

Title	The organ-protective effect of N-type Ca(2+) channel blockade.
Author(s)	Kuwahara, Koichiro; Kimura, Takeshi
Citation	Pharmacology & therapeutics (2015), 151: 1-7
Issue Date	2015-07
URL	http://hdl.handle.net/2433/200654
Right	© 2015. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/ ; The full-text file will be made open to the public on 31 July 2016 in accordance with publisher's 'Terms and Conditions for Self-Archiving'; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。 This is not the published version. Please cite only the published version.
Type	Journal Article
Textversion	author

The organ-protective effect of N-type Ca²⁺ channel blockade

Koichiro Kuwahara^{1*}, Takeshi Kimura¹

¹Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine.
Kyoto, 606-8507 Japan

***Corresponding author**

Koichiro Kuwahara, M.D., Ph.D.

Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine.

54 Shogoin Kawaharacho, Sakyo-ku, Kyoto, 606-8507 Japan

Phone: +81-75-751-4287

Fax: +81-75-771-9452

e-mail address: kuwa@kuhpo.kyoto-u.ac.jp

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-conotoxin (ω -CTX)-GVIA. Predominantly localized in the nervous system, NCCs are key regulators of neurotransmitter release. Both pharmacological blockade with ω -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 ω -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

Contents

1. Introduction
2. N-type Ca^{2+} channels and their physiological function in sympathetic nerves
3. N-type Ca^{2+} channel inhibitors
4. N-type Ca^{2+} channels and hypertension
5. Cardioprotective effect of N-type Ca^{2+} channel blockade
6. Renoprotective effect of N-type Ca^{2+} channel blockade
7. N-type Ca^{2+} channel blockade in metabolic diseases
8. Conclusions and future directions

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-conotoxin (ω -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 $\alpha 1$ subunit, which determines the major characteristics of each VDCC subtype, and the auxiliary $\alpha 2/\delta$, β and γ subunits. Among the 10 different genes encoding $\alpha 1$ subunits, which include $\alpha 1A$, $\alpha 1B$, $\alpha 1C$, $\alpha 1D$, $\alpha 1E$, $\alpha 1F$, $\alpha 1G$, $\alpha 1H$, $\alpha 1I$ and $\alpha 1S$, *CACNA1B* encodes the $\alpha 1B$ subunit, which comprises the NCC (Y. Fujita, et al., 1993; Williams, Brust, et al., 1992). The $\alpha 1B$ 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 *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 *CACNA1B*, which encodes the $\alpha 1B$ subunit of NCCs (Ino, et al., 2001). In *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 *CACNA1B*-null neurons (Ino, et al., 2001), suggesting the reduction in VDCC currents in *CACNA1B*-null SGC neurons is caused by the elimination of NCCs induced by deletion of *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 magnus* 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 GABAergic activity used to treat neuropathic pain, have affinity for the $\alpha 2\delta$ VDCC subunit and inhibit trafficking of

Cav2.2, the $\alpha 1$ pore forming unit of NCCs, from the cytoplasm to the plasma membrane (Cassidy, Ferron, Kadurin, Pratt, & Dolphin, 2014; Lee, 2013).

A dihydropyridine-type Ca^{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^{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 \pm 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, $107.3 \pm 3.4/59.8 \pm 2.6$ mmHg for wild-type mice and $111.6 \pm 3.5/59.5 \pm 2.8$ 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 *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 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 *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, *CACNA1C*, encoding the LCC $\alpha 1C$ 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 *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 *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-MVIA 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-MVIA

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.

Acknowledgements

This study was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science 26293187 and 26670400, by a grant from the Japanese Ministry of Health, Labor and Welfare, and by grants from the SENSHIN Medical Research Foundation.

Conflict of interest

The authors declare that there are no conflicts of interests.

