 nM	R-type complete block at 200 nM (Bourinet *et al.*, 2001); partial block of Na channels at 500 nM	Arroyo *et al.*, 2003	*Hysterocrates gigas*
ω-agatoxin-IIIA	K_D = 9 pM	N-type, R-type (K_D = 5–9 pM)	Yan and Adams, 2000	*Agelenopsis aperta*
ω-agatoxin-IVA	IC_{50} = 2–1000 nM	–	Mintz *et al.*, 1992a,b; Sather *et al.*, 1993; Stea *et al.*, 1994; Bourinet *et al.*, 1999; Hans *et al.*, 1999	*Agelenopsis aperta*
ω-agatoxin-IVB	K_D = 3 nM, complete block at 800 nM	–	Adams *et al.*, 1993	*Agelenopsis aperta*
ω-conotoxin CVIB	IC_{50} = 23 nM	Blocks N-type channel with an IC_{50} of 23 nM	Motin *et al.*, 2007	*Conus catus*
ω-conotoxin MVIIC	IC_{50} <0.5 μM	Blocks N-type channels with an IC_{50} of 18 nM	Sather *et al.*, 1993; McDonough *et al.*, 1996	*Conus magus*
ω-Grammtoxin-SIA	complete block at 50 nM	complete block of N-type current at 500 nM, binding to the *drkl* K^+ channel	McDonough *et al.*, 1997; Takeuchi *et al.*, 2002	*Grammostola spatulata*
ω-Lsp-IA	Partial block at 10 nM	– (?)	Pluzhnikov *et al.*, 2007	*Geolycosa sp.*
ω-PnTx3-3	79% block at 60 nM	45% block of L-type current at 80 nM	Leão *et al.*, 2000	*Phonoetrica nigriventer*

References in column 4 report activities on the P/Q-type channel. Activites on other targets are reported in the same references, unless explicitly stated in column 3. The peptide toxins were originally isolated from venom of the species stated in column 5.

ω-Agatoxins

Spider venoms are a rich source of ion channel blockers. Agatoxins comprise a group of toxins from the American funnel web spider *A. aperta* that target different classes of ion channels (Adams, 2004). Table 1 summarizes the agatoxins with P/Q channel activity. Two toxins out of this venom screen are specific for P-type channels (i.e. ω-agatoxin IVA and ω-agatoxin IVB). Both peptides share the same specificity and affinity for P-type currents, but seem to exhibit different kinetics (Adams *et al.*, 1993). ω-Agatoxin IVA blocks P-type channels in rat Purkinje neurons with an IC_{50} of 2–10 nM and only marginally affects other currents (Mintz *et al.*, 1992a,b). ω-Agatoxin IVA blocks Q-type channels less effectively than P-type channels, probably due to different spice variants encoding each subtype (Bourinet *et al.*, 1999). The cloned α_{1A} subunit may reflect the Q-type channel, which would explain the finding that the recombinant α_{1A} is much less sensitive to ω-agatoxin IVA than native P-type currents. Sather et al. (1993), for example, revealed that recombinant α_{1A} channels

are 100-fold less sensitive to ω-agatoxin IVA than P-type channels of rat cerebellar Purkinje neurons. Less sensitivity of recombinant α_{1A} channels for ω-agatoxin IVA was also described by other authors (Stea *et al.*, 1994; Bourinet *et al.*, 1999). ω-Agatoxin IVA shifts the activation curve to more positive potentials, indicating that it alters gating of the channel (Winterfield and Swartz, 2000; McDonough *et al.*, 2002). Strong depolarization steps remove the toxin from the channel (Mintz *et al.*, 1992b), indicating that the affinity of the toxin is low for the open state of the channel. In contrast to other calcium channel blockers, ω-agatoxin IVA binds outside of the pore region of the α_{1A} subunit (Winterfield and Swartz, 2000), which may explain its selectivity compared with pore blockers. The ω-agatoxin IVA receptor has been localized to the S3–S4 linker, which is also a binding site for gating modifier molecules on K^+ and Na^+ channels (Rogers *et al.*, 1996; Li-Smerin *et al.*, 2000). It has been suggested that either the hydrophobic C-terminal part of the peptide (Kim *et al.*, 1995) or charged residues in the mid-part region (Adams *et al.*, 1993) mediate activity. ω-Agatoxin-IVB is the second specific blocker of P-type currents in cerebellar Purkinje neurons with a K_D of 3 nM, and no effect on T-type, L-type or N-type calcium channels (Adams *et al.*, 1993). Also, similar to ω-agatoxin IVA, its release from the channel is strongly increased by large depolarizations steps. The only difference between the toxins is the kinetics (block by ω-agatoxin-IVB develops eightfold slower and is also reversed more slowly during washout; Adams *et al.*, 1993). The three-dimensional solution structure of both peptides has been determined by NMR: both are composed of 48 amino acids internally connected by four disulfide bonds (Adams *et al.*, 1993; Kim *et al.*, 1995). In contrast to these specific peptides, ω-agatoxin-IIIA has high affinity to all presynaptic calcium channels (N, P/Q and R) in the low picomolar range (Yan and Adams, 2000). Functionally, it exhibits only a partial block by decreasing single-channel conductance (McDonough *et al.*, 2002). It also blocks L-type channels (Mintz *et al.*, 1991; Ertel *et al.*, 1994).

Other spider toxins

P/Q blockers have been isolated from a number of spider venoms beyond *A. aperta*. ω-Grammotoxin SIA was purified from the venom of the tarantula spider *Grammostola spatulata* (Lampe *et al.*, 1993). It affects both N- and P/Q-type calcium channels (McDonough *et al.*, 1997). Isolated P-type currents in rat cerebellar Purkinje neurons were completely blocked by 50 nM ω-grammotoxin SIA, and this effect seems to be through a modification of channel gating. Resting states are stabilized by the toxin (McDonough *et al.*, 1997). Channel binding has been suggested to occur through a hydrophobic patch of the surface of ω-grammotoxin SIA, but seems not to be restricted to calcium channels (e.g. low affinity binding to K channels; Takeuchi *et al.*, 2002). A peptide homologous to ω-grammotoxin SIA, SNX482, is the 41-amino-acid toxin of the African tarantula *Hysterocrates gigas*, which has been found to block P/Q channels as well as sodium channels (Arroyo *et al.*, 2003), in addition to its earlier demonstrated effect on R-type currents (Bourinet *et al.*, 2001).

