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The organ-protective effect of N-type Ca^{2+} channel blockade

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Abstract
The six subtypes of voltage-dependent Ca\(^{2+}\) channels (VDCCs) mediate a wide range of physiological responses. N-type VDCCs (NCCs) were originally identified as a high voltage-activated Ca\(^{2+}\) channel selectively blocked by omega-connotoxin (\(\omega\)-CTX)-GVIA. Predominantly localized in the nervous system, NCCs are key regulators of neurotransmitter release. Both pharmacological blockade with \(\omega\)-CTX-GVIA and, more recently, mice lacking \(CNCNA1B\), encoding the \(\alpha\)1B subunit of NCC, have been used to assess the physiological and pathophysiological functions of NCCs, revealing in part their significant roles in sympathetic nerve activation and nociceptive transmission. The evidence now available indicates that NCCs are a potentially useful therapeutic target for the treatment of several pathological conditions. Efforts are therefore being made to develop effective NCC blockers, including both synthetic \(\omega\)-CTX-GVIA derivatives and small-molecule inhibitors. Cilnidipine, for example, is a dihydropyridine L-type VDCC blocking agent that also possesses significant NCC blocking ability. As over-activation of the sympathetic nervous system appears to contribute to the pathological processes underlying cardiovascular, renal and metabolic diseases, NCC blockade could be a useful approach to treating these ailments. In this review article, we provide an overview of what is currently known about the physiological and pathophysiological activities of NCCs and the potentially beneficial effects of NCC blockade in several disease conditions, in particular cardiovascular diseases.

Keywords: N-type Ca\(^{2+}\) channel, voltage-dependent Ca\(^{2+}\) channel, sympathetic nerve, hypertension
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1. Introduction

By mediating Ca\(^{2+}\) entry into cells, voltage-dependent Ca\(^{2+}\) channels (VDCCs) play key roles in a wide variety of physiological processes, including muscle contraction, Ca\(^{2+}\)-dependent gene transcription, neuronal excitability control and the release of neurotransmitters (Augustine, Charlton, & Smith, 1987; Miller, 1987). Based on their specific pharmacological characteristics, VDCCs have been classified into six subtypes: L, N, P, Q, R and T (Mori, et al., 1996; Varadi, Mori, Mikala, & Schwartz, 1995; Zhang, et al., 1993). T-type Ca\(^{2+}\) channels are known to be low voltage-activated channels that activate and deactivate slowly, but inactivate rapidly (Carbone & Lux, 1984; Fox, Nowycky, & Tsien, 1987; Nowycky, Fox, & Tsien, 1985). T-type Ca\(^{2+}\) channels have been implicated in repetitive firing and pacemaker activities in neurons, and in the gradual depolarization phase of sinus nodal action potentials in hearts (Mesirca, Torrente, & Mangoni, 2014; Perez-Reyes, 2003). In addition, under pathological conditions in the heart, ventricular expression of T-type Ca\(^{2+}\) channels appears to be increased and to contribute to the development of arrhythmogenicity and pathological cardiac remodeling, although there are still controversy about their specific functions (Chiang, et al., 2009; Kinoshita, et al., 2009; Kuwahara, Takano, & Nakao, 2005; Le Quang, et al., 2011; Nakayama, et al., 2009).