References

- Adams, D. J., & Berecki, G. (2013). Mechanisms of conotoxin inhibition of N-type (Ca_v2.2) calcium channels. *Biochim Biophys Acta*, 1828, 1619-1628.
- Ando, K. (2013). L-/N-type calcium channel blockers and proteinuria. *Curr Hypertens Rev*, 9, 210-218.
- Ando, K., Ueshima, K., Tanaka, S., Kosugi, S., Sato, T., Matsuoka, H., Nakao, K., & Fujita, T. (2013). Comparison of the antialbuminuric effects of L-/N-type and L-type calcium channel blockers in hypertensive patients with diabetes and microalbuminuria: the study of assessment for kidney function by urinary microalbumin in randomized (SAKURA) trial. *Int J Med Sci*, 10, 1209-1216.
- Aosaki, T., & Kasai, H. (1989). Characterization of two kinds of high-voltage-activated Ca-channel currents in chick sensory neurons. Differential sensitivity to dihydropyridines and omega-conotoxin GVIA. *Pflugers Arch*, 414, 150-156.
- Aritomi, S., Wagatsuma, H., Numata, T., Uriu, Y., Nogi, Y., Mitsui, A., Konda, T., Mori, Y., & Yoshimura, M. (2011). Expression of N-type calcium channels in human adrenocortical cells and their contribution to corticosteroid synthesis. *Hypertens Res*, 34, 193-201.
- Augustine, G. J., Charlton, M. P., & Smith, S. J. (1987). Calcium action in synaptic transmitter release. *Annu Rev Neurosci*, 10, 633-693.
- Barg, S., Galvanovskis, J., Gopel, S. O., Rorsman, P., & Eliasson, L. (2000). Tight coupling between electrical activity and exocytosis in mouse glucagon-secreting α -cells. *Diabetes*, 49, 1500-1510.
- Bond, A., & Boot, J. R. (1992). The effect of calcium channel modulators on blood pressure and pressor responses to noradrenaline in the guinea-pig. *Eur J Pharmacol*, 218, 179-181.
- Brenner, B. M., Cooper, M. E., de Zeeuw, D., Keane, W. F., Mitch, W. E., Parving, H. H., Remuzzi, G., Snapinn, S. M., Zhang, Z., Shahinfar, S., & Investigators, R. S. (2001). Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med*, 345, 861-869.
- Canale, M. P., Manca di Villahermosa, S., Martino, G., Rovella, V., Noce, A., De Lorenzo, A., & Di Daniele, N. (2013). Obesity-related metabolic syndrome: mechanisms of sympathetic overactivity. *Int J Endocrinol*, 2013, 865965.
- Carbone, E., & Lux, H. D. (1984). A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones. *Nature*, 310, 501-502.
- 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

- auxiliary $\alpha 2\delta$ -1 subunits. *Proc Natl Acad Sci U S A*, *111*, 8979-8984.
- Chiang, C. S., Huang, C. H., Chieng, H., Chang, Y. T., Chang, D., Chen, J. J., Chen, Y. C., Chen, Y. H., Shin, H. S., Campbell, K. P., & Chen, C. C. (2009). The Cav3.2 T-type Ca^{2+} channel is required for pressure overload-induced cardiac hypertrophy in mice. *Circ Res*, *104*, 522-530.
- Clasbrummel, B., Osswald, H., & Illes, P. (1989). Inhibition of noradrenaline release by omega-conotoxin GVIA in the rat tail artery. *Br J Pharmacol*, *96*, 101-110.
- Cohn, J. N., Levine, T. B., Olivari, M. T., Garberg, V., Lura, D., Francis, G. S., Simon, A. B., & Rector, T. (1984). Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med*, *311*, 819-823.
- Dooley, D. J., Lupp, A., Hertting, G., & Osswald, H. (1988). Omega-conotoxin GVIA and pharmacological modulation of hippocampal noradrenaline release. *Eur J Pharmacol*, *148*, 261-267.
- Dutar, P., Rascol, O., & Lamour, Y. (1989). Omega-conotoxin GVIA blocks synaptic transmission in the CA1 field of the hippocampus. *Eur J Pharmacol*, *174*, 261-266.
- Dzau, V. J., Antman, E. M., Black, H. R., Hayes, D. L., Manson, J. E., Plutzky, J., Popma, J. J., & Stevenson, W. (2006). The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes: part I: Pathophysiology and clinical trial evidence (risk factors through stable coronary artery disease). *Circulation*, *114*, 2850-2870.
- Epstein, M., Williams, G. H., Weinberger, M., Lewin, A., Krause, S., Mukherjee, R., Patni, R., & Beckerman, B. (2006). Selective aldosterone blockade with eplerenone reduces albuminuria in patients with type 2 diabetes. *Clin J Am Soc Nephrol*, *1*, 940-951.
- Evans, R. M., & Zamponi, G. W. (2006). Presynaptic Ca^{2+} channels--integration centers for neuronal signaling pathways. *Trends Neurosci*, *29*, 617-624.
- Fan, Y. Y., Kohno, M., Nakano, D., Ohsaki, H., Kobori, H., Suwarni, D., Ohashi, N., Hitomi, H., Asanuma, K., Noma, T., Tomino, Y., Fujita, T., & Nishiyama, A. (2010). Cilnidipine suppresses podocyte injury and proteinuria in metabolic syndrome rats: possible involvement of N-type calcium channel in podocyte. *J Hypertens*, *28*, 1034-1043.
- Fox, A. P., Nowycky, M. C., & Tsien, R. W. (1987). Single-channel recordings of three types of calcium channels in chick sensory neurones. *J Physiol*, *394*, 173-200.
- Fujii, S., Kameyama, K., Hosono, M., Hayashi, Y., & Kitamura, K. (1997). Effect of cilnidipine, a novel dihydropyridine Ca^{++} -channel antagonist, on N-type Ca^{++} channel in rat dorsal root ganglion neurons. *J Pharmacol Exp Ther*, *280*, 1184-

