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ANTAGONISTS OF NEURONAL CALCIUM CHANNELS: Structure, Function, and Therapeutic Implications[1]

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ABSTRACT

This article reviews the structural and functional diversity of neuronal calcium channels and the therapeutic potential of antagonizing such channels. Through spatial and temporal control of intracellular calcium concentration, voltage-sensitive calcium channels regulate a host of neuronal processes, including neurotransmitter secretion, electrical activity, cytoskeletal function, cell metabolism and proliferation, and gene expression. Several genes elaborate a number of calcium channel isoforms or subtypes—each tailored to specific roles in neuronal function and possessing distinct biophysical properties, distribution, modulation, and pharmacological sensitivity. This diversity has raised the possibility that subtype-specific antagonists could provide novel treatments for some neuropathologies. In fact, neuroprotective and analgesic actions of N-type channel blockers in animals appear to confirm this supposition. These properties prompted human clinical studies evaluating these agents for prevention of neuronal degeneration following ischemic brain trauma and

[1]Abbreviations used in this article: VSCC, voltage-sensitive calcium channel; GVIA, ω-conotoxin GVIA; SNX-124, ω-conotoxin GVIA; MVIIC, ω-conotoxin MVIIC; SNX-230, ω-conotoxin MVIIC; MVIIB, ω-conotoxin MVIIB; SNX-159, ω-conotoxin MVIIB; MVIIA, ω-conotoxin MVIIA; SNX-111, ω-conotoxin MVIIA; MVIID, ω-conotoxin MVIID; SNX-238, ω-conotoxin MVIID; GABA, γ-aminobutyric acid; IC_{50}, concentration to achieve 50% inhibition; ED_{50}, dose to achieve 50% effect; 4VO, four-vessel occlusion; LES, Lambert-Eaton myasthenic syndrome; SCLC, small-cell lung carcinoma.

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for relief of pain. Future medical applications for these blockers and antagonists of other channels subtypes are discussed.

INTRODUCTION AND SCOPE OF REVIEW

Classical calcium channel antagonists of the dihydropyridine, phenylalkylamine, and benzothiazepine classes have been remarkably successful in treating a variety of cardiovascular disorders through their actions on VSCC in cardiac and smooth muscle (1, 2). The recognition that dihydropyridine-sensitive calcium channels are present in the central nervous system (CNS) raised hopes that the classical calcium channel antagonists might also be useful in treating neurological disorders, such as ischemia or head trauma, in which excessive calcium influx into neurons has been implicated. However, VSCC antagonists, such as the dihydropyridines, failed to protect neurons from the consequences of ischemia (3, 4). The reason for the lack of efficacy of the classical calcium antagonists became apparent when it was recognized that neurons contain many distinct classes of calcium channels, only two of which are sensitive to classical antagonists such as dihydropyridines (5–7). Molecular cloning strategies have confirmed that multiple genes code for the proteins that form the VSCC (8–12), and selective peptide antagonists capable of distinguishing the different neuronal VSCC have recently been characterized (13–15). The availability of novel, highly selective antagonists of neuronal VSCC classes has opened the way to investigate the utility of these novel antagonists in treating a variety of pathological conditions in which neuronal VSCC may be involved. The diversity of neuronal VSCC and the structure, function, and therapeutic potential of novel VSCC antagonists are reviewed in this article. This review focuses on the novel classes of neuronal VSCC that are not sensitive to dihydropyridines (non-L-type VSCC). Particular emphasis is placed on the N-type VSCC antagonist SNX-111 because it has already entered clinical trials for the treatment of neuronal degeneration following ischemic brain trauma as well as otherwise intractable pain.

DIVERSITY OF NEURONAL VSCC

Multiple classes of VSCC have been recognized in neuronal tissue, primarily on the basis of their distinct electrophysiological properties (5–7). In addition to the low-threshold channels that mediate transient currents designated T-type, three classes of high-threshold channels, each with a distinct set of electrophysiological and pharmacological properties, were detected in neurons. These were named the L-, N- and P-type channels (5, 7, 16). L-type channels inactivate very slowly and are sensitive to dihydropyridines; N-type channels inactivate more rapidly and are blocked by GVIA; and P-type channels inac-

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tivate extremely slowly and are insensitive to both dihydropyridines and GVIA but are potently blocked by the spider venom peptide ω-AgaIVA. During the past year, two additional types of VSCC, Q-type and R-type, have been described (17, 18). The Q-type channel displays inactivation kinetics similar to those of the N-type channel but is resistant to the actions of dihydropyridines and GVIA, is blocked by AgaIVA (but less potently than AgaIVA blocks P-type channels), and is blocked by another ω-conopeptide designated MVIIC or SNX-230 (which also blocks P- and N-type channels) (13, 15). The R-type channel is resistant to all the agents mentioned above and displays more rapid inactivation than all other high-voltage–activated calcium channels in CNS neurons (17, 18).

The molecular basis of the large diversity of neuronal VSCC is beginning to emerge as a result of molecular cloning strategies. Neuronal calcium channels, as are the related skeletal muscle VSCC, are multisubunit proteins (19–21). A great deal of attention has been focused on the α_1 subunit because it has been found to be a part of every known VSCC, it appears sufficient to form the ion-permeable pore in the membrane (22), and pharmacological agents that directly affect channel function bind to this subunit (23–25). Molecular genetic studies first defined at least four genes designated as classes A, B, C, and D coding for the α_1 subunits of VSCC in neuronal tissue (12). A fifth gene, class E, has recently been identified in both mammalian (26) and electric ray (17, 27) neurons.

Investigation of the electrophysiological and pharmacological properties of the α_1 genes expressed in *Xenopus* oocytes as well as in mammalian cell lines is beginning to provide the information necessary to match the structural α_1 gene with each of the functional VSCC described so far. Thus class C and D genes (α_{1C}, α_{1D}) appear to be responsible for neuronal L-type currents (9, 28), whereas N-type VSCC are generated from the α_{1B} gene (29, 30). Identification of the α_1 gene (or genes) that gives rise to the P- and Q-type VSCC has proved less straightforward. The abundance in cerebellar Purkinje neurons of transcripts that hybridized with probes made from the α_{1A} gene sequence suggested that the α_{1A} subunit generates P-type VSCC (31, 32). However, expression of α_{1A} in oocytes generates the Q-type phenotype, which is quite different from the P-type current (33). On the other hand, the Q-type channel appears to give rise to the largest component in granule cell current (34) and displays electrophysiological and pharmacological properties that are very similar to those of the Q-type VSCC expressed in oocytes (18, 33). The R-type current appears to correspond well with the current generated by the α_{1E} gene expressed in *Xenopus* oocytes (17, 18). The gene class coding for the α_1 subunit of the T-type VSCC has not yet been identified. The functional characteristics of the different classes of neuronal VSCC and the tentative identity of the α_1 genes responsible for each functional type are summarized in Table 1.