ω-PnTx3-3, a peptide derived from the South American 'armed' spider *Phoneutrica nigriventer*, inhibits most of the isolated P/Q-type current at 60 nM in cerebellar granule neurons, but is also effective for N and L-type currents (Leão *et al.*, 2000). A second toxin was isolated from this spider (i.e. phonetoxin IIA) (Cassola *et al.*, 1998). This toxin is large with 76 amino acids and has some similarity to ω-agatoxin-III family. It irreversibly blocks recombinant P/Q- and N-type currents, and partly inhibits R-type currents (Dos Santos *et al.*, 2002). A third toxin from *P. nigriventer,* PnTx3-3, blocks L, P/Q, R and N-type channels (Vieira *et al.*, 2005). P/Q currents recombinantly expressed in cell lines were blocked with an IC_{50} of about 200 nM. A novel 47-amino-acid peptide toxin, ω-Lsp-IA, was recently identified in the venom of a *Geolycosa* sp.; it attenuates activation kinetics at 10 nM in cerebellar Purkinje neurons and has been suggested to be specific for P/Q-type currents (Pluzhnikov *et al.*, 2007).

DW13.3 is a 74-amino-acid toxin derived from *Filistata hibernalis*, which blocks all recombinant $\alpha_{1A\text{-}E}$ currents in *Xenopus* oocytes (Sutton *et al.*, 1998), most potently the α_{1A} channel with an IC_{50} of 4.3 nM. It was also observed to block ω-Agatoxin IVA-sensitive currents in cerebellar Purkinje neurons (saturation at 32–100 nM).

At this point, one may raise the question why particularly arachnids rely on P/Q channel block for prey capture and defence. Do insects have ion channels that are particularly sensitive to P/Q-modulating toxins? Many spiders hunt insects and ω-agatoxin IVA-sensitive currents have indeed been shown to occur in various insect species (Benquet *et al.*, 1999). Furthermore, functional P/Q-like currents can even be recorded in species as low as nematodes (*Caenorhabditis elegans*; Mathews *et al.*, 2003). It is possible that spiders rely on P/Q blockade to reach a larger spectrum of invertebrate animals.

ω-Conotoxins

Conotoxins are peptidic toxins derived from venomous marine cone snails. Each of the 500 *Conus* species expresses approximately 100 different conopeptides, so that a pool of more than 50 000 pharmacologically active compounds may exist (Terlau and Olivera, 2004). Most conopeptides target ion channels, some of them with high specificity, and many have been thoroughly used as research tools. ω-Conotoxins target calcium channels and largely derive from fish-hunting cone snails. A derivative of a *Conus magus* peptide ω-conotoxin MVIIA (an N-type specific inhibitor) is now clinically used under the name Prialt® (ziconotide, SNX-111) as a therapeutic for chronic pain. A number of other conopeptides are in clinical development, including an N-type channel blocker (ω-conotoxin CVID) in phase II (Han *et al.*, 2008). Medicinal chemistry efforts have enabled cyclization of conopeptides to improve bioavailability. Hence, conopeptides may in future be usable for oral drug application, opening further avenues for the development of ion channel-selective therapeutics from peptide blockers (Clark *et al.*, 2005).

Table 1 summarizes reported conopeptides with P/Q channel activity. ω-Conotoxin MVIIC, a peptide identified from a cDNA library from the venom gland of *C. magus*, inhibits calcium currents in cerebellar Purkinje cells with an IC_{50} between 1 and 10 μM (Hillyard *et al.*, 1992) and also inhibits P-type currents in hippocampal CA1 pyramidal neurons (Hillyard *et al.*, 1992). It also targets the N-type channel but does not affect the L-type channel. P-type current block by ω-conotoxin MVIIC is slower than for

N-type channels and also reverses slowly (McDonough *et al.*, 1996). In contrast to agatoxin, ω-conotoxin MVIIC blocks currents generated by the recombinantly expressed α_{1A} subunit in *Xenopus* oocytes more potently than the native current (70% block by 5 μM; Stea *et al.*, 1994; $IC_{50} < 0.15$ μM). However, the block is rather slow (Sather *et al.*, 1993). ω-Conotoxin MVIIC binds to P-type calcium channels with an affinity of 0.5 nM (estimated by McDonough *et al.*, 1996), but the specific P/Q channel blocker ω-agatoxin-IVA cannot prevent binding of ω-conotoxin MVIIC (McDonough *et al.*, 1996). The high content of basic amino acids residues in ω-conotoxins seems to mediate inhibition (Nadasdi *et al.*, 1995), while a mutation of the tyrosine residue at position 13 disrupts binding of the toxin and may be part of the toxin pharmacophore (Nielsen *et al.*, 1999a). In line with its inhibitory properties on presynaptic calcium channels, ω-conotoxin MVIIC completely prevents synaptic transmission of hippocampal CA3 neurons (Wu and Saggau, 1995).

Another ω-conotoxin exhibiting P/Q channel activity is ω-conotoxin CVIB. It reversibly inhibits both N and P/Q-type calcium channels expressed in *Xenopus laevis* oocytes with an IC_{50} of about 23 nM. The R-type current is not affected at 200–500 nM. In dorsal root ganglion cells it blocks isolated P/Q-type as well as N-type currents at 100 nM (Motin *et al.*, 2007). This P/Q-type block (but not the N-type block) is irreversible in these cells.

Other peptide toxins

Table 1 gives an overview of peptides with reported P/Q channel activity. Calcicludine, a 60 amino-acid peptide toxin isolated from the venom of the green mamba *Dendroaspis angusticeps*, blocks L-type currents recombinantly expressed in HEK293 cells but also exhibits some voltage-dependent block of P/Q- and N-type currents at 100 nM (Stotz *et al.*, 2000). In rat cerebellar Purkinje neurons, it blocks P-type currents more potently with an IC_{50} of 1–5 nM (Schweitz *et al.*, 1994), with a lower IC_{50} for L- and N-type channel block (10–100 nM). The peptide binds to olfactory bulb membranes with a K_D of 15–36 pM. Kurtoxin, derived from the scorpion venom *Parabuthus*, is interesting because it reduces high threshold calcium currents in thalamic neurons but enhances P-type currents in Purkinje cells (Sidach and Mintz, 2002).

