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Influence of ω-Conotoxin GVIA, Nifedipine and Cilnidipine on Catecholamine Release in the Rat Adrenal Medulla

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The present study was designed to establish comparatively the inhibitory effects of cilnidipine (CNP), nifedipine (NIF), and ω-conotoxin GVIA (CTX) on the release of CA evoked by cholinergic stimulation and membrane depolarization from the isolated perfused model of the rat adrenal medulla. CNP (3 μM), NIF (3 μM), and CTX (3 μM) perfused into an adrenal vein for 60 min produced greatly inhibition in CA secretory responses evoked by ACh (5.32×10^{-3} M), DMPP (10^{-4} M for 2 min), McN-A-343 (10^{-4} M for 2 min), high K^+ (5.6×10^{-2} M), Bay-K-8644 (10^{-5} M), and cyclopiazonic acid (10^{-5} M), respectively. For the CA release evoked by ACh and Bay-K-8644, the following rank order of potency was obtained: CNP > NIF > CTX. The rank order for the CA release evoked by McN-A-343 and cyclopiazonic acid was CNP > NIF > CTX. Also, the rank orders for high K^+ and for DMPP were NIF > CTX > CNP and NIF > CNP > CTX, respectively. Taken together, these results demonstrate that all voltage-dependent Ca^{2+} channels (VDCCs) blockers of cilnidipine, nifedipine, and ω-conotoxin GVIA inhibit greatly the CA release evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors and the membrane depolarization without affecting the basal release from the isolated perfused rat adrenal gland. It seems likely that the inhibitory effects of cilnidipine, nifedipine, and ω-conotoxin GVIA are mediated by the blockade of both L- and N-type, L-type only, and N-type only VDCCs located on the rat adrenomedullary chromaffin cells, respectively, which are relevant to Ca^{2+} mobilization. It is also suggested that N-type VDCCs play an important role in the rat adrenomedullary CA secretion, in addition to L-type VDCCs.

Key Words: Cilnidipine, Nifedipine, ω-conotoxin GVIA, Catecholamine release, Adrenal medulla, Voltage-dependent Ca^{2+} channels

INTRODUCTION

Generally, it has been found that adrenal CA secretion is mediated by muscarinic receptors as well as nicotinic receptors in various species (Harish et al, 1987; Nakazato et al, 1988), including the dog (Kimura et al, 1992). The adrenal medulla possesses characteristics of postganglionic sympathetic neurons, and both L- and N-type voltage-dependent Ca^{2+} channels (VDCCs) have been identified in medullary chromaffin cells (Gandia et al, 1995). Several types of VDCCs are present on adrenal chromaffin cells, but the role of each type in the secretion process of catecholamines (CA) remains controversial. Cat chromaffin cells possess L- and N-type VDCCs which each carry 50% of the Ca^{2+} current (Albillos et al, 1994), but the L-type VDCCs dominate the exocytotic process (Lopez et al, 1994a). Bovine chromaffin cells possess not only L- (Artalejo et al, 1991) and N-type VDCCs (Hans et al, 1990; Artalejo et al, 1992) but also P- (Mintz et al, 1992; Gandia et al, 1994) and Q-type VDCCs (Lopez et al, 1994b), and the L- and Q-type VDCCs dominate the exocytotic process (Lomax et al, 1997). Rat chromaffin cells possess L-, N-, P- and Q-type VDCCs (Gandia et al, 1995). Both L- and N-type Ca^{2+} currents have been shown to be recruited during exocytosis from rat chromaffin cells (Kim et al, 1995). Thus not all of the VDCCs present in chromaffin cells may contribute to the secretion of CA. Some electrophysiological data indicate that cilnidipine, unlike other DHP Ca^{2+} channel blockers might be a dual-channel antagonist for peripheral neuronal N-type and vascular L-type VDCCs (Oike et al, 1990; Fujii et al, 1997; Uneyama et al, 1997). It has been shown that the inhibition by cilnidipine of the splanchnic nerve stimulation (SNS)-induced epinephrine (EP) and norepinephrine (NE) secretion and of the muscarine-induced NE secretion in the anesthetized dogs is related to its blocking action on N-type VDCCs (Nagayama et al, 1998). Moreover, previously we demonstrated that the N-type VDCC blocker ω-conotoxin GVIA inhibits the secretion of CA induced by SNS and exogenous acetylcholine (ACh) and suggested that

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ABBREVIATIONS: CA, catecholamine; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide; CNP, cilnidipine; NIF, nifedipine; CTX, ω-conotoxin GVIA; VDCCs, voltage-dependent calcium channels; VMAT, vesicular monoamine transporter; DHP, dihydropyridine.

N-type VDCCs may contribute to the CA secretion (Kimura et al, 1994).