Table 1 Diversity of neuronal calcium channel α_1 subunits

Gene product	Electrophysiological type	Drug sensitivity[a]
α_{1A}	P/Q	AgaIVA, MVIIC
α_{1B}	N	GVIA, MVIIA, MVIIC
α_{1C}	L	DHP
α_{1D}	L	DHP
α_{1E}	R	Low concentrations of Ni^{2+}

[a]AgaIVA, ω-agatoxin IVA from *Agelenopsis aperta;* MVIIC, ω-conopeptide MVIIC from *Conus magus* (also known as SNX-230); GVIA, ω-conopeptide GVIA from *Conus geographus* (SNX-124); MVIIA, ω-conopeptide MVIIA from *Conus magus* (SNX-111); DHP, dihydropyridine.

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Four distinct genes coding for the β subunit protein (β_1, β_2, β_3, β_4) as well as at least a half dozen splice variants (β_{1a}, β_{1b}, β_{1c}, β_{2a}, β_{2b}, β_{2c}) have been reported (35–38). The α_2 and δ subunits are linked by disulfide bonds and are derived from the same gene ($\alpha_2\delta$) by posttranslational proteolytic processing (39, 40). A fifth subunit designated γ, found only in skeletal muscle so far, may also form part of the channel complex, depending on the tissue of origin (39, 40).

Elucidation of the physiological function of the novel classes of neuronal VSCC is dependent on the availability of pharmacological agents that can modulate these VSCC in a selective manner. Until recently, there was a severe paucity of such agents. Non–L-type VSCC were primarily recognized by their insensitivity to dihydropyridines, and N-type VSCC were found to be blocked by a peptide toxin isolated from the venom of a fish-eating marine snail *Conus geographus* (41). Characterization of compounds, especially peptides, found in the venoms of cone snails as well as spiders during the past five years has resulted in the discovery of several additional blockers of non–L-type VSCC (15, 42). The structural features, pharmacological properties, and medical potential of these novel antagonists of neuronal VSCC are discussed below.

STRUCTURAL FEATURES OF PEPTIDE BLOCKERS OF NEURONAL VSCC

Conopeptides

The venoms of the fish-eating marine snails of the genus *Conus* are rich in peptides that interact with high affinity and specificity with a variety of targets in the CNS (43, 44). Among these, the ω-conopeptide GVIA isolated from the venom of *C. geographus* (45) was found to be a specific blocker of N-type

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VSCC (41). GVIA has been used extensively to identify and characterize N-type VSCC in a variety of neuronal cell types (46–50). Isolation and characterization of additional members of the ω-conopeptide family from the venom of several other species of *Conus* have been particularly helpful in distinguishing the novel classes of neuronal VSCC that have been discovered by the molecular cloning strategies discussed above. Four distinct ω-conopeptides have been characterized from *Conus magus*. Two of these, MVIIA and MVIIB, were isolated directly from the venom of *C. magus* (51). Two additional peptides, MVIIC and MVIID, were discovered from sequences of genes cloned from a *C. magus* venom duct cDNA library (11, 52). MVIIA and MVIIC have been particularly useful in characterizing neuronal VSCC. Additional ω-conopeptides have been isolated from the venoms of *Conus striatus* (53), *Conus tulipa* (54), and *Conus radiatus* (54).

The primary structures of the naturally occurring ω-conopeptides derived from several species of *Conus* are shown in Table 2. The ω-conopeptides are very basic peptides, ranging in size from 24 to 29 amino acid residues. All of them contain six cysteine residues linked to form three disulfide bridges. In the case of GVIA, the disulfide bonding was shown to be between the first and fourth, the second and fifth, and the third and sixth cysteine residues (55). All the naturally occurring ω-conopeptides listed in Table 2 as well as a large number of analogues have been chemically synthesized by solid-phase peptide synthesis procedures (13, 15, 52–57). The synthetic peptides were all shown to have the same disulfide arrangement as the one in GVIA (D Chung, S Gaur, J Bell, J Ramachandran & L Nadasdi, unpublished data). The biochemical, pharmacological, and morphological studies reported in the recent literature have used well-characterized synthetic ω-conopeptides that have been designated by SNX numbers. This nomenclature is also given in Table 2. Thus SNX-124 refers to synthetic GVIA, SNX-111 to synthetic MVIIA, SNX-230 to synthetic MVIIC, and so on. Because each of the ω-conopeptides can form three disulfide bonds in 15 different ways, the linear sequences represented by the designations MVIIA, MVIIC, etc are incomplete representations of these compounds. The SNX numbers refer to compounds with a given sequence folded in a unique disulfide arrangement. Thus SNX-111 refers to a peptide with the amino acid sequence corresponding to MVIIA and folded to form Cys1-Cys4, Cys2-Cys5, and Cys3-Cys6 disulfide bridges.

Besides the invariant cysteine residues, glycine in position 5 appears to be the only residue that is conserved. The ω-conopeptides are generally very polar and highly water soluble. The solution structure of GVIA (SNX-124) has been determined using two-dimensional NMR spectroscopy with the aid of distance geometry and restrained molecular dynamics calculations (58–60). The conformation of the peptide backbone is highly constrained by the three disulfide bonds and by several hydrogen bonds forming an antiparallel triple stranded

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Table 2 Sequences of naturally occurring ω-conopeptides

Name[a]	Sequence[b]	Species
SNX-124 (GVIA)	C K S X G S S C S X T S Y N C C R – S C N X Y T K R C Y	*Conus geographus*
SNX-178 (GVIIA)	C K S X G T X C S R G M R D C C T – S C L L Y S N K C R R Y	*C. geographus*
SNX-111 (MVIIA)	C K G K G A K C S R L M Y D C C T G S C – R – S G K C	*Conus magus*
SNX-159 (MVIIB)	C K G K G A S C H R T S Y D C C T G S C N R – – G K C	*C. magus*
SNX-230 (MVIIC)	C K G K G A P C R K T M Y D C C S G S C G R – R G K C	*C. magus*
SNX-238 (MVIID)	C Q G R G A S C R K T M Y N C C S G S C N R – – G R C	*C. magus*
SNX-157 (SVIA)	C R S S G S X C G V T S I – C C – G R C – – Y R G K C T	*Conus striatus*
SNX-183 (SVIB)	C K L K G Q S C R K T S Y D C C S G S C G R – S G K C	*C. striatus*
SNX-185 (TVIA)	C L S X G S S C S X T S Y N C C R – S C N X Y S R K C	*Conus tulipa*

[a] SNX numbers refer to synthetically prepared peptides.

[b] All synthetic peptides are amidated at the carboxy terminus. The peptides GVIA, MVIIA, and MVIIB are known to be amidated at the carboxy terminus in the *Conus* venom. Whether or not any of the other naturally occurring peptides in *Conus* venoms are also amidated is not known. X = hydroxyproline. Dashes indicate gaps introduced to optimize sequence alignments.

β-sheet. The structure contains four tight turns and is well defined over its entire length.