The other five VDCCs are high voltage-activated (HVA) channels, which are activated through membrane depolarization to approximately -40 mV (Mori, et al., 1996). Among these, the N-type calcium channel (NCC) is a HVA Ca\(^{2+}\) channel selectively blocked by omega-connotoxin (\(\omega\)-CTX)-GVIA (Olivera, et al., 1985). NCCs are expressed in presynaptic nerve terminals, where they, along with P/Q-type Ca\(^{2+}\) channels and probably, to a lesser extent, R-type Ca\(^{2+}\) channels, regulate release of
neurotransmitters from synaptic vesicles (Dutar, Rascol, & Lamour, 1989; Evans & Zamponi, 2006; Hirning, et al., 1988; Ishibashi, Rhee, & Akaike, 1995; Ishikawa, Kaneko, Shin, & Takahashi, 2005; Kamp, et al., 2005). Experiments using ω-CTX-GVIA indicate that NCCs are important mediators of neurotransmitter release in both the central and peripheral nervous systems (Clasbrummel, Osswald, & Illes, 1989; Dutar, et al., 1989; Hirning, et al., 1988; Ishibashi, et al., 1995; Pruneau & Angus, 1990). In central neurons, for example, NCCs are critically involved in the release of several neurotransmitters, including glutamate (Luebke, Dunlap, & Turner, 1993), γ-aminobutyric acid (GABA) (Luebke, et al., 1993), acetylcholine (Herdon & Nahorski, 1989; Wessler, Dooley, Werhand, & Schlemmer, 1990), dopamine (Dooley, Lupp, Hertting, & Osswald, 1988; Horne & Kemp, 1991; Turner, Adams, & Dunlap, 1993; Woodward, Rezazadeh, & Leslie, 1988) and noradrenaline (Komuro & Rakic, 1992). Likewise, in peripheral neurons, such as autonomic and motor neurons, and in spinal cord neurons, NCCs mediate release of neurotransmitters from nerve terminals (Hirning, et al., 1988).

HVA Ca\(^{2+}\) channels are composed of the α1 subunit, which determines the major characteristics of each VDCC subtype, and the auxiliary α2/δ, β and γ subunits. Among the 10 different genes encoding α1 subunits, which include α1A, α1B, α1C, α1D, α1E, α1F, α1G, α1H, α1I and α1S, CACNA1B encodes the α1B subunit, which comprises the NCC (Y. Fujita, et al., 1993; Williams, Brust, et al., 1992). The α1B subunit is expressed widely in the nervous system, as suggested by experiments using ω-CTX-GVIA (Mills, et al., 1994; Takemura, Kiyama, Fukui, Tohyama, & Wada, 1989; Whorlow, Loiacono, Angus, & Wright, 1996). Although ω-CTX-GVIA has been used to elucidate physiological function of NCCs, ω-CTX-GVIA is a relatively large polypeptide whose distribution in tissue is somewhat limited, and it also appears to inhibit certain neuronal LCCs (Aosaki & Kasai, 1989; Williams, Feldman, et al., 1992). As an alternative, genetic
deletion of \textit{CACNA1B} is a direct means of defining the physiological function of NCCs (Ino, et al., 2001). Using both these pharmacological and genetic approaches, the physiological and pathophysiological functions of NCCs have been investigated. This article reviews what is currently known about the activities of NCCs and the potential organ-protective effects of NCC inhibition in several diseases conditions, focusing in particular on cardiovascular diseases and related disorders.

2. N-type calcium channels and their physiological function in sympathetic nerves

The physiological functions of NCCs have been studied using ω-CTX-GVIA and by generating mice lacking \textit{CACNA1B}, which encodes the α1B subunit of NCCs (Ino, et al., 2001). In \textit{CACNA1B}-null superior cervical ganglion (SCG) neurons, VDCC current density is significantly lower than in wild-type SCG neurons. In addition, ω-CTX-GVIA-sensitive NCC currents are nearly absent in \textit{CACNA1B}-null neurons (Ino, et al., 2001), suggesting the reduction in VDCC currents in \textit{CACNA1B}-null SCG neurons is caused by the elimination of NCCs induced by deletion of \textit{CACNA1B}. It also indicates that no other VDCC subtype compensates for the loss of NCCs SCG neurons.