Low molecular weight calcium channel blockers

In drug development, low molecular weight blockers are usually preferred, as they exhibit several advantages over peptide blockers: First of all, compounds can be selected for tissue penetration, distribution and pharmacokinetics. Peptides usually have extremely low penetration of tissue barriers, which is especially important for CNS indications where the blood–brain barrier often prevents accessibility of the target. Thus, small molecules have a higher potential for improvement of structure–activity relationship. Unfortunately, there is no small molecule known, which is specific to P/Q-type channels. Table 2 provides an overview of the low molecular weight compounds with reported activities for P/Q-type channels. Probably the best-examined small molecule P/Q channel modulator is the cycline-dependent kinase (CDK) inhibitor roscovitine (seliciclib). Roscovitine has been tested for clinical efficacy in a phase II cancer trial (Aldoss *et al.*, 2009). Yan *et al.* (2002) showed that roscovitine enhanced P/Q-type calcium tail currents with an IC_{50} of about 20 μM in isolated neostriatal interneurons. This effect was the result of slowed deactivation kinetics. P/Q channel modulation was independent of CDK inhibition. Consequently, roscovitine enhances presynaptic vesicle release in cultured neurons. A subsequent study elaborated on the kinetics of this modulation and found that roscovitine slows deactivation of all recombinantly expressed presynaptic calcium channels (P/Q, N and R) in stably transfected cell lines (Buraei *et al.*, 2006), albeit at high concentrations (EC_{50} for P/Q: 120 μM). Recently, Buraei and Elmslie (2008) showed that R-roscovitine exhibits both agonist and antagonist effects on all presynaptic calcium channels. Agonist properties were observed at a lower concentration (28 μM) than the antagonistic effect (130 μM). The agonism is specific for the stereoisomer and less pronounced for S-roscovitine and is determined by the residue on the C2 position of the molecule. The antagonism by R-roscovitine was state-dependent, with higher potency at depolarized potentials. Such bidirectional regulation has also been described for two antiepileptic drugs (benidipine and cilnidipine). Interestingly, a bidirectional modulation of ion channels is brought about by a number of conditions, such as changes in the holding potential (Kass, 1987). It is also not restricted to low molecular weight compounds (Koch *et al.*, 2004; Mezler *et al.*, 2012a) and may simply be observed by changing the expression system (Mezler *et al.*, 2012a). Cho and Meriney (2006) reported a 427% attenuation of the deactivation kinetics of calcium currents by roscovitine in *Xenopus* motorneurons and consequently increased transmitter release. Apparently, the enhancement of the tail current in such a system predominates, perhaps as the tail current comprises most of the total current during the brief time of an action potential. Such enhanced presynaptic function by roscovitine may cause excitotoxicity, as shown in cultured neurons (Monaco and Vallano, 2005).

A second compound causing current enhancement of P/Q-type calcium currents is the β-adrenoceptor agonist isoprenaline. The effect on P/Q is mediated by a cAMP cascade (Huang *et al.*, 1996). It causes an increase in the excitatory postsynaptic potential in rat amygdale slices, which can be blocked by ω-agatoxin IVA (Huang *et al.*, 1996). A direct enhancement of ω-agatoxin-sensitive calcium currents by 15 μM isoprenaline was subsequently shown (Huang *et al.*, 1998).

Interestingly, a number of NMDA receptor antagonists have P/Q channel activity: Eliprodil has been reported to block P-type currents in cerebellar Purkinje neurons (Biton *et al.*, 1995). The IC_{50} for P-type channel block was 1.94 μM, which is in the range of the IC_{50} for N and L-type channels. The block was not state-dependent. The NMDA receptor antagonist antazoline reversibly blocks P/Q-type channels with an IC_{50} of 10 μM. This block was state-dependent (Milhaud *et al.*, 2002). It has been suggested that such block may contribute to the neuroprotective properties of imidazolines. In this respect, it should be mentioned that the clinically used NMDA receptor blocker memantine also inhibits P/Q-type/N-type currents (Lu *et al.*, 2010). It is assumed that the therapeutic effect of memantine is mediated by a partial

Table 2

Reported LMW blockers with P/Q-type channel activity (in alphabetical order)

Compound	P/Q channel activity	Activity on other targets	Reference
A-1048400	IC_{50} = 1.3 μM (inactivated state), 16 μM (hyperpolarized state)	N-type: IC_{50} = 0.8–4.1 μM; T-type channel: IC_{50} = 0.9–2.6 μM	Scott *et al.*, 2012
Amlodipine	IC_{50} = 3–11.5 μM	L-type channel (IC_{50} = 1.2–4.2 μM); N-type (IC_{50} = 0.14–7.9 μM)	Furukawa *et al.*, 1999
Antazoline	IC_{50} = 10 μM	Antagonizes NMDA receptors (IC_{50} 4 μM; Milhaud *et al.*, 2002)	Milhaud *et al.*, 2002
Barnidipine	IC_{50} = 13.1–213 μM	L-type channel (IC_{50} = 1.2–3.1 μM); N-type (IC_{50} = 7.1–1370 μM)	Furukawa *et al.*, 1999
Cilnidipine	IC_{50} = 20.8–58.5 μM	L-type channel (IC_{50} = 5.3–12.7 μM); N-type (IC_{50} = 4.2–39.4 μM)	Furukawa *et al.*, 1999
Diltiazem	IC_{50} = 97 μM; IC_{50} = 169 μM;	L-type channel (IC_{50} = 33.3 μM –40 μM; Diochot *et al.*, 1995; Hockerman *et al.*, 2000)	Ishibashi *et al.*, 1995; Hockerman *et al.*, 2000
Dodecylamine	IC_{50} = 2.1 nM	Blocks L-type channels with an IC_{50} of 100 nM, N-type channels 1.8 μM, R-type channels 2.0 μM	Beedle and Zamponi, 2000
Eliprodil	IC_{50} = 1.9 μM	Antagonizes NMDA receptors with an IC_{50} of 670 nM and N and L-type currents with an IC_{50} of 1.48 μM (Biton *et al.*, 1994)	Biton *et al.*, 1995
Flunarizine	IC_{50} = 1.77–11 μM	hERG (IC_{50} = 5.7 nM) Na channels (use-dependent block at 100 nM; Trepakova *et al.*, 2006), T-type (IC_{50} = 0.1–19); L-type channels (IC_{50} 0.1–11 μM), N-type channel 0.8 μM	Geer *et al.*, 1993; Ye *et al.*, 2011
Fluspirilene	IC_{50} = 6 μM	Nanomolar affinity for D2 receptors (Schotte *et al.*, 1996); N-type: 2 μM; 90% block of T-type current at 1 μM (Enyeart *et al.*, 1992)	Sah and Bean, 1994
Gabapentin	IC_{50} = 98 μM; reduces P/Q-type mediate effect on EPSCs at 20 μM; binds to the α2δ subunit; chronic application modulates P/Q-type inactivation kinetics >0.3 μM; attenuates P/Q-mediated noradrenalin release	L-type (partial block at 100 μM); reduces N-type mediate effect on epscs at 20 μM	Dooley *et al.*, 2002; Fink *et al.*, 2002; Kang *et al.*, 2002; Sutton *et al.*, 2002; Oka *et al.*, 2003a,b; Cunningham *et al.*, 2004
Halothane	Partial block at 0.59 mM	Na channels (IC_{50} = 0.75 mM; Rehberg *et al.*, 1996); enhancement of $GABA_A$ receptor-mediated chloride currents (Jones *et al.*, 1992); N-type, R-type, L-type: Partial block at 0.59 mM	Kamatchi *et al.*, 1999; Kameyama *et al.*, 1999
Isoflurane	Partial block at 0.7 mM	Na channels (IC_{50} = 0.85 mM; Rehberg *et al.*, 1996); enhancement of $GABA_A$ receptor-mediated chloride currents (Jones *et al.*, 1992); N-type, R-type, L-type: Partial block at 0.7 mM	Kamatchi *et al.*, 1999
Isoproterenol	Enhancement of P/Q currents at 15 μM	β-adrenoreceptor ($K_d = 1.7 \times 10^{-7}$; Brown *et al.*, 1976)	Huang *et al.*, 1996; 1998
Lamotrigine	Partial block at 30 μM	Partial block of N-type channels at 30 μM; partial blockade of sodium channels at 1 μM (Stefani *et al.*, 1997)	Stefani *et al.*, 1996a
Levetiracetam	Partial block at 100 μM	Partial block of N-type at 100 μM; binds to SV2A (Lynch *et al.*, 2004)	Pisani *et al.*, 2004