Spider Venom Peptides

The strategy of prey capture through the actions of a mixture of toxins directed toward multiple molecular targets is also widely prevalent in arthropods. Among spiders, the American funnel-web spider *Agelenopsis aperta* has been most comprehensively investigated (36, 61–63). The venom of *A. aperta* contains three groups of neurotoxins, one of which, the ω-agatoxins, is composed of peptide antagonists of VSCC. The ω-agatoxins have been further classified into four types: Types I and II block insect neuromuscular transmission, and Types III and IV block ^{45}Ca influx into rat brain synaptosomes and have no effect on the insect neuromuscular junction. ω-AgaIIIA is a polypeptide composed of 76 amino acid residues with 12 cysteines (64) that form six disulfide bridges. AgaIIIA turns out to be a nonselective blocker of L-, N-, and P-type VSCC (65). The most selective spider venom peptides are ω-AgaIVA and ω-AgaIVB, and P- and Q-type antagonists. These are closely related peptides of 48 amino acid residues with 8 cysteines in identical locations (14, 66). AgaIVA blocks P-type VSCC in Purkinje neurons with high affinity and has no effect on L- and N-type VSCC. It blocks Q-type VSCC with lower affinity and has been highly useful in characterizing amino acid neurotransmitter release, which seems to be regulated primarily by P- and Q-type VSCC (see below). Both AgaIVA and AgaIVB have been chemically synthesized, and preliminary structural studies of AgaIVB by two-dimensional NMR have been reported (66). The disulfide bonding in AgaIVA and AgaIVB is similar to that of the ω-conopeptides, with a small additional disulfide-bonded loop residing inside the large loop of the third disulfide bond (66).

PHARMACOLOGICAL PROPERTIES OF NEURONAL VSCC ANTAGONISTS

Characteristics and Location of Binding Sites

The interaction of the synthetic ω-conopeptides SNX-111 (MVIIA) and SNX-230 (MVIIC) with rat brain synaptosomal preparations has been investigated with the aid of well-characterized ^{125}I (radioactive) and ^{127}I (nonradioactive) derivatives of the tyrosine in position 13 in these two peptides (67). Saturation analysis showed that ^{125}I–SNX-111 and ^{125}I–SNX-230 bound to two distinct classes of high-affinity sites with apparent dissociation constants (K_d) of 9 pM and 11 pM and capacities of 0.54 pmol mg^{-1} protein and 2.2 pmol mg^{-1} protein, respectively. Kinetic studies revealed that both peptides exhibited high association rates as well as rapid dissociation rates. In contrast, ^{125}I–SNX-124

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(GVIA) displayed extremely slow dissociation from rat brain synaptosomes. Competition binding experiments with ^{125}I–SNX-111 and ^{125}I–SNX-124 established that both of them bind on the same site, namely N-type VSCC, which has previously been clearly established as the target of SNX-124 (GVIA) (41, 46–48).

The site detected by the binding of ^{125}I–SNX-230 is distinct from N-type VSCC, since SNX-111 has very low affinity (K_i = 135 nM) in competition studies. Based on recent reports (34) of a novel high-voltage–activated calcium channel in rat cerebellar granule neurons that is resistant to blockers of L-, N-, and P-type VSCC but is highly sensitive to SNX-230, Kristipati et al (67) suggested that the ^{125}I–SNX-230–binding site may represent a novel type of VSCC, which has been termed the Q-type. Alternatively, this high-affinity SNX-230–binding site may represent a VSCC subtype yet to be characterized electrophysiologically—a so-called O-type (68). Introduction of iodine into the two peptides decreased the affinity for the N-type VSCC and increased the affinity for the Q-type (or O-type) VSCC defined by the binding of ^{125}I–SNX-230. Thus SNX-259 (^{127}I–SNX-111) competed for ^{125}I–SNX-111 with an elevenfold lower affinity than SNX-111 but displaced ^{125}I–SNX-230 with threefold higher affinity than SNX-111. Similarly SNX-260 (^{127}I–SNX-230) was fourfold less potent than SNX-230 in displacing ^{125}I–SNX-111 but twice as potent in displacing ^{125}I–SNX-230. These results further substantiate the structural differences between N-type and the Q- or O-type VSCC.

Studies with ^{125}I derivatives of the spider venom peptides AgaIIIA and AgaIVA have been less informative. Compared to the very high affinity displayed by the ω-conopeptides (~10 pM), the ^{125}I-AgaIVA preparation detected binding sites with IC_{50} of 50 nM in rat brain membranes (68). Whether these sites correspond to P-type VSCC that are blocked effectively by AgaIVA is unclear.

The neuroanatomical distribution of neuronal VSCC is being elucidated with the aid of radioactive derivatives of the novel selective antagonists as well as antibodies capable of recognizing unique segments of the α_1 subunits of the VSCC. Both ^{3}H-nitrendipine (69) and monoclonal antibodies against dihydropyridine-sensitive calcium channels from skeletal muscle (19) have shown that L-type VSCCs are primarily localized to cell bodies and dendrites of neurons in the CNS of the rat. Receptors for ^{125}I-GVIA (N-type VSCC) were found in regions enriched in synaptic connections (70, 71). An antibody that recognized the α_1 subunit of N-type VSCC was predominantly localized to discrete dendritic regions that probably correspond to synapses (72). Gohil et al (73) reported the distribution of binding sites for ^{125}I–SNX-111 (N-type VSCC) and ^{125}I–SNX-230 (Q-type or O-type VSCC?). The highest binding densities for both ligands were seen in areas rich in synaptic connections, such as the oriens, radiatum, and molecular layers of the hippocampus. In most

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areas of the brain, binding of the two ligands overlapped. However, in the glomeruli of the olfactory bulb, binding of ^{125}I–SNX-230 was undetectable, in contrast to the high density of ^{125}I–SNX-111 binding. In cervical spinal cord both ligands bound to the dorsal-most two or three layers of Rexed, whereas only ^{125}I–SNX-230 bound to lower layers. In general, the density of ^{125}I–SNX-230 binding was higher in cerebellum compared to that in forebrain; this general distribution of density was reversed for ^{125}I–SNX-111. Because saturating concentrations of SNX-111 did not affect the pattern of ^{125}I–SNX-230 binding, these binding sites are distinct from those of ^{125}I–SNX-111, even in regions where they are colocalized macroscopically. These studies further reinforce that N-type VSCC recognized with high affinity by ^{125}I–SNX-111 and the VSCC recognized by ^{125}I-SNX-230 are distinct entities.

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Regulation of Transmitter Release

Recent studies suggest that the currently known VSCC ligands may define sets of homologous, yet structurally distinct, binding sites that occur across most or all of the high-voltage–activated VSCC subtypes. A well-studied example is the AgaIIIA-MVIIC-GVIA "macrosite" that is present on all high-voltage–activated VSCC tested thus far (42, 68). AgaIIIA exhibits nanomolar affinity for this site on L-, N-, P-, and O- and/or Q-type VSCC, whereas GVIA appears to be considerably more sensitive to the detailed structural differences, or "microsites," within the macrosite of each channel subtype and binds with high affinity to only the N-type VSCC. MVIIC displays intermediate discrimination, binding to N-, P-, and O- and/or Q-types, but not the L-type, with high affinity. Thus it is conceivable that receptor macrosites homologous to those for AgaIVA on P- and Q-type VSCCs exist on other subtypes. Moreover, if currently cryptic sites for some or all of the classic organic L-type VSCC antagonists are conserved on non-L subtypes, the potential for developing novel VSCC-directed therapeutics is manifold. The amino acid sequences of domains that contribute to the binding sites of dihydropyridines and phenylalkylamines in the L-type calcium channels are well conserved in the non-L-type channels (see 73a).