It has been observed that ω-CTX-GVIA inhibits neurotransmitter release from cultured rat sympathetic neurons and in anesthetized cat heart, and suppresses sympathetic nerve-mediated positive-inotropic effects in isolated guinea pig atria, which suggests NCCs participate in the regulation of sympathetic nerve activity (Hirning, et al., 1988; Hong & Chang, 1995; Serone & Angus, 1999; Toth, Bindokas, Bleakman, Colmers, & Miller, 1993; Vega, De Pascual, Bulbena, & Garcia, 1995; Yahagi, Akiyama, & Yamazaki, 1998; Yamazaki, et al., 1997). Consistent with those findings, it was also observed that the positive inotropic effect is substantially inhibited (from 35% to 8% of basal condition) in isolated atria from NCC knockout (KO) mice. Assuming that the
magnitude of the positive inotropic response reflects the amount of norepinephrine released from sympathetic nerve endings, these results imply that neurotransmitter release from sympathetic nerve terminals is predominantly governed by NCCs. The fact that the negative inotropic response remains intact in isolated atria from NCC KO mice indicates that channels other than NCCs contribute to parasympathetic nerve activity (Mori, et al., 2002). With the exception of an altered response in nociception (Hatakeyama, et al., 2001; Kim, et al., 2001), NCC KO mice show no functional or anatomical abnormalities in the brain (Ino, et al., 2001), indicating the dispensable role of NCCs in the normal development of the central nervous system. A study that addressed the developmental alterations in the VDCC types governing neurotransmitter release at various central synapses showed that P/Q-type channels predominantly mediate synaptic transmission in adult mammalian neurons, which may underlie the finding that NCCs are not essential for the normal features of central nervous system activity in adult mice (Iwasaki, Momiyama, Uchitel, & Takahashi, 2000). On the other hand, the evidence from NCC KO mice demonstrates the essential role played by NCC in regulating sympathetic nervous system activity.

3. N-type calcium channel inhibitors

The NCC blocker ω-CTX-GVIA is a 27-amino acid peptide isolated from venom of the marine cone snail Conus geographus (Olivera, et al., 1985). Likewise, ω-CTX-MVIIA and -CVID isolated from the venom of Conus magnum and Conus catus, respectively, also block NCCs. A synthetic ω-CTX MVIIA derivative, known as SNX-111 or ziconotide, has been approved by the U.S. FDA for treatment of refractory pain. In addition, gabapentin and pregabalin, two GABA analogues without GABAnergic activity used to treat neuropathic pain, have affinity for the α2δ VDCC subunit and inhibit trafficking of
Cav2.2, the α1 pore forming unit of NCCs, from the cytoplasm to the plasma membrane (Cassidy, Ferron, Kadurin, Pratt, & Dolphin, 2014; Lee, 2013).

A dihydropyridine-type Ca\textsuperscript{2+} channel antagonist, cilnidipine, has been shown to not only block LCCs but to effectively suppress NCC activity at sub-micromolar concentrations (Uneyama, et al., 1997). Uneyama compared the inhibitory effects of various dihydropyridines on cardiac LCCs in isolated ventricular myocytes with those on NCCs in rat SCG neurons (Uneyama, Uchida, Konda, Yoshimoto, & Akaike, 1999). They showed that at a concentration of 1 µM all dihydropyridines, except cilnidipine, exert little if any inhibitory effect on NCCs. In dorsal root ganglion neurons, by contrast, cilnidipine exerted similar inhibitory effects on both LCC and NCC currents, but had no effect on P/Q-type Ca\textsuperscript{2+} channel currents (Fujii, Kameyama, Hosono, Hayashi, & Kitamura, 1997). This inhibitory effect of cilnidipine on NCC currents was further confirmed in human neuroblastoma cells (Takahara, et al., 2003).