Table 2

Continued

Compound	P/Q channel activity	Activity on other targets	Reference
LY393615	IC_{50} = 4 μM	N-type (IC_{50} = 1.9 μM); R-type (IC_{50} = 5.2 μM)	O'Neill *et al.*, 2001
Memantine	100 μM memantine abolishes P/Q-type channel mediated glutamate release	Blocks NMDA receptor currents (IC_{50} = 0.47–0.93 μM; Bresink *et al.*, 1996); 100 μM memantine abolishes N-type channel mediated glutamate release	Lu *et al.*, 2010
Neuromed 2	IC_{50} = 4.5 μM	N-type (IC_{50} 0.12–0.16 μM); T-type (81 nM); L-type (IC_{50} 133 μM)	Yamamoto and Takahara, 2009
Neuromed 5	IC_{50} = 1.659 μM	N-type (13–60 nM); T-type (0.27–0.579 μM); L-type (144 μM)	Yamamoto and Takahara, 2009
Nicardipine	IC_{50} = 21.1–85 μM	L-type (IC_{50} = 9.6–24 μM); N-type (IC_{50} = 59.9 μM-7.6 mM)	Furukawa *et al.*, 1999
Nimodipine	IC_{50} = 200–500 nM (little activity on P/Q detected by Furukawa *et al.*, 1999)	L-type channel (IC_{50} = 1 μM);	Diochot *et al.*, 1995; Mansvelder *et al.*, 1996
NMED-160 (Neuromed 1)	IC_{50} = 0.12–0.65 μM	N-type (IC_{50} = 0.04–0.2 μM); L-type (IC_{50} = 0.3–0.5 μM)	Yamamoto and Takahara, 2009
R-roscovitine	Decreases step current and enhances tail currents (EC_{50} = 120 μM)	Decreases step current and enhances tail currents of N-type and R-type calcium channels with EC_{50} of 54 μM; inhibits L-type current, K-currents and Na currents without tail enhancement; Inhibitor of cdc2, cdk2, cdk2 and cdk5 (IC_{50} = 0.2–0.7 μM; Meijer *et al.*, 1997)	Buraei *et al.*, 2006; Buraei and Elmslie, 2008
TROX-1	IC_{50} = 0.4 μM	N and R-type channels (IC_{50} = 0.4 μM)	Abbadie *et al.*, 2010
Verapamil	Full block at 50 μM; IC_{50} = 62 μM	L-type channel (IC_{50} = 4 μM); N-type channel (full block at 40 μM)	Diochot *et al.*, 1995; Ishibashi *et al.*, 1995; Dobrev *et al.*, 1999
α-Eudesmol	IC_{50} = 3.6 μM	N-type current: IC_{50} = 6.6 μM;	Asakura *et al.*, 1999; Horak *et al.*, 2009

References in column 4 report activities on the P/Q-type channel. Activites on other targets are reported in the same references, unless explicitly stated in column 3.

block of NMDA-receptor currents, thereby inhibiting excitotoxicity (Rogawski and Wenk, 2003). One may speculate that a presynaptic calcium channel block could contribute to a common silencing of overactive synapses. In this respect, a state-dependent block of presynaptic calcium channels may leave normal transmission unaltered, while buffering tonic glutamatergic transmission.

Dodecylamine is another low molecular weight blocker with activity for P/Q. It inhibits recombinantly-expressed P/Q-type currents with an IC_{50} of 2.1 μM, but it is not specific (Beedle and Zamponi, 2000). The block is use-dependent and restricted to the open state.

It should be mentioned that ethanol, although at very high concentrations, inhibits P/Q-type currents (Solem *et al.*, 1997). Although the principal pharmacological effect of ethanol is likely to be on other systems in the CNS, it would be worth examining whether alcohol-induced ataxia could be a result of P/Q channel blockade. The possibility that ataxia is caused by P/Q-channel loss-of-function in several genetic models has been well described in the literature.

Calcium channel blockers in development

The development of calcium channel blockers, particularly of the N-type, has been recently inspired by the FDA approval of the peptidic calcium channel blocker ziconotide (Piralt®). This peptide is a synthetic form of ω-conotoxin MVIIA derived from *C. magus* and blocks N-type calcium channels on nociceptive A-δ and C nerve fibre endings in lamina I and II of the spinal cord dorsal horn. Ziconotide is efficacious in opioid-resistant pain as well as other severe pain states (Pexton *et al.*, 2011). There does not seem to be development of tolerance (Webster *et al.*, 2009), underlining its advantage over opioids especially for non-cancer patients. However, there are multiple issues regarding adverse effects and the route of administration: First of all, ziconotide is a water-soluble and polar molecule with high molecular weight and thus has limited tissue penetration. Systemic administration inhibits noradrenaline release at sympathetic neurons and therefore exhibits systemic adverse effects like blood pressure changes. It has little effect on parasympa-