Perhaps no other function of VSCC in the nervous system is more important than the control of the calcium-dependent secretion of neurotransmitters from presynaptic nerve terminals to initiate synaptic transmission. The bulk of this task is apparently handled by the N-, P-, and Q-type VSCC, while T- and L-types appear to make little direct contribution to the process (however, see 73b). Thus it is probably more than coincidental that most naturally occurring VSCC antagonists are specifically directed against the N-P-Q subfamily of calcium channels; there are essentially no known naturally occurring antagonists that specifically block T-type or L-type VSCC.

Through the use of subtype-specific VSCC antagonists, it is now clear that

the N, P, and Q subtypes support the release of distinct but overlapping pools of neurotransmitters. This differential distribution of presynaptic VSCC subtypes cuts across transmitter classes as well as regional localization of synapses within the nervous system (reviewed in 42). The differential regulation of release of distinct pools of transmitter forms the basis for development of safe and effective neuroactive therapeutic agents directed against VSCC subtypes. The subtype-specific VSCC antagonists described in this review provide some of the reagents necessary to advance this development.

The inhibitory effects of N-, P-, and Q-type VSCC antagonists in a variety of systems have been comprehensively reviewed (42). Here, we focus on recent progress in elucidating some of the details of the differential regulation of transmitter release by VSCC subtypes.

Turner et al (74, 75) have shown that an AgaIVA-sensitive VSCC triggers the release of ^{3}H-glutamate and ^{3}H-dopamine evoked by K^+ depolarization from rat striatal synaptosomes. These investigators designated this AgaIVA-sensitive VSCC as the P-type. A fraction of dopamine release, but not glutamate release, was also blocked by GVIA and thus is controlled by N-type channels. With strong depolarizations, where neither peptide was very effective alone, a combination of AgaIVA and GVIA produced a synergistic inhibition of 60–80% of dopamine release. These results suggested that P- and N-type VSCC coexist in dopaminergic terminals to regulate neurosecretion. That is, strong depolarizations are likely to activate sufficient numbers of both channel subtypes so that calcium entry through either VSCC subtype alone can sustain maximal dopamine release. When both subtypes are blocked, however, a profound inhibition of secretion results. A similar situation appears to prevail in glutamatergic terminals. The partial block of glutamate release by AgaIVA was more efficacious at weaker levels of depolarization but essentially had no effect when the K^+ concentration was raised. These results suggested that a P-type channel and a non-N-, non-P-type channel coexist in glutamatergic terminals to promote release. A component (~20%) of dopamine release also resistant to both GVIA and AgaIVA was observed, suggesting that an additional non-N-, non-P-type VSCC subtype coexists with N- and P-type VSCC to regulate release of this transmitter as well. Turner et al propose that the coexistence of multiple VSCC subtypes on single populations of nerve terminals lends flexibility to the regulation of transmitter release.

Similar conclusions were drawn by Gaur et al (76) from comparisons of the inhibitory effects of the N-type VSCC blocker SNX-111 (MVIIA), the P-type blocker AgaIVA, and the P- and/or Q-type antagonist MVIIC (SNX-230) on the K^+-evoked release of ^{3}H-D-aspartate (a nonmetabolizable analogue of glutamate), ^{3}H-GABA, and ^{3}H-norepinephrine from rat hippocampal slices. MVIIC (SNX-230) blocked both D-asp and GABA release completely, whereas AgaIVA blocked them potently but partially (approximately 50%),

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and SNX-111 had no effect up to 10 μM. The potency of D-asp block by MVIIC (SNX-230) is in the range of its potency for inhibiting both electrophysiologically defined P-type and Q-type VSCC currents in cerebellar granule cells (18, 33). Moreover, the potency of AgaIVA is similar to its potency for blocking P-type currents in Purkinje neurons (14). These results suggest that glutamate release is mediated by two VSCC subtypes, a P-type and a non-N-, non-P-type, perhaps Q-type, VSCC (18, 34, 77) (see below). How this VSCC subtype corresponds with the non-N-, non-P-type VSCC that Turner et al (75) found to mediate a portion of glutamate release from brain synaptosomes remains to be determined. The potency of inhibition of GABA release by AgaIVA and by MVIIC (SNX-230) is somewhat lower than that for D-asp block. Whether or not this reflects a fundamental difference between the two VSCC subtypes that trigger glutamate release and the two that mediate GABA release requires further investigation.

Gaur et al found that K-evoked norepinephrine release is also supported by at least two VSCC subtypes (76). The N-type blockers GVIA and SNX-111 (MVIIA) block norepinephrine potently but partially, whereas SNX-230 blockade is complete but biphasic. AgaIVA also blocks norepinephrine release potently but partially. These results suggest that one of these VSCC subtypes is an N-type and that the other is a novel non-N-type that is extremely sensitive to SNX-230 (MVIIC) and AgaIVA (IC_{50} = 20 pM for both peptides). Two questions arise that are, for the moment, unanswered: Is this VSCC subtype the recently proposed O-type channel that has been suggested to correspond to the high-affinity SNX-230 (MVIIC)–binding site in rat brain synaptic membranes (68)? And, how does this SNX-230 (MVIIC)– and AgaIVA-sensitive VSCC relate to the P-like VSCC that triggers dopamine release observed by Turner et al (75)?

The sum of the block of norepinephrine release by AgaIVA alone (65%) and SNX-111 (MVIIA) alone (75%) was greater than 100%. The simplest explanation for these observations is that both the N-type and the non-N-type [O-type? (68)] VSCC contribute to release of a common pool of norepinephrine, supporting the notion that multiple VSCC subtypes coexist on nerve terminals to effect transmitter release. Pair-wise combinations of the three ligands indicated that at least three pharmacologically distinct components comprise norepinephrine release in the hippocampus; in addition to the two cited above, there appears to be a small component that is resistant to SNX-111 (MVIIA) and SNX-230 (MVIIC) but sensitive to AgaIVA (76).

Takahashi & Momiyama (78) have also obtained evidence for multiple VSCC subtypes mediating transmitter release at central synapses by investigating the effects of GVIA and AgaIVA on electrically stimulated postsynaptic currents recorded from thin slices of rat central nervous system. GABAergic inhibitory postsynaptic currents (IPSCs) evoked in cerebellar neurons by stim-

ulating Purkinje cell axons were completely and irreversibly blocked by AgaIVA (IC_{50} of ~10 nM) but partially (~50%) and irreversibly blocked by GVIA. As discussed above, because the sum of the inhibition of release by each of the two subtype-specific antagonists alone exceeded 100%, these results are consistent with a P-type VSCC and an N-type VSCC coexisting on the same set of nerve terminals to mediate GABA secretion. Similar results were obtained and similar conclusions drawn from experiments with IPSCs evoked at glycinergic synapses in spinal dorsal horn and with excitatory postsynaptic potentials at glutamatergic synapses on hippocampal CA1 pyramidal cell bodies.