4. N-type calcium channels and hypertension

Sympathetic nerve activity is a major contributor to the occurrence of hypertension (Julius, Schork, & Schork, 1988). NCC inhibition would therefore be expected to exert a hypotensive effect (Figure 1). Consistent with that idea, administration of ω-CTX-GVIA induces hypotension in some animal models (Bond & Boot, 1992; Pruneau & Angus, 1990). Unexpectedly, however, Ino et al. reported that NCC KO mice show elevated arterial blood pressures and heart rates (Ino, et al., 2001). In that study, the mean arterial blood pressure and heart rate were 102±4.3 mmHg and 714±11.5 bpm (means±SEM), respectively, in NCC KO mice, whereas they were 77±3.9 mmHg and 625.4±20.0 bpm, in wild-type mice. Moreover, administration of ω-CTX-GVIA significantly reduced both arterial blood pressure and heart rate in wild-type mice (decreased by 22.6±2.6 mmHg
and 158.4±41.3 bpm, respectively), but exerted only marginal effects on arterial blood pressure and heart rates in NCC KO mice (decreased by 2.4±1.0 mmHg and 10.3±7.0 bpm, respectively). In the wild-type mice, increases in mean arterial pressure elicited via a carotid baroreflex induced by bilateral carotid artery occlusion were significantly suppressed by treatment of ω-CTX-GVIA, but in NCC KO mice carotid baroreflex-mediated increases in mean arterial pressure were impaired and unaffected by ω-CTX GVIA. These results suggest that carotid baroreflex function is primarily mediated by NCCs in wild-type mice, and that baroreflex function is greatly impaired in NCC KO mice (Ino, et al., 2001). However, the molecular mechanism responsible for the paradoxical elevation of basal arterial blood pressure in NCC KO mice described in this report remains unclear. Furthermore, Saegusa et al. reported that blood pressures and heart rates in NCC KO mice are equivalent to those in control wild-type mice (blood pressures, 107.3±3.4/59.8±2.6 mmHg for wild-type mice and 111.6±3.5/59.5±2.8 mmHg for NCC KO mice; heart rates, 564.3±21.9 bpm for wild-type mice and 547.0±25.8 bpm for NCC KO mice) (Saegusa, et al., 2001). In addition, another group reported that heart rates were lower in NCC KO mice than wild-type mice (659±13 bpm vs. 712±15 bpm) (Murakami, et al., 2007). In our recent study, systolic blood pressures and heart rates did not significantly differ between NCC heterozygotic KO mice and wild-type mice, but systolic blood pressure was lower in the KO than wild-type mice (91.25±2.78 mmHg vs. 101.25±7.26 mmHg) (Yamada, et al., 2014). The reason for the inconsistency among these results is not known, but mouse backgrounds and/or experimental conditions could contribute to differences in the blood pressure phenotype. At present, the contribution of NCC activity to physiological blood pressure regulation remains unclear.

In addition to the potential contribution of NCC expressed in the sympathetic nerve to blood pressure regulation, recently it has been reported that NCC is also expressed in
vascular endothelial cells (Nishida, et al., 2013). Angiotensin II-induced, oxidative stress-related impairment of endothelium-dependent relaxation of thoracic aorta was significantly attenuated in aorta from NCC KO mice. In addition, cilnidipine, a dual NCC and LCC blocker, but not amlodipine, prevented angiotensin II-induced endothelial dysfunction. NCC expressed in the vascular endothelial cells may also contribute to the regulation of vascular function by modifying endothelial function.