thetic nerves (Wermeling, 2005). Thus, intrathecal administration is mandatory. Yet, even with this application route, there are common central side effects like memory impairment, dizziness or speech disorders, leading to dropout rates of up to 39% in clinical studies (Ellis *et al.*, 2008; Webster *et al.*, 2009). Recently, a number of companies tried to overcome these hurdles by the development of state-dependent, low molecular weight compounds with improved pharmacokinetic and side effect profiles. Small molecules would be accessible to chemical optimization processes for improving structure–property relationships (SPR), facilitating oral availability and tissue distribution. A recent trend is the development of state-dependent channel blockers that are designed to only inhibit voltage-gated ion channels at inactivated state. It is thought that these molecules prevent excessive neurotransmission of cells under pain conditions, while leaving normal synaptic function unaltered. Thus, there is the hope for small molecule calcium channel blockers that can be applied systemically with a low side effect profile. These improvements, however, were accompanied by lack of selectivity, especially against the P/Q-type channel (Yamamoto and Takahara, 2009). As a result, most small molecule 'N-type channel blockers' in pharmaceutical development are mixed N-P/Q-type blockers (see Table 2 for an overview). Neuromed Pharmaceuticals recently disclosed their compound NMED-160, which blocks N-type and P/Q-type channels in the low nanomolar range. NMED-160 is the only small molecule molecular weight blocker in clinical trials [Neuromed, Merck give up on new pain drug. *Philadelphia Business Journal*. August 2007. Available from: http://wwwizjournalscom/philadelphia/stories/2007/08/06/daily17html (Last access April 2011)]. Channel block by NMED-160 seems to be use-dependent (McNaughton *et al.*, 2008), which should provide an advantage over non-state-dependent peptides. A number of compounds have been developed fairly recently, some of which have been shown to affect P/Q-type channels (Neuromed 2–7; reviewed by Yamamoto and Takahara, 2009). Merck developed a substituted *N*-triazole oxindole (TROX-1), which is orally available and showed efficacy in a number of pain models (Abbadie *et al.*, 2010). TROX-1 is potent and state-dependent (IC_{50} = 400 nM). However, in dorsal root ganglion cells, it blocks all Ca_V2 channels, including the P/Q-type channel, and it also inhibits recombinantly expressed P/Q channels with a potency similar to that for the N-type channel. Elli Lilly published an N-type blocker (LY393615) with an IC_{50} for N-type channels of 1.9 μM (recombinantly expressed in HEK293 cells), which blocks P/Q channels with similar potency (IC_{50} for P/Q: 4 μM in isolated Purkinje cells; O'Neill *et al.*, 2001). Abbott Laboratories recently published a state-dependent, orally available calcium channel blocker, which does not affect the L-type calcium channel (A-1048400; Scott *et al.*, 2012). The IC_{50} for the P/Q-type channel is 16.3 μM at a hyperpolarized state and 1.3 μM at an inactivated state. This compound also potently blocks N-type, R-type and T-type channels (IC_{50} at inactivated state: 0.8, 0.9 and 1.6 μM respectively). Current drug discovery efforts focusing on the discovery of P/Q-type channel blockers for CNS disorders may provide us with more selective, small molecule blockers for P/Q-type channels (Mezler *et al.*, 2012b).

Clinically used therapeutics that block calcium channels

A number of therapeutics modulate P/Q channels, although the therapeutic effect is thought to be mediated by other targets. A precise understanding of the respective target profile is often missing and may be valuable for the development of more selective compounds with fewer adverse effects. Table 3 gives an overview of clinically used compounds with P/Q-type channel activity.

Calcium antagonists. In 1964, Albrecht Fleckenstein showed that verapamil mimics the effect of Ca^{2+} removal on electrically-stimulated guinea pig papillary muscle (Fleckenstein-Grün, 1994). He created the name 'calcium antagonists' to separate the principle as an alternative to β-receptor blockade and confirmed the idea of calcium channel blockade by voltage-clamp analysis. Shortly afterwards, Bayer AG developed a highly potent calcium channel blocker, Bay a 1040, which was later named nifedipine. In the following years, a large number of calcium antagonists with distinct properties were identified by the pharmaceutical industry, belonging to different classes: benzothiazepines and phenylalkylamines. Some, like verapamil, have inotropic, chronotropic and dromotropic effects besides their vasodilator properties, whereas nifedipine was largely a vasodilator. Calcium antagonists are used clinically for the treatment of hypertension, coronary heart disease and cardiac arrhythmia. Their principal mode of action is the inhibition of L-type calcium channels in smooth muscle cells (including those of coronary arteries), leading to a block of excitation–contraction coupling and a relaxation of the vasculature. They also inhibit L-type channel-mediated calcium influx into cardiomyocytes and thus inhibit the cardiac action potential. The cardiac pacemaker activity may be brought about by the inhibition of calcium channels (including the T-type channel) in the sinoatrial node as well as the atrioventricular node. After identification and cloning of other VGCC, many therapeutically used calcium antagonists have been evaluated for efficacy on these channels, and it has become clear that many calcium antagonists are not selective for the L-type channel (Fujii *et al.*, 1997; Furukawa *et al.*, 1997).

For example, verapamil, nicardipine and nimodipine block ω-conotoxin GVIA-insensitive and ω-agatoxin IVA-sensitive currents in dorsal root ganglion cells, indicating N- and P/Q-blockade (Diochot *et al.*, 1995). Effective concentrations were in the micromolar range and several-fold higher than for L-type block. Verapamil and diltiazem block P-type currents in cerebellar Purkinje neurons (Ishibashi *et al.*, 1995), and diltiazem may shift the P-type inactivation curve. It was later shown that verapamil blocks P-type currents as well as other high voltage-gated calcium currents in rat striatal slices. Diltiazem also blocks P-type currents in this system (Dobrev *et al.*, 1999). Diltiazem also blocks P/Q-type channels recombinantly expressed in HEK293 cells, although it is five-fold less potent than on L-type channels (Hockerman *et al.*, 2000). Interestingly, when P/Q-type channels containing a nine-amino acid sequence specific for the dihydropyridine binding site were expressed, diltiazem reached the same potency at P/Q-type channels as at L-type channels. Mansvelder et al. (1996) published a study showing that ω-conotoxin GVIA and ω-Agatoxin IVA-sensitive currents are

Table 3

Clinically used compounds with P/Q channel activity (in alphabetical order)

Compound	Primary indication(s)	Suggested primary target mediating clinical efficacy	Reference
Amlodipine	Hypertension, angina pectoris	L-type channel	Haria and Wagstaff, 1995; Scholz, 1997
Barnidipine	Hypertension	L-type channel	Liau 2005
Diltiazem	Hypertension, angina pectoris, cardiac arrhythmias	L-type channel	McAuley and Schroeder, 1982
Flunarizine	Migraine	VGCC, Na^+ channels	Amery, 1983; Ye *et al.*, 2011
Fluspirilene	Schizophrenia	Dopamine D_2 receptors	Galizzi *et al.*, 1986
Gabapentin	Epilepsy, neuropathic pain	α2δ subunit of VGCC	Striano and Striano, 2008
Halothane	Inhalation anaesthesia	Multiple modes of action	Krnjević, 1992
Isoflurane	Inhalation anaesthesia	Multiple modes of action	Krnjević, 1992
Isoprenaline	Bradycardia, heart block, asthma	β-adrenoceptor	Ahlquist, 1976
Lamotrigine	Epilepsy	Na^+ channels, VGCC	Rogawski and Löscher, 2004; Elger and Schmidt, 2008
Levitiracetam	Epilepsy	VGCC, SV2	Elger and Schmidt, 2008
Memantine	Alzheimer's disease	NMDA receptor	Rogawski and Wenk, 2003
Mibefradil	Hypertension	T-type channel	Glasser, 1998
Nicardipine	Hypertension	L-type channel	Pepine and Lambert, 1990
Nimodipine	Cerebral vasospasm after subarachnoid haemorrhage	L-type channel	Tomassoni *et al.*, 2008
Verapamil	Cardiac arrhythmias, hypertension, angina pectoris	L-type channel	Rosen *et al.*, 1975; Scholz, 1997

Listed are the primary indications as well as targets suggested to mediate the therapeutic effect. References in column 4 report the pharmacological mechanism suggested to mediate the therapeutic effect.

blocked in rat melanotropic cells by the two dihydropyridines nimodipine and nitrendipine with an IC_{50} of 200–500 nM. This indicates that both nimodipine and nitrendipine affect N- and P/Q-type currents with appreciable potencies.