Are presynaptic VSCC subtypes in the same terminal actually under differential regulation, and is this differential regulation reflected in the modulation of transmitter release? Wheeler et al attempted to answer these questions by studying the glutamatergic synapse between hippocampal CA3 and CA1 neurons (77). They showed that about 50% of the electrically evoked synaptic transmission between these neurons is blocked by GVIA and therefore mediated by N-type calcium channels.[2] The remainder of the CA3-CA1 transmission is mediated by channels whose pharmacology resembles that of the Q-type channel current in cerebellar granule cells (18, 34) and that are encoded by the α_{1A} subunit gene expressed in oocytes (33). High concentrations of AgaIVA alone maximally blocked about 75% of transmission. Thus selective blockade of N- or Q-type channels produced reductions in synaptic strength whose sum exceeded 100%. As in the other systems described above, the simplest explanation is that multiple VSCC subtypes coexist on nerve terminals to elicit neurotransmitter release.

Wheeler et al then showed that after complete blockade of N-type channels, transmission was strongly modulated by stimulation of metabotropic glutamate, $GABA_B$, adenosine, or acetylcholine receptors or protein kinase C levels by the appropriate agonists (77). The modulatory effects observed were much the same as those previously shown for N-type VSCC (81–83). Thus differential regulation as a rationale for the coexistence of more than one VSCC subtype on a single terminal remains to be demonstrated.

Taken together, these findings show that neurotransmitter release depends on multiple subtypes of calcium channels and that the complement of channels

[2]These observations of partial GVIA sensitivity of glutamate release agree with those of Takahashi & Momiyama (78) but appear to be at odds with those of Turner et al (74, 75) and Gaur et al (76). This apparent discrepancy may be due to the particular population of synapses in each of the studies. Alternatively, K^+ depolarization [used by Turner et al (74, 75) and Gaur et al (76)] is milder, yet much longer than electrical depolarization [used by Takahashi & Momiyama (78) and Wheeler et al (77)]; thus the recruitment of VSCC over time may be very different as a result of the different methods of depolarization [see, however, Burke et al (79)]. Momiyama & Takahashi (80) also discuss the differential effects of K^+ depolarization and electrical stimulation on release.

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may vary from one population of synapses to another. Such complexity in regulation of synaptic transmission by VSCC subtypes presents numerous opportunities for therapeutic intervention.

THERAPEUTIC IMPLICATIONS

The various VSCC-regulated components of transmitter release, electrical signaling in cell bodies and dendrites, and intracellular calcium signaling, which contribute to numerous higher-order physiological processes, are each potential targets for therapeutic intervention. The structural diversity of VSCC subtypes has set the stage for their differentiation by pharmacological agents. In this section, drugs currently in development as non-L-type VSCC antagonists are summarized and some of the directions and key issues for future development are discussed. Because N-type antagonists are furthest along in drug development, the progress of this class of antagonists is highlighted.

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Antihypertension

N-type VSCC antagonists potently block release of norepinephrine from terminals of sympathetic nerves (84, 85). Therefore, these agents may find use as novel therapies for some forms of hypertension (86). Both SNX-111 (MVIIA) and GVIA block pressor responses elicited by electrical sympathetic nerve stimulation in pithed rats but, in the same preparation, do not prevent increases in blood pressure evoked by exogenously administered norepinephrine; these actions are consistent with inhibition of presynaptic norepinephrine release as the site of action (87–90). Bolus intravenous injections of SNX-111, as well as other ω-conopeptides, lower arterial blood pressure in intact rats in a dose-dependent manner. The dose-response curve is biphasic, reflecting a combination of sympathetic blockade at low doses and activation of histamine release from mast cells at higher doses (87). The sympatholytic actions are long-lived; a single intravenous dose blocks pressor responses in the pithed rat for 15 hours or more. The histaminic hypotension is prevented by pretreatment with H1 and H2 histamine receptor antagonists or by administering SNX-111 slowly by continuous infusion at rates that do not challenge mast cells (87). Intracerebroventricular injection of SNX-111, GVIA, and other N channel–blocking ω-conopeptides induce tachycardia and mild elevations in mean arterial blood pressure, supporting the peripheral nervous system as the site of hypotensive action of this class of drug (87, 91).

Neuroprotection

GLOBAL ISCHEMIA Activation of excitatory amino acid transmitter receptors and intracellular accumulation of calcium are strongly implicated in the pro-

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motion of neuronal degeneration following brain ischemia (92). Thus VSCC blockers, especially those that block excitatory transmitter release, might be expected to be neuroprotective following ischemic insult to the brain. Early tests of this hypothesis employing L-type VSCC blockers produced variable and inconsistent findings, generally indicating that the L channel antagonists have little or no neuroprotective activity (3, 4, 93). In some of the first assessments of neuroprotective activity of N channel antagonists, Madden et al (94) found that GVIA provided no significant neuroprotection in vitro to cultured rat cortical neurons subjected to hypoxic challenge or in vivo to rabbit spinal cord neurons that suffered transient spinal ischemia. In contrast, more recent studies using the ω-conopeptides have shown that selective N-type VSCC blockers are neuroprotective in rodent models of both global and focal brain ischemia.

SNX-111 (MVIIA) has been tested in the rat 4VO model of transient global forebrain ischemia (56, 95). A single bolus intravenous injection of peptide at times from 0 to 24 h but not 48 h after occlusion decreased damage to pyramidal neurons in the hippocampal CA1 subfield. Interestingly, the apparent neuroprotective potency of SNX-111 is increased if administration of the compound is delayed. When hippocampal damage is compared in SNX-111–treated 4VO animals after 5 or 12 days of survival, damage scores are comparably reduced at both time points, indicating that SNX-111 prevents, and not simply delays, ischemia-related neuronal degeneration. SNX-111 is also neuroprotective in the rat 4VO model at submicrogram doses when administered intracerebroventricularly, suggesting (*a*) that the site of action of the drug is in the brain and (*b*) that a small but biologically significant fraction of the compound administered intravenously crosses the blood-brain barrier. Because N-type VSCC blockers are hypothermic agents and hypothermia itself is neuroprotective, normothermia was carefully maintained during all of the rat global ischemia experiments described in this section.

Continuous, constant-rate intravenous infusion over 24 h starting at 1 h postocclusion also enhances the apparent neuroprotective potency of SNX-111 (ED_{50} = 0.4 mg kg^{-1}) compared to bolus administration (96). With intravenous infusion for 24 h, neuroprotective doses are achieved without eliciting tremor (see below) or histamine-dependent hypotension (see section on antihypertension, above). In the rat two-vessel-plus-hypotension model, SNX-111 infused over 15 min 6 h postischemia also decreased neuronal necrosis in neocortex and CA1 hippocampus, demonstrating that the neuroprotective activity of N-type VSCC antagonists is widespread and not confined to a relatively small population of neurons in the hippocampus (97).