5. Cardioprotective effect of N-type Ca^{2+} channel blockade

As overactivation of sympathetic nerve activity underlies the development of several cardiovascular disorders, one might expect that the sympatholytic action of NCC inhibitors would exert a cardioprotective effect (Cohn, et al., 1984; Julius, 1993; Spalding, et al., 1998) (Figure 1). For example, the cardioprotective action of cilnidipine, which blocks both NCCs and LCCs, has been evaluated in a rabbit model of myocardial infarction. It was found that myocardial interstitial norepinephrine levels during ischemia/reperfusion, the size of myocardial infarction, and the incidence of ventricular premature contractions were all reduced in animals treated with cilnidipine (Nagai, et al., 2005). Enhanced sympathetic activity also appears to be an important factor contributing to the sudden arrhythmic death associated with chronic heart failure. This is evidenced by the finding that treating chronic heart failure with β-blockers reduces the incidence of sudden arrhythmic death in patients with chronic heart failure and reduced ejection fraction (Jafri, 2004). In addition, we evaluated the contribution of NCCs to lethal arrhythmias associated with chronic heart failure using a mouse model of non-ischemic cardiomyopathy, the cardiac-specific dominant-negative mutant of neuron-restrictive silencer factor (NRSF) transgenic (dnNRSF-Tg) mouse (Kuwahara, et al., 2003; Yamada, et al., 2014). dnNRSF-Tg mice develop cardiomyopathy at around 8 weeks of age and
then die suddenly due to lethal arrhythmias. We treated dnNRSF-Tg mice with cilnidipine, a dual NCC/LCC blocker, or with nitrendipine, a more selective LCC channel blocker, and compared the effects on cardiac phenotypes of each drug. Among the untreated control group, nitrendipine group and cilnidipine group, only cilnidipine-treated mice showed a reduced incidence of malignant arrhythmias and improved survival rates. On the other hand, the cilnidipine dose used in this study had no effect on cardiac structure or systolic function. Heart rate variability, a marker of the balance of autonomic nervous system activities, was significantly disturbed in dnNRSF-Tg mice. As heart rate variability predominantly correlates with parasympathetic activities in mice, this indicates reduced parasympathetic nervous system activities in these mice (Just, Faulhaber, & Ehmke, 2000; Kinoshita, et al., 2009). Furthermore, in dnNRSF-Tg mice urinary norepinephrine levels were significantly increased, which is indicative of the increased sympathetic nervous system activities in these mice. Cilnidipine treatment mitigated these abnormalities in dnNRSF-Tg mice, whereas nitrendipine did not. Genetic titration of NCCs in dnNRSF-Tg mice, achieved by crossing dnNRSF-Tg with CACNA1B-null mice, also restored cardiac autonomic balance, reduced the incidence of malignant arrhythmias and improved survival. The precise mechanisms by which NCC inhibition improved parasympathetic activity in these mice model of chronic heart failure are not clear at present. However, there are accumulating data indicating that sympathetic nervous system and parasympathetic nervous system interacts via multiple mechanisms at both the central and peripheral levels of the neurexis. NCC inhibition-induced reduction of sympathetic activity may affect these interactions, ameliorating the reduction in parasympathetic activity observed in dnNRSF-Tg.

These results imply the pivotal role played by NCCs in mediating the sympathetic nervous system activation that leads to the occurrence of malignant arrhythmias in failing
hearts (Nattel, 2014). Intriguingly, although pharmacological inhibition of NCCs using cilnidipine did not ameliorate the reduction in cardiac function seen in dnNRSF-Tg mice, genetic deletion of NCCs blocked the deterioration of cardiac function. The reasons for this difference in the effects on cardiac function are not known. One possibility is that the relatively low dose of cilnidipine used in this study was not sufficient to prevent the decline in cardiac function. Another possibility is that the NCC inhibition achieved through \textit{CACNA1B} knockdown was more prolonged and more constant than that achieved with cilnidipine, which was not started until the mice were 8 weeks of age in this study (Yamada, et al., 2014). In addition, the inhibitory effect of cilnidipine on NCCs expressed in the brain may also differ from the effect of genetic titration because cilnidipine has little ability to cross the blood-brain barrier (Watanabe, Dozen, & Hayashi, 1995).