A comparative study with 10 dihydropyridines was performed on different calcium channels recombinantly expressed in *Xenopus* oocytes (Furukawa *et al.*, 1999). Nifedipine, nilvadipine, barnidipine, nimodipine, nitrendipine, amlodipine, nicardipine, benidipine, felodipine and cilnidipine all showed appreciable block of L-type channels at 10 μM. Of these, amlodipine, benidipine, cilnidipine, nicardipine and barnidipine also blocked P/Q- and N-type calcium channels. The P/Q channel block by amlodipine, nicardipine and barnidipine was voltage-dependent. Amlodipine was most potent with an IC_{50} of 3 μM at depolarized states (vs. 11.5 μM at hyperpolarized state). Similar potencies were observed for benidipine, cilnidipine and barnidipine. Some calcium antagonists, like cilnidipine, exhibited a similar block of L-, P/Q- and N-type currents at 10 μM. In contrast to the two studies on native currents described above (Diochot *et al.*, 1995; Mansvelder *et al.*, 1996), nimodipine had only a minor and nitrendipine no effect on P/Q-type channels. An explanation for this contrasting result could be the use of recombinant versus native test systems.

Taken together, these data indicate that several classes of therapeutically used 'calcium antagonists' are not specific to the L-type channel of smooth muscle, but also affect other calcium channels including the P/Q-type channel. Further studies need to examine whether their activity on N- and P/Q-type calcium channels explains some of the clinical findings, especially the efficacy in some neurological disorders discussed below.

We recently reported that Aβ globulomer, an oligomeric peptide with a toxic epitope found in AD patients, increases P/Q-type calcium currents recombinantly expressed in *Xenopus* oocytes (Mezler *et al.*, 2012a). A similar increase in P/Q-type channel activity has also been observed by Ramsden *et al.* (2002) and MacManus *et al.* (2000) in cultured neurons, albeit with less relevant Aβ preparations. It has been suggested Aβ oligomers enhance calcium channel flux through P/Q-type channels. Tonically overactive P/Q-type channels at central synapses may cause excessive glutamate release in affected brain regions, leading to excitotoxic cell death (Mezler *et al.*, 2012a). Such neuronal decline should be prevented by P/Q-type channel block. The neuroprotective effect of P/Q-type channel blockers has been thoroughly described in the literature (e.g. Small *et al.*, 1995; Asakura *et al.*, 1997). A few reports state that nimodepine is protective against AD (Tollefson, 1990; Grobe-Einsler and Traber, 1992), although the effect is minimal. Some efficacy of nimodipine in dementia trials was also stated in a Cochrane Review (López-Arrieta and Birks, 2002). Clinical improvement of cognitive decline was observed after treatment with nicardipine (Amenta *et al.*,

2009). In a nucleus basalis lesion model in rats, verapamil was efficacious in the behavioural outcome (Popović *et al.*, 1997).

P/Q block by verapamil may also explain why a particular type of stroke caused by a mutation in the gene for the P/Q-type calcium channel responds to treatment with verapamil (case report: Knierim *et al.*, 2011). This type of recurrent stroke is associated with seizures and may be prevented by P/Q channel inhibition because of a down-regulation of neuronal firing.

A number of calcium antagonists that are not classically related to the L-type channel block, also show P/Q channel activity. Mibefradil is considered to be a selective T-type calcium channel blocker and is used clinically for the treatment of hypertension. It also has been shown to exhibit some activity for the N-type and P/Q-type channel (Viana *et al.*, 1997).

Flunarizine is a mixed sodium and calcium channel blocker clinically used for the treatment of migraine. Flunarizine blocks P-type currents in neocortical slices, thereby preventing potassium-stimulated calcium influx with an IC_{50} of 11 μM (Geer *et al.*, 1993). In cultured cortical neurons, the calcium channel block was more potent with an IC_{50} of 1.77 μM, which is similar to the potency at the sodium channel (IC_{50} 0.94 μM; Ye *et al.*, 2011). Certain types of familiar migraines are caused by mutations in the CACNA1A subunit of the P/Q-type channel. Expression of these mutants in transgenic mice leads to enhanced P/Q channel activity and consequently facilitation of cortical spreading depression (Tottene *et al.*, 2009), which is thought to underlie migraine aura. The phenomena of spreading depression can be blocked by ω-agatoxin IVA (Kunkler and Kraig, 2004). It has been shown that flunarizine enhances the threshold for cortical spreading depression (Wauquier *et al.*, 1985), and one may speculate its preventive effect in migraine can at least in part be attributed to block of P/Q-type channels in the brain. Familiar migraines caused by P/Q mutations also seem to respond to verapamil (Yu and Horowitz, 2003), as do other migraine types (Solomon *et al.*, 1983; Markley *et al.*, 1984).

In summary, there is evidence that certain clinically used calcium antagonists show therapeutic benefit for some neurological diseases that might be linked to P/Q-type calcium channels. If the clinical effects can be attributed to their shared efficacy on P/Q channels, an improvement in P/Q channel specificity as well as target availability may improve their efficacy for these diseases and may reduce their side effects. As all the substances discussed above affect multiple targets, none of these structures may serve as a real lead compound for the development of selective P/Q-type channel blockers.

Antiepileptic drugs. Epileptic seizures are generally caused by a shift in the excitation/inhibition balance in cortical networks towards excitation. This involves enhanced neurotransmission at glutamatergic synapses, which is at least in part mediated by P/Q-type channels (Qian and Noebels, 2001). There is increasing evidence that VGCC, including P/Q-type channels, contribute to idiopathic generalized epilepsies (Zamponi *et al.*, 2010). Mutations in P/Q-type calcium channels have also been linked to absence seizures. Some anti-epileptic drugs interact with the α2δ accessory subunit of VGCC (Vohora *et al.*, 2010). For some, a direct P/Q-type current modulation has been shown. Levetiracetam has been demonstrated to inhibit high-voltage-gated calcium currents in hippocampal pyramidal neurons in slices (Niespodziany *et al.*, 2001). It has also recently been shown to block excitatory postsynaptic potentials in granule cells in slices specifically by blocking the P/Q-type calcium current (Lee *et al.*, 2009). Levetiracetam reduces N- and P/Q-type currents in isolated neocortical neurons without affecting sodium currents at 100 μM, a concentration that attenuates the paroxysmal depolarization shift in a neocortical slice model of epilepsy (Pisani *et al.*, 2004). Lamotrigine also inhibits high-threshold voltage-gated calcium currents with an IC_{50} of 12.3 μM in isolated rat pyramidal neurons (Stefani *et al.*, 1996a), which is attributed to N- and P-type blockade. Older antiepileptic drugs like carbamazepin and oxcarbamazepine are also active on high threshold calcium currents (Stefani *et al.*, 1995; Zhu *et al.*, 2002), and it has been suggested that carbamazepine has P/Q-activity (Zhu *et al.*, 2002). Some antiepileptics like felbamate also affect high VGCCs, but not the P/Q-type calcium channel (Stefani *et al.*, 1996b), whereas other anti-epileptics have little effect on high-threshold VGCC (phenytoin; Stefani *et al.*, 1997). Valproate does not seem to have any effect on presynaptic calcium channels, even at very high concentrations (up to 1.5 mM; Sitges *et al.*, 2007).