Taken together, the above results suggest that in these models the point in the neurodegenerative process susceptible to N-type calcium channel blockers occurs at about 24 h after the ischemic insult. The rank order of neuroprotective

potency for a series of ω-conopeptides is essentially the same as the rank order of affinity of these compounds for binding to N-type channels, consistent with N channels as the site of neuroprotective action for this class of compound (T Singh, K Valentino, B Hoffman, G Miljanich, L Nadasdi & J Ramachandran, unpublished results).

The N-type channel blocker GVIA has been tested in the gerbil model of transient global ischemia and found to protect CA1 neurons from degeneration (98). In contrast, the same investigators showed that the peptide failed to protect hippocampal neurons against quinolinic acid–induced neurotoxicity. Because quinolinic acid is a glutamate (NMDA) receptor agonist, Yamada et al (98) suggest that the neuroprotective effect of N channel antagonists results from its inhibition of excessive release of neurotransmitters, including excitatory amino acids, during ischemia.

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FOCAL ISCHEMIA Several studies have shown that N-type VSCC antagonists are neuroprotective in focal models of cerebral ischemia (99–101). For example, SNX-111 decreased neocortical infarction volumes by approximately 40% in spontaneously hypertensive rats subjected to permanent occlusion of the right common carotid artery and transient occlusion of the right middle cerebral artery when administered one hour after the onset of ischemia. Xue et al (99) obtained similar outcomes in normotensive rats. As with the rat global ischemia studies described above, normothermia was carefully maintained in all focal model studies described in this section.

Continuous infusion of SNX-111 for 3 h beginning 20 min prior to the ischemic insult reduced cortical infarct volumes by approximately 60% compared to vehicle controls in rats subjected to 60 min of left common carotid artery occlusion and permanent ligation of the right cerebral and common carotid arteries (101). Mean arterial blood pressure was reduced by approximately 25% by drug infusion prior to and during occlusion and to a lesser extent after termination of drug infusion following occlusion. No significant differences in cerebral blood flow between drug-treated and saline-treated animals were observed. Occlusion induced an increase in extracellular glutamate levels in vehicle-treated animals that reached a peak of 36-fold over preocclusion levels 50 min into the ischemic episode. The time course of glutamate levels in SNX-111-treated animals was similar, except that the levels at all time points were significantly reduced compared to saline-treated animals. Takizawa et al (101) suggest that SNX-111 achieves its neuroprotective effects in focal ischemia through inhibition of the release of excitatory amino acids.

TOXICOLOGY The potent and unusual motor effects elicited by N-type VSCC antagonists were recognized early on in the effort to isolate and characterize

these compounds (102). Within 10–20 min after intravenous injection of SNX-111, rats develop dose-dependent body tremors and rotatory nystagmus (90). These effects are reversible but persist for many hours after injection of the drug.

In cynomolgus monkeys, no motor effects were seen when SNX-111 was infused intravenously at 3.5 mg kg^{-1} over 6 h, and the drug was well tolerated with rare tremors when administered at 1.5 mg kg^{-1} over 24 h for 14 days (90). Mild, intermittent tremors were observed following infusion of 5 mg kg^{-1} over 24 h, with more severe effects at higher doses (up to 175 mg kg^{-1} over 6 h and 25 mg kg^{-1} over 24 h). (Note that the ED_{50} for neuroprotection in rats subjected to transient global ischemia is 0.4 mg kg^{-1} over 24 h; see above.) Intracerebroventricular injection into rats or mice of much smaller doses (i.e. submicrogram amounts) of GVIA or SNX-111, for example, also induces dose-dependent whole-body tremors and rotatory nystagmus. These results strongly suggest that, as are the neuroprotective effects, these motor effects are centrally mediated.

N-type VSCC antagonists also induce hypothermia in a dose-dependent manner, whether administered intravenously or intracerebroventricularly. Thus this effect also appears to be centrally mediated. The intravenous doses required to achieve both the motor and hypothermic effects are roughly three to four orders of magnitude greater than the intracerebroventricular doses that promote the same effects. This difference is consistent with the blood-brain barrier permeability of these peptides. That is, over a large dose range, roughly 0.1 to 0.01% of the total administered dose gains access to the central compartment (R Newcomb, J Bell & J Ramachandran, unpublished results).

The safety profile of SNX-111 administered by continuous intravenous infusion has been evaluated in both single-dose and repeated-dose (14-day) studies in rats and monkeys (90). Clinical signs in both species consisted of tremor, incoordination, and moderate decreases in heart rate and blood pressure. These effects reversed spontaneously upon termination of single-dose administration or, in repeated-dose studies, either remained constant in severity and frequency or tended to dissipate with dosing. There was no histopathological evidence of toxicity in a variety of internal organs examined after either single-dose (monkeys: 3.5–175 mg kg^{-1} over 6 h; rats: 10–40 mg kg^{-1} over 24 h) or repeated-dose (monkeys: 1.5–25 mg kg^{-1} over 24 h; rats: 5–40 mg kg^{-1} over 24 h) SNX-111 administration.

PHARMACOKINETICS The pharmacokinetics of SNX-111, when administered for 24 h by continuous, constant-rate infusion, were determined in rats and monkeys (90). For the rat study, doses of 10 mg kg^{-1} over 24 h and 40 mg kg^{-1} over 24 h were used. Plasma SNX-111 levels were measured by radioimmunoassay, and plasma-concentration-versus-time data were evaluated by

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a two-compartment pharmacokinetic model. Peptide concentrations approached apparent steady state after 2 h of infusion and were 1.2 mg liter^{-1} and 4 mg liter^{-1} for the 10 mg kg^{-1} over 24 h and 40 mg kg^{-1} over 24 h doses, respectively. The steady-state volume of distribution and the total body clearance were 0.3 liter kg^{-1} and 0.4 liter kg^{-1} h^{-1}, respectively. For both dose groups, the disposition function for SNX-111 could be fitted with two exponential components. The faster phase (0.4 h plasma half-life) represented 96% of the area under the curve of the unit impulse disposition function, while the slower phase (4.6 h plasma half-life) contributed 4%.

In cynomolgus monkeys (90), continuous, constant-rate intravenous infusion of SNX-111 over 24 h was performed at doses of 11.2 mg kg^{-1} and 25 mg kg^{-1}. Plasma SNX-111 levels approached steady state within 4 h, and apparent steady-state concentrations were 1.4 and 3.2 mg liter^{-1} for the lower and higher doses, respectively. As with the rat, the disposition function could be fitted with two exponential components. The elimination-phase half-life of the major component is 0.7 h, whereas that of the minor component is 6.5 h. The steady-state volume of distribution and the total body clearance are 0.4 liter kg^{-1} and 0.33 liter kg^{-1} h^{-1}, respectively.