1191.

- Fujita, T., Ando, K., Nishimura, H., Ideura, T., Yasuda, G., Isshiki, M., Takahashi, K., & Cilnidipine versus Amlodipine Randomised Trial for Evaluation in Renal Disease Study, I. (2007). Antiproteinuric effect of the calcium channel blocker cilnidipine added to renin-angiotensin inhibition in hypertensive patients with chronic renal disease. *Kidney Int*, *72*, 1543-1549.
- Fujita, Y., Mynlieff, M., Dirksen, R. T., Kim, M. S., Niidome, T., Nakai, J., Friedrich, T., Iwabe, N., Miyata, T., Furuichi, T., & et al. (1993). Primary structure and functional expression of the omega-conotoxin-sensitive N-type calcium channel from rabbit brain. *Neuron*, *10*, 585-598.
- Gromada, J., Bokvist, K., Ding, W. G., Barg, S., Buschard, K., Renstrom, E., & Rorsman, P. (1997). Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the Ca²⁺ current and the number of granules close to the L-type Ca²⁺ channels. *J Gen Physiol*, *110*, 217-228.
- Hatakeyama, S., Wakamori, M., Ino, M., Miyamoto, N., Takahashi, E., Yoshinaga, T., Sawada, K., Imoto, K., Tanaka, I., Yoshizawa, T., Nishizawa, Y., Mori, Y., Niidome, T., & Shoji, S. (2001). Differential nociceptive responses in mice lacking the α_{1B} subunit of N-type Ca²⁺ channels. *Neuroreport*, *12*, 2423-2427.
- Hayashi, K., Wakino, S., Sugano, N., Ozawa, Y., Homma, K., & Saruta, T. (2007). Ca²⁺ channel subtypes and pharmacology in the kidney. *Circ Res*, *100*, 342-353.
- Herdon, H., & Nahorski, S. R. (1989). Investigations of the roles of dihydropyridine and omega-conotoxin-sensitive calcium channels in mediating depolarisation-evoked endogenous dopamine release from striatal slices. *Naunyn Schmiedebergs Arch Pharmacol*, *340*, 36-40.
- Hirning, L. D., Fox, A. P., McCleskey, E. W., Olivera, B. M., Thayer, S. A., Miller, R. J., & Tsien, R. W. (1988). Dominant role of N-type Ca²⁺ channels in evoked release of norepinephrine from sympathetic neurons. *Science*, *239*, 57-61.
- Holmer, S. R., Kaissling, B., Putnik, K., Pfeifer, M., Kramer, B. K., Riegger, G. A., & Kurtz, A. (1997). Beta-adrenergic stimulation of renin expression in vivo. *J Hypertens*, *15*, 1471-1479.
- Holz, G. G. t., Dunlap, K., & Kream, R. M. (1988). Characterization of the electrically evoked release of substance P from dorsal root ganglion neurons: methods and dihydropyridine sensitivity. *J Neurosci*, *8*, 463-471.
- Hong, S. J., & Chang, C. C. (1995). Calcium channel subtypes for the sympathetic and parasympathetic nerves of guinea-pig atria. *Br J Pharmacol*, *116*, 1577-1582.
- Horne, A. L., & Kemp, J. A. (1991). The effect of omega-conotoxin GVIA on synaptic transmission within the nucleus accumbens and hippocampus of the rat in vitro.