Gabapentin and pregabalin comprise a class of molecules that bind to the α2δ accessory subunit of VGCC with nanomolar affinities (Suman-Chauhan *et al.*, 1993; Gee *et al.*, 1996). It is thus not surprising that there are multiple studies showing a modulation of VGCC by those drugs. Some authors consider them to be selective for VGCC (reviewed by Sills, 2006). Both, gabapentin and pregabalin, at μM concentrations, attenuate neurotransmitter release in cortical slices by inhibition of P/Q-type channels (Dooley *et al.*, 2002). In cortical synaptosomes, gabapentin also blocks the ω-agatoxin IVA-sensitive increase in potassium-induced calcium levels in the μM range (Fink *et al.*, 2002). In addition, it blocks P/Q-type (and N-type) channels in rat cerebrocortical slices with an IC_{50} (for P/Q) of 98 μM (Oka *et al.*, 2003a). In another study, Oka et al. (2003b) analysed the effect of gabapentin on depolarization-evoked NOS activity in primary cortical neurons. High concentrations of gabapentin (100 μM) reduced depolarization-induced NOS activity by blockade of P/Q-type and L-type (not N-type) calcium channels. Gabapentin reduced presynaptic vesicle release at low μM concentrations, preferably acting via P/Q-type channel block (Cunningham *et al.*, 2004). Gabapentin also reduced EPSCs and IPSCs in spinal cord, with an IC_{50} of 23 nM, by reducing P/Q-type calcium currents (Bayer *et al.*, 2004). Whole-cell recordings from dorsal root ganglion cells revealed that gabapentin blocks all N-, P/Q- and L-type channels (Sutton *et al.*, 2002), although the P/Q block appears to be the smaller part. Kang *et al.* (2002), when recording from P/Q channels recombinantly expressed in *Xenopus* oocytes, found that chronic but not acute treatment with gabapentin induced a dose-dependent decrease in P/Q-type current inactivation. Inactivation kinetics were modified at concentrations starting at 300 nM. In this respect, it should be noted that the α2δ subunit has been shown to modulate calcium channel kinetics (Qin *et al.*, 1998).

As described above, both familiar migraines as well as Aβ pathology involve a gain-of-function in the P/Q-type current. Thus, it may not come as a surprise that some anti-epileptics are efficacious in these conditions. Piracetam (Croisile *et al.*, 1993) and levetiracetam (Cumbo and Ligori, 2010) are also efficacious in AD patients. Treament with levetiracetam has been correlated with improved cognitive performance, whereas piacetam seems to slow down cognitive decline. Similarly, levetiracetam appears to be beneficial in patients with migraine (Pizza *et al.*, 2011). A number of studies also report efficacy of gabapentin as a prophylactic in migraine (e.g. Di Trapani *et al.*, 2000; Mathew *et al.*, 2001), which may be explained by the ability of the calcium channel blocker gabapentin to prevent cortical spreading depression (Hoffmann *et al.*, 2010).

Mood stabilizers. Calcium channel blockers have been suggested to be beneficial in the treatment of bipolar disorder (Levy and Janicak, 2000). While for some drugs of this class the efficacy has been questioned, data for nimodipine have appeared promising (Pazzaglia *et al.*, 1998). This could be attributed to a better brain penetration by nimodipine as opposed to other calcium channel blockers. It is also possible that nimodipine exhibits different modulatory effects on calcium channels. Whether block of P/Q-type calcium channels contributes to the mood stabilizing properties is unclear. Some data suggest that modulation of presynaptic calcium influx may positively influence bipolar disorder. For example, Chen *et al.* (2010) implicate presynaptic glutamate release in the pathophysiology of bipolar disorder. Patients with bipolar disorder have been shown to have an increased neurotransmission in the anterior cingulate cortex (Eastwood and Harrison, 2010). Animal studies are needed to clarify whether a specific block of P/Q-type channels could ameliorate symptoms of bipolar disorder.

Anaesthetics. Volatile anaesthetics inhibit P/Q-type currents in the higher μM range, although the block is not specific for this channel isoform. Both isoflurane and halothane increase the rate of inactivation in P/Q-type currents recombinantly expressed in *Xenopus* oocytes (Kamatchi *et al.*, 1999). Isoflurane also inhibits P/Q-type, N-type and L-type calcium currents in dorsal root ganglion cells (Kameyama *et al.*, 1999) and probably in hippocampal pyramidal cells (Study, 1994). Volatile anaesthetics including halothane prevent glutamate release evoked in synaptosomes via a presynaptic mechanism (Schlame and Hemmings, 1995).

It is generally thought that volatile anaesthetics affect multiple targets in the brain with low potency, including $GABA_A$ receptors and VGCC. Thus, it is likely that P/Q channel block has – if at all – a minor contribution to the general anaesthetic state.

Antipsychotics. A number of dopamine receptor antagonists exhibit P-type channel activity, unrelated to a specific structure (Sah and Bean, 1994). Diphenylbutylpiperidines have long been known to exhibit calcium channel activity (Gould *et al.*, 1983) and compromise the activity of the most potent calcium channel blockers (Sah and Bean, 1994). They bind to calcium channels with affinities in the low nM range (Gould *et al.*, 1983). Of all these compounds, fluspirilene is the most potent P-type current blocker, with an IC_{50} of 6 μM. This block is not specific to P-type channels but also affects N-, L- and T-type channels (Sah and Bean, 1994). At a higher concentration (30 μM), most neuroleptic drugs, including chlorpromazine and haloperidol, have considerable activity on P-type currents. However, these concentrations may not be relevant for antipsychotic activity. As some neuroleptics show the ability to act as a calmodulin antagonist, the effect of fluspirilene was studied in the presence of the calcium chelator BAPTA, but this did not change its potency. Its effect was also not mediated by neurotransmitter activation of G-proteins (that are known to modulate P-type channels). However, the block of P-type currents by fluspirilene was voltage- and frequency dependent (Sah and Bean, 1994). Hence, the binding site of fluspirilene seems to be different from that of ω-agatoxin-IVA and also different from that of the pore blocker Cd^{2+}.