CLINICAL STUDIES The N-type VSCC antagonist SNX-111 is neuroprotective in both global and focal models of ischemia and is effective when administered many hours after the ischemic insult. Of all the neuroprotective agents identified thus far, only the AMPA-type glutamate receptor antagonists have shown similarly robust neuroprotective properties in a variety of studies (103). As a result, SNX-111 is the first neuronal VSCC blocker to enter human clinical trials (104). SNX-111 was evaluated in 40 healthy volunteers in a randomized, double-blind, placebo-controlled, escalating-dose, safety and tolerability study of four doses—0.3, 1.0, 3.3, and 10 μg kg^{-1} over 24 h—of SNX-111 administered by continuous, constant-rate intravenous infusion. SNX-111 produced symptomatic orthostatic hypotension without reflex tachycardia as a dose-related pharmacological effect. All subjects receiving the highest dose exhibited this effect. Modest decreases in recumbent systolic and diastolic blood pressure and increases in heart rate relative to the placebo control group were observed, but no pharmacologic support of blood pressure was required. With the exception of orthostatic hypotension, SNX-111 was well tolerated. Reported adverse events included dizziness, syncope, bradycardia, nasal congestion, conjunctival hyperemia, and nausea. Cognitive and affective testing suggested drug-induced fatigue and tension. Not surprisingly, the cardiovascular findings in this study reflect peripheral sympathetic blockade (see section on antihypertension, above).

The results demonstrate that SNX-111 is biologically active at N-type VSCC in humans. Overall, SNX-111 was shown to be safe and well tolerated in the

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recumbent position. Because the patient populations targeted for treatment with SNX-111 are hospitalized, comatose, or minimally responsive, and confined to bed, orthostatic hypotension is not viewed as a clinically significant, dose-limiting adverse effect. Further studies in healthy volunteers will examine the tolerability of higher doses that approximate doses effectively neuroprotective in animals (90, 104).

Analgesia

As detailed in the section on regulation of neurotransmitter release, N-type VSCC antagonists inhibit synaptic transmission at a subset of central and peripheral synapses. N-type VSCCs are highly localized to the superficial layers of the dorsal horn (laminae I and II), which receive dense innervation from the terminations of Aδ and C nociceptive afferents in the spinal cord (73; see above). These observations suggested that, by blocking transmitter release from these inputs, N channel antagonists such as SNX-111 might be antinociceptive but possess few adverse effects, especially if administered intraspinally.

Consistent with this hypothesis, the N-type VSCC blocker SNX-111 does indeed potently suppress evoked pain behavior in rats when administered spinally (intrathecally). For example, SNX-111 has been subjected to the formalin test of nociception, which models both acute pain and persistent pain. Intrathecal bolus administration of SNX-111 significantly attentuates nociceptive behavior in dose-dependent fashion with an IC_{50} between 10 and 100 ng (105, 106). Interestingly, the P- and/or Q-type VSCC blocker SNX-230 [in spite of dense receptor binding in spinal laminae I and II (73)] has no effect on nociception up to a 1 μg bolus dose (106). However, another P- and/or Q-type blocker, AgaIVA, potently blocks formalin-induced pain behavior, as do the nonselective VSCC blockers La^{3+} and Nd^{3+}. In contrast to SNX-111, AgaIVA was found to be highly toxic. The L-type VSCC antagonists—such as nifedipine, verapamil, and diltiazem—are considerably less potent. Nociceptive behavior was also markedly attenuated when low doses of SNX-111 were administered over 72 h by continuous intrathecal infusion. With bolus administration or continuous infusion, SNX-111 is two to three orders of magnitude more potent than morphine under the same test conditions. At lower antinociceptive doses, SNX-111 does not produce detectable adverse behavioral effects. At higher doses, motor effects similar to those observed following intracerebroventricular injection (see above) are observed.

Unlike the opioid agonists, chronic administration of SNX-111 does not lead to the development of tolerance, or desensitization. For example, after two days of continuous infusion with morphine, formalin-evoked nociceptive responses are significantly decreased in rats, and after seven days of infusion, nociceptive responses are no different from those of saline-infused animals, which is indicative of development of complete morphine tolerance. In con-

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trast, after continuous administration of SNX-111 over seven days, formalin-induced nociception remains maximally attenuated throughout (106). Similar results are observed in the hot plate test of nociception. Furthermore, morphine-tolerant rats show no cross-tolerance to SNX-111 (86).

The antinociceptive effects of SNX-111, either administered as a bolus or as a continuous infusion over many days, are completely reversible. The rank order of potencies of conopeptides in blocking nociceptive behavior in the formalin test correlates very well with the rank order of affinities for N-type VSCC binding in rat brain synaptic membranes. (106, 107)

N-type VSCC antagonists are also antinociceptive in rodent models of peripheral neuropathic pain. For example, intrathecal bolus injection of as little as 0.3 μg of SNX-111 acutely blocks tactile allodynia in rats subjected to unilateral ligation of the L5 and L6 spinal nerves just distal to the dorsal root ganglia (108). A longer-lasting, reversible blockade of tactile allodynia is achieved when the compound is administered by continuous intrathecal infusion. Under the same conditions, L-type and P-type VSCC blockers are without effect. These results demonstrate that SNX-111 represents a novel class of potential analgesic agents for treating sufferers of chronic nociceptive and neuropathic pain and possesses promising pharmacological properties distinct from those of the opiates.

Autoimmune Diagnostics

VSCC ligands are proving useful as diagnostic indicators of certain autoimmune neurologic syndromes. In some of these disorders, autoantibodies to VSCC are the pathogenic effectors of impaired synaptic transmission, and in others they are novel serological markers of an associated carcinoma. For example, SCLC is variably associated with a spectrum of paraneoplastic disorders, including several neurological syndromes thought to result from host anticancer immune responses directed against neuron-like components of the SCLC tumor cells. A prototypic disorder is the LES (109). The skeletal muscle weakness and autonomic secretomotor defects that are characteristic of LES appear to result from IgG-mediated down-regulation of presynaptic VSCC in peripheral cholinergic neurons. The disorder typically remains undiagnosed for many months or years, because its definitive diagnosis requires clinical neurophysiological testing by a skilled electromyographer.

Cultured SCLC cells express neuronal-type VSCC activity that is antagonized to varying degrees by IgG from LES patients and less so by GVIA and dihydropyridines (110–112). Analysis of high-affinity binding of GVIA demonstrated the presence of low levels of N-type VSCC on these cells (113). Furthermore, mRNA transcripts for α_{1A} (P- and/or Q-type), α_{1B} (N-type), and α_{1D} (L-type) VSCC subunits have been detected in SCLC cell lines (112–114). It has been proposed that the corresponding channel proteins (perhaps in

mutated form) expressed in the SCLC tumors provide the antigenic determinants for eliciting the anti-VSCC autoantibodies that mediate the autoimmune symptomatology of LES (114). Lennon et al (115) recently found that SCLC cells from patients with and without LES possess high-affinity receptors for MVIIC (the N-, P-, and/or Q-type VSCC blocker) that are distinct from N-type VSCC (115). Sera from 94% of LES patients (64 were tested) with and without a diagnosis of cancer immunoprecipitated solubilized human MVIIC-receptor complexes (116; VA Lennon, TJ Kryzer, GE Griesmann, P O'Suilleabhain, AJ Windebank, A Woppmann, GP Miljanich & EH Lambert, in preparation). Control sera were essentially seronegative (in patients with epilepsy, none out of 20 was positive; in patients with myasthenia gravis, 1 out of 21 was positive). In contrast, use of GVIA-labeled N-type VSCC in similar assays yielded only 75% seropositivity for LES patients with lung cancer, uniform seronegativity for LES patients with other types of cancer, and only 43% seropositivity for LES patients without evidence of cancer (117). The overall seropositivity rate of 94% for all LES patients strongly implicates autoantibodies of P- and/or Q-type VSCC specificity in the pathogenesis of LES. These results are consistent with the finding that the VSCC mediating evoked acetylcholine release and synaptic transmission at the skeletal neuromuscular junction is of the P- and/or Q-type (reviewed in 42). The cited results obtained with solubilized neuronal channels labeled with MVIIC provide the basis for a rapid, convenient immunoprecipitation assay for differentiating LES from symptomatically similar syndromes such as myasthenia gravis.