- Br J Pharmacol*, 103, 1733-1739.
- Ino, M., Yoshinaga, T., Wakamori, M., Miyamoto, N., Takahashi, E., Sonoda, J., Kagaya, T., Oki, T., Nagasu, T., Nishizawa, Y., Tanaka, I., Imoto, K., Aizawa, S., Koch, S., Schwartz, A., Niidome, T., Sawada, K., & Mori, Y. (2001). Functional disorders of the sympathetic nervous system in mice lacking the α_{1B} subunit (Cav 2.2) of N-type calcium channels. *Proc Natl Acad Sci U S A*, 98, 5323-5328.
- Ishibashi, H., Rhee, J. S., & Akaike, N. (1995). Regional difference of high voltage-activated Ca^{2+} channels in rat CNS neurones. *Neuroreport*, 6, 1621-1624.
- Ishikawa, T., Kaneko, M., Shin, H. S., & Takahashi, T. (2005). Presynaptic N-type and P/Q-type Ca^{2+} channels mediating synaptic transmission at the calyx of Held of mice. *J Physiol*, 568, 199-209.
- Iwasaki, S., Momiyama, A., Uchitel, O. D., & Takahashi, T. (2000). Developmental changes in calcium channel types mediating central synaptic transmission. *J Neurosci*, 20, 59-65.
- Jafri, S. M. (2004). The effects of beta blockers on morbidity and mortality in heart failure. *Heart Fail Rev*, 9, 115-121.
- Julius, S. (1993). Corcoran Lecture. Sympathetic hyperactivity and coronary risk in hypertension. *Hypertension*, 21, 886-893.
- Julius, S., Schork, N., & Schork, A. (1988). Sympathetic hyperactivity in early stages of hypertension: the Ann Arbor data set. *J Cardiovasc Pharmacol*, 12 Suppl 3, S121-129.
- Just, A., Faulhaber, J., & Ehmke, H. (2000). Autonomic cardiovascular control in conscious mice. *Am J Physiol Regul Integr Comp Physiol*, 279, R2214-2221.
- Kamp, M. A., Krieger, A., Henry, M., Hescheler, J., Weiergraber, M., & Schneider, T. (2005). Presynaptic 'Ca_v2.3-containing' E-type Ca channels share dual roles during neurotransmitter release. *Eur J Neurosci*, 21, 1617-1625.
- Katayama, K., Nomura, S., Ishikawa, H., Murata, T., Koyabu, S., & Nakano, T. (2006). Comparison between valsartan and valsartan plus cilnidipine in type II diabetics with normo- and microalbuminuria. *Kidney Int*, 70, 151-156.
- Kerr, L. M., Filloux, F., Olivera, B. M., Jackson, H., & Wamsley, J. K. (1988). Autoradiographic localization of calcium channels with [¹²⁵I]ω-conotoxin in rat brain. *Eur J Pharmacol*, 146, 181-183.
- Kim, C., Jun, K., Lee, T., Kim, S. S., McEnery, M. W., Chin, H., Kim, H. L., Park, J. M., Kim, D. K., Jung, S. J., Kim, J., & Shin, H. S. (2001). Altered nociceptive response in mice deficient in the α_{1B} subunit of the voltage-dependent calcium channel. *Mol Cell Neurosci*, 18, 235-245.
- Kinoshita, H., Kuwahara, K., Takano, M., Arai, Y., Kuwabara, Y., Yasuno, S., Nakagawa,