It has also been found that adrenal CA secretion is mediated by muscarinic receptors in various species (Douglas & Poisner, 1965; Harish et al, 1987; Nakazato et al, 1988; Kimura et al, 1992). Concerning the role of VDCCs in the muscarinic receptor-mediated CA secretion, observations obtained with L-type VDCC blockers in vitro studies remain controversial. Verapamil, an L-type VDCC blocker, does not affect muscarine-induced CA secretion from perfused rat adrenal gland (Harish et al, 1987). In contrast, isradipine, another L-type VDCC blocker, inhibits the methacholine-induced CA secretion from cat chromaffin cells (Uceda et al, 1992). However, little is known about the involvement of N-type VDCCs in the muscarinic receptor-mediated CA secretion (Uceda et al, 1994). Therefore, the present study was designed to investigate the effects of cilnidipine, a dual blocker of both L- and N-type VDCCs, nifedipine, an L-type selective VDCC blocker, and ω-conotoxin GVIA, an N-type selective VDCC blocker, on the secretion of CA from the isolated perfused model of the rat adrenal gland in response to ACh, a direct membrane depolarizer, high potassium, a selective neuronal nicotinic agonist 1,1-dimethyl-4-phenyl-piperazinium (DMPP), and a selective muscarinic M_1, McN-A-343 to elucidate the functional role of VDCCs in controlling the adrenal CA secretion.

METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180 to 250 g, were anesthetized with thiopental sodium (40 mg/kg) intraperitoneally. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and placing three hook retractors exposed the left adrenal gland and surrounding area. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations. A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at $37\pm1^{\circ}C$ (Fig. 1).

Perfusion of adrenal gland

The adrenal glands were perfused by means of a peristaltic pump (WIZ Co., USA) at a rate of 0.3 ml/min. The perfusion was carried out with Krebs-bicarbonate solution of following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The solution was constantly bubbled with 95% O_2+5% CO_2 and the final pH of the solution was maintained at 7.4~7.5. The solution contained disodium EDTA (10 μg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of CA.

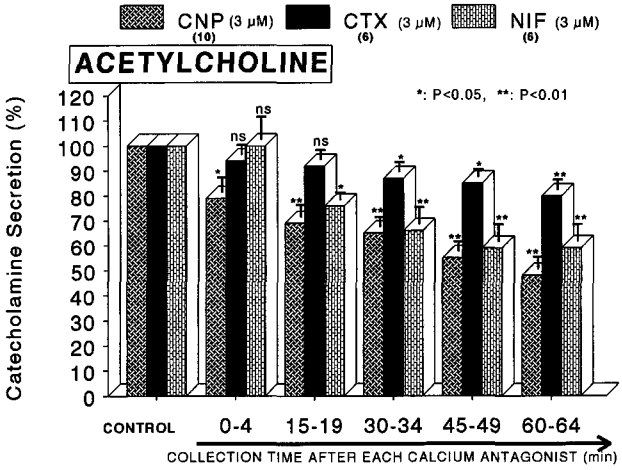

Fig. 1. Time course effect of cilnidipine (CNP), ω-contoxin GVIA (CTX) and nifedipine (NIF) on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. The CA secretion by a single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with CNP (3 μM), CTX (3 μM) and NIF (3 μM) for 60 min, respectively, as indicated at an arrow mark. Numbers in the parenthesis indicate number of rat adrenal glands. Vertical bars on the columns represent the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from the adrenal gland (% of control). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control with the pretreated group with CNP (3 μM), CTX (3 μM) and NIF (3 μM) for 60 min, respectively. ACh-induced perfusate was collected for 4 minutes. *$p<0.05$, **$p<0.01$. ns: statistically not significant.

Drug administration

The perfusions of DMPP (100 μM), McN-A-343 (100 μM) for 2 minutes, Bay-K-8644 (10 μM) and cyclopiazonic acid (10 μM) for 4minutes were made into perfusion stream, respectively. A single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml was injected into perfusion stream via a three-way stopcock.

In the preliminary experiments it was found that upon administration of the above drugs, secretory responses to ACh, KCl, McN-A-343, Bay-K-8644 and cyclopiazonic acid returned to pre-injection level in about 4 min, but the responses to DMPP in 8 min.

Collection of perfusate

As a rule, prior to stimulation with various secretagogues, perfusate was collected for 4 min to determine the spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, collection of the perfusates was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated sample's was

collected for 4 to 8 min. The amounts secreted in the background sample have been subtracted from that secreted from the stimulated sample to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effects of Ca^{2+} channel antagonists on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing Ca^{2+} channel antagonists for 60 min immediately after the perfusate was collected for a certain minute (background sample). And then the medium was changed to the one containing the stimulating agent and the perfusates were collected for the same period as that for the background sample. Generally, the adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

CA content of perfusate was measured directly by the fluorometric method of Anton & Sayre (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981) using fluorospectrophotometer (Kontron Co. Italy).