The renin-angiotensin II-aldosterone system (RAAS) plays an important role in the development of cardiovascular diseases. One recent report showed that cilnidipine, but not amlodipine, suppresses angiotensin II-induced aldosterone production in cultured adrenal cells (Aritomi, et al., 2011). In this report, adrenal cells were shown to express NCCs, and angiotensin II-induced production of aldosterone was inhibited in the presence of \textit{ω}-CTX-GVIA or cilnidipine, suggesting the involvement of NCCs in aldosterone secretion from adrenal cells. In addition to its direct inhibitory effect on aldosterone production, NCC blockade may also affect RAAS activity through inhibition of sympathetic nerve activity. Renin secretion from juxtaglomerular cells is regulated in part by renal sympathetic activity. For instance, β-adrenergic stimulation is known to be a powerful stimulus for renin secretion and renin gene expression in juxtaglomerular cells \textit{in vivo} (Holmer, et al., 1997). Dihydropyridine LCC blockers can also stimulate renin production in juxtaglomerular cells (Schricker, et al., 1996; Stornello, et al., 1983). In the
spontaneously hypertensive rat (SHR)/Ism model, cilnidipine treatment had no effect on plasma renin activity or angiotensin II levels, whereas amlodipine increased both. Furthermore, cilnidipine and ω-CTX-GVIA each suppressed plasma aldosterone levels, but amlodipine did not (Konda, et al., 2009). These suppressive effects on RAAS activity may also contribute to the favorable effects of NCC blockade on cardiovascular diseases (Figure 1).

6. Renoprotective effect of N-type Ca\(^{2+}\) channel blockade

In kidney, \textit{CACNA1C}, encoding the LCC \(\alpha1\)C subunit, is preferentially expressed in glomerular afferent arterioles, but not in efferent arterioles (Hayashi, et al., 2007). Consequently, LCC blockers such as nifedipine cause a greater increase in the glomerular filtration rate than in renal plasma flow, and thus increase the filtration fraction (Nagahama, Hayashi, Fujiwara, Ozawa, & Saruta, 2000). By contrast, sympathetic innervation is distributed along both the afferent and efferent arterioles, so that NCC blockade may dilate both afferent and efferent arterioles (Hayashi, et al., 2007; Kon, 1989) (Figure 1). Cilnidipine, a dual LCC/NCC blocker, predominantly affects the afferent arterioles in isolated perfused hydronephrotic kidneys (Nagahama, et al., 2000), but in the canine kidney \textit{in vivo}, cilnidipine elicited substantial dilation of both afferent and efferent arterioles (Hayashi, et al., 2007). These results suggest that cilnidipine can dilate both afferent and efferent arterioles by blocking NCC expressed in sympathetic nervous system in the \textit{in vivo} settings. The predominance of the effect of LCC blockers on glomerular afferent arterioles could cause glomerular hypertension resulting in renal injury. By contrast, Ca\(^{2+}\) channel blockers acting on both afferent and efferent arterioles theoretically mitigate glomerular hypertension and thus may exert a beneficial effect on the progression of renal injury. Supporting this possibility, cilnidipine reduces glomerular
capillary pressure, afferent and efferent arteriolar resistances, urinary albumin excretion and glomerular volume, as well as plasma norepinephrine levels in animal renal injury models (Konda, Enomoto, Matsushita, Takahara, & Moriyama, 2005; Zhou, Ono, Ono, & Frohlich, 2002). In addition, in the SHR/ND mcr-cp model of metabolic syndrome, cilnidipine suppressed proteinuria and podocyte injury to a greater degree than did amlodipine (Fan, et al., 2010).

The RAAS suppression induced by NCC blockade may also contribute to its renoprotective effect. RAAS makes a critical contribution to the development of proteinuria and chronic kidney injury (Ando, 2013). Several clinical trials have shown the renoprotective effect of RAAS inhibitors such as ACE inhibitors and angiotensin II AT1 receptor blockers in patients with diabetic or non-diabetic nephropathy (Brenner, et al., 2001; Lewis, Hunsicker, Bain, & Rohde, 1993; Lewis, et al., 2001; Wright, et al., 2002). In addition, mineral corticoid receptor antagonists have been shown to ameliorate urinary protein and the progression of kidney damage in human clinical studies (Epstein, et al., 2006; White, et al., 2003).