- Y., Nakanishi, M., Harada, M., Fujiwara, M., Murakami, M., Ueshima, K., & Nakao, K. (2009). T-type Ca^{2+} channel blockade prevents sudden death in mice with heart failure. *Circulation*, *120*, 743-752.
- Kojima, S., Shida, M., & Yokoyama, H. (2004). Comparison between cilnidipine and amlodipine besilate with respect to proteinuria in hypertensive patients with renal diseases. *Hypertens Res*, *27*, 379-385.
- Komatsu, M., Yokokawa, N., Takeda, T., Nagasawa, Y., Aizawa, T., & Yamada, T. (1989). Pharmacological characterization of the voltage-dependent calcium channel of pancreatic B-cell. *Endocrinology*, *125*, 2008-2014.
- Komuro, H., & Rakic, P. (1992). Selective role of N-type calcium channels in neuronal migration. *Science*, *257*, 806-809.
- Kon, V. (1989). Neural control of renal circulation. *Miner Electrolyte Metab*, *15*, 33-43.
- Konda, T., Enomoto, A., Aritomi, S., Niinuma, K., Koganei, H., Ogawa, T., & Nitta, K. (2009). Different effects of L/N-type and L-type calcium channel blockers on the renin-angiotensin-aldosterone system in SHR/Izm. *Am J Nephrol*, *30*, 155-161.
- Konda, T., Enomoto, A., Matsushita, J., Takahara, A., & Moriyama, T. (2005). The N- and L-type calcium channel blocker cilnidipine suppresses renal injury in dahl rats fed a high-sucrose diet, an experimental model of metabolic syndrome. *Nephron Physiol*, *101*, p1-13.
- Kuwahara, K., Saito, Y., Takano, M., Arai, Y., Yasuno, S., Nakagawa, Y., Takahashi, N., Adachi, Y., Takemura, G., Horie, M., Miyamoto, Y., Morisaki, T., Kuratomi, S., Noma, A., Fujiwara, H., Yoshimasa, Y., Kinoshita, H., Kawakami, R., Kishimoto, I., Nakanishi, M., Usami, S., Saito, Y., Harada, M., & Nakao, K. (2003). NRSF regulates the fetal cardiac gene program and maintains normal cardiac structure and function. *EMBO J*, *22*, 6310-6321.
- Kuwahara, K., Takano, M., & Nakao, K. (2005). Pathophysiological significance of T-type Ca^{2+} channels: transcriptional regulation of T-type Ca^{2+} channel-regulation of CACNA1H by neuron-restrictive silencer factor. *J Pharmacol Sci*, *99*, 211-213.
- Le Quang, K., Naud, P., Qi, X. Y., Duval, F., Shi, Y. F., Gillis, M. A., Comtois, P., Tardif, J. C., Li, D., Levesque, P. C., Dobrev, D., Charpentier, F., & Nattel, S. (2011). Role of T-type calcium channel subunits in post-myocardial infarction remodelling probed with genetically engineered mice. *Cardiovasc Res*, *91*, 420-428.
- Lee, S. (2013). Pharmacological Inhibition of Voltage-gated Ca^{2+} Channels for Chronic Pain Relief. *Curr Neuropharmacol*, *11*, 606-620.
- Lewis, E. J., Hunsicker, L. G., Bain, R. P., & Rohde, R. D. (1993). The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The

- Collaborative Study Group. *N Engl J Med*, 329, 1456-1462.
- Lewis, E. J., Hunsicker, L. G., Clarke, W. R., Berl, T., Pohl, M. A., Lewis, J. B., Ritz, E., Atkins, R. C., Rohde, R., Raz, I., & Collaborative Study, G. (2001). Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med*, 345, 851-860.
- Luebke, J. I., Dunlap, K., & Turner, T. J. (1993). Multiple calcium channel types control glutamatergic synaptic transmission in the hippocampus. *Neuron*, 11, 895-902.
- Maggi, C. A., Tramontana, M., Cecconi, R., & Santicoli, P. (1990). Neurochemical evidence for the involvement of N-type calcium channels in transmitter secretion from peripheral endings of sensory nerves in guinea pigs. *Neurosci Lett*, 114, 203-206.
- Mesirca, P., Torrente, A. G., & Mangoni, M. E. (2014). T-type channels in the sino-atrial and atrioventricular pacemaker mechanism. *Pflugers Arch*, 466, 791-799.
- Miller, R. J. (1987). Multiple calcium channels and neuronal function. *Science*, 235, 46-52.
- Mills, L. R., Niesen, C. E., So, A. P., Carlen, P. L., Spigelman, I., & Jones, O. T. (1994). N-type Ca^{2+} channels are located on somata, dendrites, and a subpopulation of dendritic spines on live hippocampal pyramidal neurons. *J Neurosci*, 14, 6815-6824.
- Mori, Y., Mikala, G., Varadi, G., Kobayashi, T., Koch, S., Wakamori, M., & Schwartz, A. (1996). Molecular pharmacology of voltage-dependent calcium channels. *Jpn J Pharmacol*, 72, 83-109.
- Mori, Y., Nishida, M., Shimizu, S., Ishii, M., Yoshinaga, T., Ino, M., Sawada, K., & Niidome, T. (2002). Ca^{2+} channel α_{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.
- Murakami, M., Ohba, T., Wu, T. W., Fujisawa, S., Suzuki, T., Takahashi, Y., Takahashi, E., Watanabe, H., Miyoshi, I., Ono, K., Sasano, H., Ito, H., & Iijima, T. (2007). Modified sympathetic regulation in N-type calcium channel null-mouse. *Biochem Biophys Res Commun*, 354, 1016-1020.
- Nagahama, T., Hayashi, K., Fujiwara, K., Ozawa, Y., & Saruta, T. (2000). Characterization of the renal action of pranidipine in the rat. *Arzneimittelforschung*, 50, 248-253.
- Nagai, H., Minatoguchi, S., Chen, X. H., Wang, N., Arai, M., Uno, Y., Lu, C., Misao, Y., Onogi, H., Kobayashi, H., Takemura, G., Maruyama, R., Fujiwara, T., & Fujiwara, H. (2005). Cilnidipine, an N+L-type dihydropyridine Ca channel blocker, suppresses the occurrence of ischemia/reperfusion arrhythmia in a rabbit model