A volume of 0.2 ml of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by secretogagues used in the present work was high enough to obtain readings several folds greater than the reading of control samples (unstimulated). The sample blanks were also lowest for perfusates of stimulated and non-stimulated samples. The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statistical analysis

The statistical significance between groups was determined by utilizing the Student's t-test. A p-value of less than 0.05 was considered to represent statistically significant changes unless specifically noted in the text. Values given in the text refer to means and the standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computer program described by Tallarida & Murray (1987).

Drugs and their sources

The following drugs were used: cilnidipine hydrochloride (UCB Japan Co., Japan), nifedipine hydrochloride, acetylcholine hydrochloride, 1,1-dimethyl-4- phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, nicotine tartrate, methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-2-trifluoromethylphenyl) pyridine-5- carboxylate (BAY-K8644), nicotine tartrate (Sigma Chemical Co., USA), cyclopiazonic acid and [3-(m-chloro-phenyl-carbamoyl-oxy]-2-butynyl rimethyl ammonium chloride [McN-A-343] (RBI, USA), and ω -conotoxin GVIA (Bachem, Bubendorf, Switzerland). Drugs were dissolved in distilled water (stock) and diluted to the normal Krebs solution as required except Bay-K- 8644, nifedipine and clinidipine, which were dissolved in 99.5% ethanol and diluted appropriately (final concentration of alcohol was less than 0.1%). Bay-K-8644 and clinidipine are stored under dark light before use. Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

Effects of cilnidipine, ω-conotoxin GVIA and nifedipine on CA secretion evoked by acetylcholine from th perfused rat adrenal glands

After the initial perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 21±2 ng/2 min (n=8). Previously, it is also demonstrated that the exogenous ACh-induced CA secretion is inhibited by ω-conotoxin GVIA, an N-type VDCC blocker as well as nifedipine and verapamil, L-type VDCC blockers in anesthetized dogs (Kimura et al, 1994). Therefore, it was decided initially to examine the effects of cilnidipine, ω-conotoxin GVIA and nifedipine on ACh-evoked CA secretion from the isolated perfused rat adrenal glands. ACh was given at 15 min-intervals. Cilnidipine, ω-conotoxin GVIA and nifedipine were present for 60 min, respectively.

In the present study, it was found that cilnidipine, ω-conotoxin GVIA and nifedipine themselves did not produce any effect on the spontaneous CA release (data not shown). When ACh (5.32×10^{-3} M) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 330±30 ng for 4 min. However, after the simultaneous perfusion with cilnidipine (3×10^{-6} M), ACh-stimulated CA secretion was relatively time-dependently inhibited to 48% of the control release (100%) from 10 adrenal glands as shown in Fig. 1. Also, in the presence of ω-conotoxin GVIA (3×10^{-6} M) for 60 min, ACh-evoked

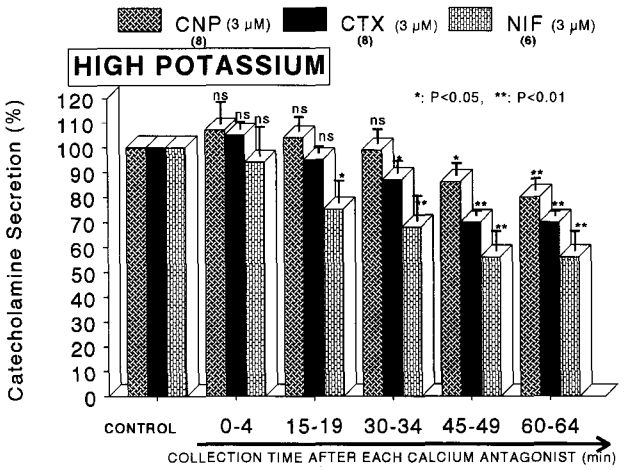

Fig. 2. Time course effect of cilnidipine (CNP), ω-contoxin GVIA (CTX) and nifedipine (NIF) on the secretory responses of catecholamines (CA) evoked by high potassium from the isolated perfused rat adrenal glands. The CA secretion by a single injection of high K^+ (5.6×10^{-2} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with CNP (3 μM), CTX (3 μM) and NIF (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 1. High K^+-induced perfusate was collected for 4 minutes. *$p<0.05$, **$p<0.01$. ns: statistically not significant.