Future Prospects

N-TYPE Based on positive results in animal models of global and focal ischemia, the ω-conopeptide SNX-111 is in clinical trials for preventing neuronal degeneration following ischemic insult. Phase I studies (safety in normal human volunteers) are in progress, and no unexpected toxicities have emerged. Phase II (tolerability and efficacy in a relatively small group of human patients) will now likely ensue for cardiac bypass surgery, head trauma, cardiac arrest, and/or stroke.

ω-Conopeptides are extremely potent analgesics in animal models and possess a pharmacological profile that distinguishes them from other analgesic agents such as the opiates. In particular, chronic administration does not appear to lead to the development of tolerance, or desensitization, which is characteristic of the opiates. Human trials of intrathecally administered SNX-111 for treating severe, persistent pain refractory to opiates have now commenced (R Luther, personal communication).

Peptides are appropriate chemical forms for some therapeutic applications but not others. For chronic indications or for those requiring efficient blood-

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brain barrier permeability, for example, a small organic N channel antagonist would likely be more desirable. Natural products are one potential source of chemical leads for this purpose. For example, daurisoline, an alkaloid extracted from the gingko tree, has been reported to block N-type VSCC with micromolar affinity (118, 119). Some aminoglycosides are also moderately potent N-type channel blockers (120). However, the subtype specificities of these agents have not been well characterized. In addition, aminoglycoside chemistry provides a daunting challenge for the medicinal chemist. Another potential approach is the design and synthesis of peptidomimetics based on three-dimensional conopeptide structures.

Chronic treatment of psychiatric or neurodegenerative disorders by currently available N channel antagonists using the usual routes of administration may be somewhat limited due to sympathetic and parasympathetic blockade by these compounds—unless central N-type VSCCs and peripheral N-type VSCCs are found to be pharmacologically distinguishable. Thus far, the evidence to support a difference is scant.

R-TYPE The R channel subtype (26) has not been definitively associated with transmitter release in any neural system and, therefore, may be largely somatic and/or dendritic. Northern analysis showed that the α_{1E} transcript is expressed throughout the nervous system. Its regional distribution within the brain has been reported using in situ hybridization techniques and is widespread. A specific antagonist for the R subtype has yet to be discovered. Such a ligand is needed to elucidate the functions of this subtype and to reveal potential therapeutic applications for drugs directed against it. One advantage of selective R-type antagonists seems to be the lack of reports of significant populations of this channel in the peripheral nervous system. Thus the possibility of a selective therapeutic with salutary central efficacy but little or no adverse action peripherally may be reasonably entertained, and chronic treatment of psychiatric and/or neurodegenerative disorders is conceivable.

P- AND/OR Q-TYPE P- and/or Q-type channels differentially mediate a large fraction of release of various transmitters and thus are potential therapeutic targets. However, both intracerebroventricular and intravenous administration of P- and/or Q-type blockers such as AgaIVA or MVIIC (SNX-230) are potently lethal. Might a P-specific or Q-specific ligand be less toxic than the less-selective ligands presently known and, in addition, have therapeutic utility? Such highly specific ligands are needed to answer this question. As mentioned above, because MVIIC binds with high affinity to the P- and/or Q-type VSCC that are the target of the autoimmunity underlying the pathology of LES, it may have applications as a diagnostic probe for LES and other autoimmune disorders directed against P- and/or Q-type channels.

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INHIBITORS OF SUBUNIT-SUBUNIT INTERACTIONS As summarized in the section on diversity of neuronal VSCC, neuronal VSCC are multimeric, consisting of, at least, closely associated α_1, α_2, δ, and β subunits. Studies of the effects of various combinations of the pore-bearing α_1 subunit with the other subunits show that these other subunits can markedly alter activation and inactivation kinetics (121). Therefore, sites of subunit-subunit interactions are potential targets for therapeutic intervention either as agonists or antagonists of channel activity.

β subunits can affect the coupling of gating charge movement to channel opening in L-type VSCC (122). For α_{1C} subunits expressed in *Xenopus* oocytes, coexpression of β_{2a} increases the efficiency of this coupling fivefold. This increase suggests that this β subunit lowers the energy barrier for opening the channel pore and thereby increases peak calcium currents. Significant progress has been made recently in identifying the specific domains on both the α_1 subunit and the β subunit that mediate their interaction. Pragnell et al (123) have shown that the β subunit binds to the cytoplasmic linker between transmembrane homology domains I and II on α_{1S}, α_{1C}, α_{1A}, and α_{1B} subunits. A sequence motif, QQ-E- -L-GY- -WI- -E, conserved among all six known α_1 subunits, appears to be specifically responsible for β subunit binding. Mutations within this motif reduce the effects of coexpressed β subunit on channel kinetics in oocytes. By screening epitope libraries of α_1 sequence fragments, it was found that peptides as small as 50 amino acids retain the ability to interact with the β subunit, suggesting that the design and synthesis of specific peptide inhibitors of the α_1-β interaction are feasible. The functional effects of the sequence fragments generated were not assessed in this study.

On the other side of the α_1-β interaction, a stretch of amino acids in the sequence of β subunits sufficient for binding of β to α_1 has been identified (124). Mutations in this region abolish α_1 binding as well as the biophysical effects of β on barium currents in oocytes. This 30–amino acid domain is located in a region that is highly conserved among the four known β subtypes. Coexpression of β subunit sequence fragments as small as 35 amino acids that encompassed this domain stimulated current density, similar to the effects of full-length β. This result demonstrates that synthetic channel agonists (and perhaps antagonists) targeted to the β-binding site on α_1 are more than a theoretical concept. Because the site of α_1-β interaction is intracellular, small organic compounds that readily permeate the neuronal membrane will likely be required in order to demonstrate therapeutic promise in animal models of disease as well as to achieve therapeutic utility in humans.

In the future, the therapeutic potential of inhibiting or activating VSCC will expand as the subtler forms of molecular diversity within each subunit subtype are revealed. Thus splice variants, posttranslational processing, and multiplicity of subunit association could confer differential pharmacological sensitivities that may be exploited for medical benefit.

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