- of myocardial infarction. *Hypertens Res*, 28, 361-368.
- Nakayama, H., Bodi, I., Correll, R. N., Chen, X., Lorenz, J., Houser, S. R., Robbins, J., Schwartz, A., & Molkentin, J. D. (2009). α_1G -dependent T-type Ca^{2+} current antagonizes cardiac hypertrophy through a NOS3-dependent mechanism in mice. *J Clin Invest*, 119, 3787-3796.
- Nattel, S. (2014). N-type calcium channel blockade: a new approach to preventing sudden cardiac death? *Cardiovasc Res*, 104, 1-2.
- Nishida, M., Ishikawa, T., Saiki, S., Sunggip, C., Aritomi, S., Harada, E., Kuwahara, K., Hirano, K., Mori, Y., & Kim-Mitsuyama, S. (2013). Voltage-dependent N-type Ca^{2+} channels in endothelial cells contribute to oxidative stress-related endothelial dysfunction induced by angiotensin II in mice. *Biochem Biophys Res Commun*, 434, 210-216.
- Nowycky, M. C., Fox, A. P., & Tsien, R. W. (1985). Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature*, 316, 440-443.
- Olivera, B. M., Gray, W. R., Zeikus, R., McIntosh, J. M., Varga, J., Rivier, J., de Santos, V., & Cruz, L. J. (1985). Peptide neurotoxins from fish-hunting cone snails. *Science*, 230, 1338-1343.
- Penn, R. D., & Paice, J. A. (2000). Adverse effects associated with the intrathecal administration of ziconotide. *Pain*, 85, 291-296.
- Perez-Reyes, E. (2003). Molecular physiology of low-voltage-activated t-type calcium channels. *Physiol Rev*, 83, 117-161.
- Pruneau, D., & Angus, J. A. (1990). Omega-conotoxin GVIA, the N-type calcium channel inhibitor, is sympatholytic but not vagolytic: consequences for hemodynamics and autonomic reflexes in conscious rabbits. *J Cardiovasc Pharmacol*, 16, 675-680.
- Ramanadham, S., & Turk, J. (1994). ω -Conotoxin inhibits glucose- and arachidonic acid-induced rises in intracellular $[Ca^{2+}]$ in rat pancreatic islet beta-cells. *Cell Calcium*, 15, 259-264.
- Rose, G. W., Kanno, Y., Ikebukuro, H., Kaneko, M., Kaneko, K., Kanno, T., Ishida, Y., & Suzuki, H. (2001). Cilnidipine is as effective as benazepril for control of blood pressure and proteinuria in hypertensive patients with benign nephrosclerosis. *Hypertens Res*, 24, 377-383.
- Saegusa, H., Kurihara, T., Zong, S., Kazuno, A., Matsuda, Y., Nonaka, T., Han, W., Toriyama, H., & Tanabe, T. (2001). Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type Ca^{2+} channel. *EMBO J*, 20, 2349-2356.
- Santicioli, P., Del Bianco, E., Tramontana, M., Geppetti, P., & Maggi, C. A. (1992). Release of calcitonin gene-related peptide like-immunoreactivity induced by electrical field stimulation from rat spinal afferents is mediated by conotoxin-