Cilnidipine also exerts an anti-proteinuric effect in hypertensive patients with the kidney disease (Kojima, Shida, & Yokoyama, 2004; Rose, et al., 2001), and reduces urinary albumin in patients with type II diabetic nephropathy treated with an angiotensin receptor blocker (Katayama, et al., 2006). In the multicenter, open-label, randomized Cilnidipine versus Amlodipine Randomized Trial for Evaluation in Renal Disease (CARTER) trial, cilnidipine was superior to amlodipine for preventing the progression of proteinuria in hypertensive patients with chronic kidney disease who were already receiving a renin-angiotensin system (RAS) inhibitor (T. Fujita, et al., 2007). These studies demonstrate the potential renoprotective effects of cilnidipine. However, in the recent SAKURA trial, in which the anti-albuminuric effects of cilnidipine and amlodipine
in RAS inhibitor-treated diabetic patients with microalbuminuria were compared, cilnidipine did not show greater renoprotection than amlodipine (Ando, et al., 2013). Thus cilnidipine may only reduce urinary protein or albumin levels more effectively than LCC blockers in hypertensive patients with non-diabetic chronic kidney disease.

7. N-type Ca\(^{2+}\) channel blockade in metabolic diseases

Metabolic syndrome is a cluster of abnormalities, including hyperglycemia, central obesity, dyslipidemia and hypertension. Because several aspects of the ailment appear to be associated with sympathetic overactivation (Canale, et al., 2013), it is plausible that modulating sympathetic nerve activity is important for effective management of metabolic syndrome.

Insulin secretion from β-cells and glucagon secretion from α-cells in the pancreatic islets of Langerhans are both initiated by Ca\(^{2+}\) influx, which is mediated in part through NCCs (Barg, Galvanovskis, Gopel, Rorsman, & Eliasson, 2000; Gromada, et al., 1997; Komatsu, et al., 1989; Ramanadham & Turk, 1994; Taylor, et al., 2005; Vignali, Leiss, Karl, Hofmann, & Welling, 2006; Yang & Berggren, 2005). NCC KO mice fed a normal diet show improved glucose tolerance without changes in insulin sensitivity, while NCC KO mice fed a high-fat diet exhibit less body weight gain than control wild-type mice (Takahashi, et al., 2005).

8. N-type calcium channel in pain transmission and its blockade in refractory pain

Using both pharmacological and genetic approaches, it has been shown that NCCs play an important role in pain pathways. For example, ω-CTX-GVIA blocks the release of calcitonin gene-regulated peptide (CGRP) and substance P from primary afferent nerves, suggesting NCCs contribute to nociceptive transmission (Holz, Dunlap, & Kream, 1988;
Maggi, Tramontana, Cecconi, & Santicioli, 1990; Santicioli, Del Bianco, Tramontana, Geppetti, & Maggi, 1992). Supporting this possibility, autoradiography using radiolabeled ω-CTX-GVIA revealed spinal localization of NCCs in the surface laminae of the dorsal horn, where primary afferent nerves terminate (Kerr, Filloux, Olivera, Jackson, & Wamsley, 1988; Takemura, et al., 1989). In addition, selective NCC blockade or genetic deletion of NCCs provides analgesia in animal pain models (Adams & Berecki, 2013). In NCC KO mice, NCC currents are almost completely abolished in DRG neurons, nociceptive responses are significantly reduced (Hatakeyama, et al., 2001), and neuropathic pain is greatly reduced (Saegusa, et al., 2001). Taken together, these results suggest NCC inhibition can be beneficial in reducing pathological pain, and in 2004 the U.S. FDA approved a synthetic ω-CTX-MVIIA derivative, ziconotide, for refractory pain (Lee, 2013). However, the use of ziconotide has been limited due to its narrow therapeutic window, uncomfortable intrathecal administration, severe side effects and cost of production (Penn & Paice, 2000). To overcome these limitations, much effort is being made to develop other, less toxic, peptide neurotoxins or systemically available small molecules that inhibit NCCs for pain control (Yamamoto & Takahara, 2009).