Herbal medication. Some plant extracts that are used as prophylactics for migraine (Diener *et al.*, 2004; Lipton *et al.*, 2004) also inhibit P/Q-type channels. α-Eudesmol (*Eucalyptus williamsiania*) and petasins (*Petasites hybridus*) state-dependently inhibit recombinant P/Q-type currents (Horak *et al.*, 2009). In rat cerebellar Purkinje cells, eudesmol (*Juniperus virginiana*) inhibits P/Q-type currents with an IC_{50} of 3.6 μM (Asakura *et al.*, 1999). However, whether the ability of these substances to affect P/Q-type currents is therapeutically relevant is questionable.

Is there a path for the development of a selective P/Q-type channel blocker?

One of the challenges in drug discovery is the development of a lead compound with sufficient selectivity for the target. In this respect, VGCCs may be at the extreme end of the spectrum, as selectivity for some types appears to be extremely difficult to obtain. Consequently, there are few fully selective calcium channel blockers. For the development of selective P/Q-type channel blockers, sparse information on structure–activity relationships and perhaps limited high-throughput electrophysiogical methods may have hampered the development of the appropriate lead molecule in the past. The pharmaceutical industry has initiated drug discovery programmes for low molecular weight N-type channel blockers, which was inspired by the approval of the selective peptide N-type channel blocker ziconotide for chronic pain. Yet, no small lead molecule with appreciable selectivity for a presynaptic calcium channel has been forwarded into clinical trials.

Recently, some progress has been made by the pharmaceutical industry to separate N- and P/Q-type blockade from L-type channel activity. Abbott Laboratories, for example, recently presented a small lead molecule – A-1048400 – with high potency for the N-, P/Q- and T-type channel, but which is largely devoid of L-type channel activity (Scott *et al.*, 2012). Separation from L-type channel activity was also reached by Neuromed Pharmaceuticals (see Table 2). Anecdotal reports also indicate separation of N and P/Q channel activity. Neuromed have described a number of compounds that show some selectivity for the N-type versus the P/Q-type channel (e.g. Neuromed 5). Beedle and Zamponi (2000) report that a

small molecule – dodecylamine – is largely selective for P/Q-type channels. However, these reports do not provide a clear structure–activity relationship for a development path for small P/Q channel blockers.

An alternative would be a development programme based on one of the two selective peptide P/Q-type channel blockers, ω-agatoxin IVA and ω-agatoxin IVB – in analogy to the N-type blocker ziconotide (Schmidtko *et al.*, 2010). For a number of reasons, ω-agatoxins themselves may not be suitable for CNS therapeutics: firstly, their pharmacokinetic properties are not suitable for oral administration and sufficient brain availability is still an illusion. The latter would be required for the treatment of potentially P/Q channel-related disorders like migraine or AD. Medicinal chemistry approaches have recently succeeded in modifying peptide toxins by cyclization to improve their biophysical properties (for review, see Craik and Adams, 2007). Yet, good bioavailability of peptide toxins is still a challenge, and these molecules do not penetrate the blood–brain barrier. Secondly, administration of ω-agatoxin may have strong adverse effects, as it blocks the channel largely irreversibly (unblock occurs only at large depolarizations; Adams *et al.*, 1993). However, structural information from the binding domain of ω-agatoxin IVA and ω-agatoxin IVB may be a basis for the development of selective and more appropriate peptide analogues. The C-terminal domain of ω-agatoxin IVA has been identified as the active peptide part for P/Q channel blockade (Kim *et al.*, 1995). Some information on active parts of the peptide has also been obtained for ω-agatoxin IVB (Adams *et al.*, 1993). Narrowing down an amino acid sequence to the minimal active motif may provide a basis for the development of peptidomimetics with appropriate pharmacokinetic properties. For ω-conotoxins, channel activity has been to some degree attributed to two conserved amino acid residues Tyr^{13} and Lys^{2} (Sato *et al.*, 1993; Kim *et al.*, 1994), together with other residues in loop 2 and 4 (for overview, see Lewis *et al.*, 2012). Lys^{10} and Arg^{22}, as well as a number of positively charged residues in loops 2 and 4, seem to influence subtype selectivity for the P/Q-type channel (Haack *et al.*, 1993; Nielsen *et al.*, 1999b). There have been encouraging reports on N-type peptide mimetics showing that the development of a pharmacophore in drug discovery based on toxin peptide information is principally possible. Baell *et al.* (2004) generated peptide mimetics for N-type channel blockers based on the peptide information from ω-conotoxin GVIA. One analogue, compound 4a, mimics three side chains of this peptide and potently blocks N-type calcium channels in the micromolar range. It also retains some selectivity over the P/Q-type channel (approx. 20-fold). Parke-Davis (now Pfizer) designed a small-molecule N-type channel blocker mimicking three residues of ω-conotoxin MIIA (Menzler *et al.*, 2000). Further development also resulted in the generation of an orally-available small-molecule blocker with improved physicochemical properties (e.g. Hu *et al.*, 2000).

A different approach to achieving a selective P/Q-type channel blocker is to address the binding site for selective P/Q channel blockers at the channel. For ω-agatoxin IVA, at least (Winterfield and Swartz, 2000), and possibly kurtoxin (Sidach and Mintz, 2002), this site has been localized and is thought to be the S3-S4 linker at the outer mouth of the pore. A displacement assay using a selective ω-agatoxin-based radioligand could be implemented into a high throughput screening. However, this requires the selective small molecules to be actually available in synthetic compound libraries.

Conclusions

The aim of this review was to provide an overview of the vast number of compounds that modulate the P/Q-type channel. Currently, there are only two selective molecules available, which are peptide blockers derived from spider venom. All other compounds discussed here are nonselective, and their activity on other targets is often higher than that on P/Q-type channels. Yet, the knowledge of the distinct profile of each of those compounds is necessary to interpret and design experiments, and perhaps to analyse clinical studies. Knowledge about the spectrum of targets of each of the classical calcium antagonists may also challenge the view that all of the observed effects in animal models and clinical trials are mediated by L-type channel blockade.

Currently, there is not sufficient information on structure–activity relationships available for a focused development of P/Q channel blockers. Recent advances in high-throughput electrophysiological techniques may facilitate screening for small molecules with higher selectivity. Perhaps one may draw hope from peptide chemistry efforts to create P/Q-type specific peptide mimetics with improved pharmacokinetic profiles.

The development of P/Q-type selective tool compounds and lead molecules with sufficient bioavailability and brain penetration will clearly remain a challenge.

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Statement of conflicts of interest

None.

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