- sensitive calcium channels. *Neurosci Lett*, 136, 161-164.
- Schricker, K., Hamann, M., Macher, A., Kramer, B. K., Kaissling, B., & Kurtz, A. (1996). Effect of amlodipine on renin secretion and renin gene expression in rats. *Br J Pharmacol*, 119, 744-750.
- Serone, A. P., & Angus, J. A. (1999). Role of N-type calcium channels in autonomic neurotransmission in guinea-pig isolated left atria. *Br J Pharmacol*, 127, 927-934.
- Spalding, A., Vaitkevicius, H., Dill, S., MacKenzie, S., Schmaier, A., & Lockette, W. (1998). Mechanism of epinephrine-induced platelet aggregation. *Hypertension*, 31, 603-607.
- Stornello, M., Di Rao, G., Iachello, M., Pisani, R., Scapellato, L., Pedrinelli, R., & Salvetti, A. (1983). Hemodynamic and humoral interactions between captopril and nifedipine. *Hypertension*, 5, III154-156.
- Takahara, A., Fujita, S., Moki, K., Ono, Y., Koganei, H., Iwayama, S., & Yamamoto, H. (2003). Neuronal Ca²⁺ channel blocking action of an antihypertensive drug, cilnidipine, in IMR-32 human neuroblastoma cells. *Hypertens Res*, 26, 743-747.
- Takahashi, E., Ito, M., Miyamoto, N., Nagasu, T., Ino, M., & Tanaka, I. (2005). Increased glucose tolerance in N-type Ca²⁺ channel $\alpha 1B$ -subunit gene-deficient mice. *Int J Mol Med*, 15, 937-944.
- Takemura, M., Kiyama, H., Fukui, H., Tohyama, M., & Wada, H. (1989). Distribution of the ω -conotoxin receptor in rat brain. An autoradiographic mapping. *Neuroscience*, 32, 405-416.
- Taylor, J. T., Huang, L., Keyser, B. M., Zhuang, H., Clarkson, C. W., & Li, M. (2005). Role of high-voltage-activated calcium channels in glucose-regulated beta-cell calcium homeostasis and insulin release. *Am J Physiol Endocrinol Metab*, 289, E900-908.
- Toth, P. T., Bindokas, V. P., Bleakman, D., Colmers, W. F., & Miller, R. J. (1993). Mechanism of presynaptic inhibition by neuropeptide Y at sympathetic nerve terminals. *Nature*, 364, 635-639.
- Turner, T. J., Adams, M. E., & Dunlap, K. (1993). Multiple Ca²⁺ channel types coexist to regulate synaptosomal neurotransmitter release. *Proc Natl Acad Sci U S A*, 90, 9518-9522.
- Uneyama, H., Takahara, A., Dohmoto, H., Yoshimoto, R., Inoue, K., & Akaike, N. (1997). Blockade of N-type Ca²⁺ current by cilnidipine (FRC-8653) in acutely dissociated rat sympathetic neurones. *Br J Pharmacol*, 122, 37-42.
- Uneyama, H., Uchida, H., Konda, T., Yoshimoto, R., & Akaike, N. (1999). Selectivity of dihydropyridines for cardiac L-type and sympathetic N-type Ca²⁺ channels. *Eur J Pharmacol*, 373, 93-100.