9. Conclusions and future directions

In this review, we summarized the physiological and pathophysiological actions of NCCs and the potentially protective effect of their blockade in several pathological conditions. NCCs are predominantly localized in the nervous system, where they are key mediators of neurotransmitter release. Both pharmacological and genetic inhibition revealed that NCCs are essential for proper sympathetic nerve activation and nociceptive transmission, which suggests NCCs could be a useful therapeutic target in several pathological conditions. As mentioned in the previous section, ziconotide, a synthetic ω-CTX-MVIIA
derivative, has been approved by the U.S. FDA for chronic pain management, but its use is limited by undesirable characteristics, such as its narrow therapeutic window, uncomfortable intrathecal administration and severe side effects. Other synthetic ω-CTX derivatives and small-molecule inhibitors are currently under development, mainly for the treatment of chronic and neuropathic pain (Adams & Berecki, 2013). Cilnidipine, a dihydropyridine LCC blocker, also blocks NCC activity (Uneyama, et al., 1997). Overactivation of sympathetic nervous system is known to be involved in the development of hypertension and related cardiovascular, kidney and metabolic disorders. NCC blockade exerts a suppressive effect on RAAS activation, which is critically involved in the development of these conditions (Dzau, et al., 2006). Thus NCC blockade alone or in conjunction with LCC blockade may be beneficial in patients with hypertension and cardiovascular and metabolic diseases, which is supported by observations in several animal models. In addition, the CARTER trial, in which the abilities of cilnidipine and amlodipine to prevent the progression of proteinuria were compared in hypertensive patients with chronic kidney disease and already having received a RAS inhibitor, showed that cilnidipine was superior to amlodipine (T. Fujita, et al., 2007). But in the recent SAKURA trial, in which the anti-albuminuric effects of cilnidipine and amlodipine were compared in diabetic patients with microalbuminuria having been treated with a RAS inhibitor, cilnidipine failed to show greater renoprotective effects than amlodipine (Ando, et al., 2013). Further clinical studies will be needed to translate the promising results from animal studies into clinical practice.
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Conflict of interest

The authors declare that there are no conflicts of interests.
References


Cassidy, J. S., Ferron, L., Kadurin, I., Pratt, W. S., & Dolphin, A. C. (2014). Functional exofacially tagged N-type calcium channels elucidate the interaction with


Fujii, S., Kameyama, K., Hosono, M., Hayashi, Y., & Kitamura, K. (1997). Effect of cilnidipine, a novel dihydropyridine Ca$^{2+}$-channel antagonist, on N-type Ca$^{2+}$ channel in rat dorsal root ganglion neurons. *J Pharmacol Exp Ther*, 280, 1184-


Kinoshita, H., Kuwahara, K., Takano, M., Arai, Y., Kuwabara, Y., Yasuno, S., Nakagawa,


Mori, Y., Nishida, M., Shimizu, S., Ishii, M., Yoshinaga, T., Ino, M., Sawada, K., & Niidome, T. (2002). Ca$^{2+}$ channel $\alpha_{1B}$ subunit (Cav 2.2) knockout mouse reveals a predominant role of N-type channels in the sympathetic regulation of the circulatory system. *Trends Cardiovasc Med.*, 12, 270-275.


pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA*, 288, 2421-2431.


Figure Legend

Figure 1. Effects of N-type Ca$^{2+}$ channel inhibition on the cardiovascular system
Increase in heart rate
Increase in arrhythmias
Increase in contraction
Pathological cardiac remodeling

Vasoconstriction
Increase in blood pressure

Decrease in renal blood flow ($\alpha_1$)
Renin secretion ($\beta_1$)