- Varadi, G., Mori, Y., Mikala, G., & Schwartz, A. (1995). Molecular determinants of Ca²⁺ channel function and drug action. *Trends Pharmacol Sci*, *16*, 43-49.
- Vega, T., De Pascual, R., Bulbena, O., & Garcia, A. G. (1995). Effects of omega-toxins on noradrenergic neurotransmission in beating guinea pig atria. *Eur J Pharmacol*, *276*, 231-238.
- Vignali, S., Leiss, V., Karl, R., Hofmann, F., & Welling, A. (2006). Characterization of voltage-dependent sodium and calcium channels in mouse pancreatic A- and B-cells. *J Physiol*, *572*, 691-706.
- Watanabe, K., Dozen, M., & Hayashi, Y. (1995). [Effect of cilnidipine (FRC-8653) on autoregulation of cerebral blood flow]. *Nihon Yakurigaku Zasshi*, *106*, 393-399.
- Wessler, I., Dooley, D. J., Werhand, J., & Schlemmer, F. (1990). Differential effects of calcium channel antagonists (omega-conotoxin GVIA, nifedipine, verapamil) on the electrically-evoked release of [³H]acetylcholine from the myenteric plexus, phrenic nerve and neocortex of rats. *Naunyn Schmiedebergs Arch Pharmacol*, *341*, 288-294.
- White, W. B., Duprez, D., St Hillaire, R., Krause, S., Roniker, B., Kuse-Hamilton, J., & Weber, M. A. (2003). Effects of the selective aldosterone blocker eplerenone versus the calcium antagonist amlodipine in systolic hypertension. *Hypertension*, *41*, 1021-1026.
- Whorlow, S. L., Loiacono, R. E., Angus, J. A., & Wright, C. E. (1996). Distribution of N-type Ca²⁺ channel binding sites in rabbit brain following central administration of omega-conotoxin GVIA. *Eur J Pharmacol*, *315*, 11-18.
- Williams, M. E., Brust, P. F., Feldman, D. H., Patthi, S., Simerson, S., Maroufi, A., McCue, A. F., Velicelebi, G., Ellis, S. B., & Harpold, M. M. (1992). Structure and functional expression of an omega-conotoxin-sensitive human N-type calcium channel. *Science*, *257*, 389-395.
- Williams, M. E., Feldman, D. H., McCue, A. F., Brenner, R., Velicelebi, G., Ellis, S. B., & Harpold, M. M. (1992). Structure and functional expression of alpha 1, alpha 2, and beta subunits of a novel human neuronal calcium channel subtype. *Neuron*, *8*, 71-84.
- Woodward, J. J., Rezazadeh, S. M., & Leslie, S. W. (1988). Differential sensitivity of synaptosomal calcium entry and endogenous dopamine release to omega-conotoxin. *Brain Res*, *475*, 141-145.
- Wright, J. T., Jr., Bakris, G., Greene, T., Agodoa, L. Y., Appel, L. J., Charleston, J., Cheek, D., Douglas-Baltimore, J. G., Gassman, J., Glasscock, R., Hebert, L., Jamerson, K., Lewis, J., Phillips, R. A., Toto, R. D., Middleton, J. P., Rostand, S. G., African American Study of Kidney, D., & Hypertension Study, G. (2002). Effect of blood

- pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA*, 288, 2421-2431.
- Yahagi, N., Akiyama, T., & Yamazaki, T. (1998). Effects of omega-conotoxin GVIA on cardiac sympathetic nerve function. *J Auton Nerv Syst*, 68, 43-48.
- Yamada, Y., Kinoshita, H., Kuwahara, K., Nakagawa, Y., Kuwabara, Y., Minami, T., Yamada, C., Shibata, J., Nakao, K., Cho, K., Arai, Y., Yasuno, S., Nishikimi, T., Ueshima, K., Kamakura, S., Nishida, M., Kiyonaka, S., Mori, Y., Kimura, T., Kangawa, K., & Nakao, K. (2014). Inhibition of N-type Ca²⁺ channels ameliorates an imbalance in cardiac autonomic nerve activity and prevents lethal arrhythmias in mice with heart failure. *Cardiovasc Res*, 104, 183-193.
- Yamamoto, T., & Takahara, A. (2009). Recent updates of N-type calcium channel blockers with therapeutic potential for neuropathic pain and stroke. *Curr Top Med Chem*, 9, 377-395.
- Yamazaki, T., Kawada, T., Akiyama, T., Kitagawa, H., Takauchi, Y., Yahagi, N., & Sunagawa, K. (1997). omega-Conotoxin GVIA and desipramine insensitive norepinephrine efflux from cardiac sympathetic nerve terminal. *Brain Res*, 761, 329-332.
- Yang, S. N., & Berggren, P. O. (2005). Beta-cell CaV channel regulation in physiology and pathophysiology. *Am J Physiol Endocrinol Metab*, 288, E16-28.
- Zhang, J. F., Randall, A. D., Ellinor, P. T., Horne, W. A., Sather, W. A., Tanabe, T., Schwarz, T. L., & Tsien, R. W. (1993). Distinctive pharmacology and kinetics of cloned neuronal Ca²⁺ channels and their possible counterparts in mammalian CNS neurons. *Neuropharmacology*, 32, 1075-1088.
- Zhou, X., Ono, H., Ono, Y., & Frohlich, E. D. (2002). N- and L-type calcium channel antagonist improves glomerular dynamics, reverses severe nephrosclerosis, and inhibits apoptosis and proliferation in an l-NAME/SHR model. *J Hypertens*, 20, 993-1000.

Figure Legend

Figure 1. Effects of N-type Ca^{2+} channel inhibition on the cardiovascular system

Figure 1