CA secretory response was inhibited to 80% of the control release, although it was not affected for 0~19 min period (Fig. 1). Nifedipine (3×10^{-6} M), a selective L-type Ca^{2+} channel antagonist, also inhibited ACh-evoked CA release by 59% of the corresponding control, as shown in Fig. 1.

Effects of cilnidipine, ω-conotoxin GVIA and nifedipine on CA secretion evoked by high potassium from the perfused rat adrenal glands

Also, it has been found that direct membrane-depolarizing agent like KCl stimulates sharply CA secretion. High K^+ (5.6×10^{-2} M) in a volume of 0.05 ml was given into the rat adrenal medulla before the treatment with Ca^{2+} channel antagonists evoked the CA secretion of 182±18 ng for 4 min. In the present work, high K^+ (5.6×10^{-2} M)-stimulated CA secretion after the pretreatment with lower dose of cilnidipine (3×10^{-6} M) was not affected for 0~34 min period. However, following 45 min of cilnidipine-treatment it was inhibited to 80% of the corresponding control secretion (100%) from 8 glands, as shown in Fig. 2. Also, ω-conotoxin GVIA (3×10^{-6} M) and nifedipine (3×10^{-6} M), given for 60 min, reduced high potassium-evoked CA secretory response to 70% and 56% of the corresponding control responses (100%), respectively, although they were not affected for early period (Fig. 2).

Effects of cilnidipine, ω-conotoxin GVIA and nifedipine on CA secretion evoked by DMP from the perfuse rat adrenal glands

When perfused through the rat adrenal gland, DMPP (10^{-4} M), which is a selective neuronal nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (361±28 ng for 0~8 min). As shown in Fig. 3, DMPP-stimulated CA secretion following the loading with cilnidipine (3×10^{-6} M) for 60 min was relatively time-dependently inhibited to 41% of the control secretion (100%) from 10 adrenal glands. In the presence of ω-conotoxin GVIA (3×10^{-6} M), a selective N-type Ca^{2+} channel antagonist, for 60 min, DMPP-evoked CA secretory response was inhibited to 69% of the control release, as shown in Fig. 3. Nifedipine (3×10^{-6} M) also reduced markedly DMPP-evoked CA secretion by 32% of the corresponding control (Fig. 3).

Effects of cilnidipine, ω-conotoxin GVIA and nifedipine on CA secretion evoked by McN-A-343 from th perfused rat adrenal glands

In awaked rats, McN-A-343 induced hypertension and tachycardia, which are antagonized by propranolol (Martin, 1996). Adrenal demedullation had no effect on the tachycardia, whereas treatment with guanethidine suppressed both tachycardia and hypertension (Martin, 1996). It has been shown that muscarinic stimulation generates a depolarizing signal, which triggers the firing of action potentials, resulting in the increased CA release in the rat chromaffin cells (Akaike et al, 1990) and the perfused rat adrenal gland (Lim & Hwang, 1991). Therefore, it was of interest to examine the effects of cilnidipine, ω-conotoxin GVIA and nifedipine on CA secretion evoked by McN-A-343, which is a selective muscarinic M1-receptor agonist (Hammer & Giachetti, 1982), in the isolated perfused rat adrenal glands.

As illustrated in Fig. 4, McN-A-343 (10^{-4} M), perfused into an adrenal vein for 4 min before the treatment with Ca^{2+} channel antagonists caused an increased CA secretion by 140±6 ng (0~4 min). However, in the presence of cilnidipine (3×10^{-6} M), the CA secretory response evoked

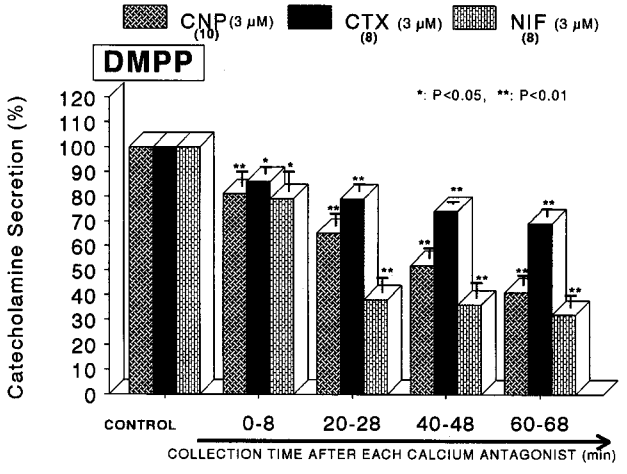

Fig. 3. Time course effect of cilnidipine (CNP), ω-contoxin GVIA (CTX) and nifedipine (NIF) on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. The CA secretion by a perfusion of DMPP (10^{-4} M) for 2 min was evoked at 20 min intervals after preloading with CNP (3 μM), CTX (3 μM) and NIF (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 1. DMPP-induced perfusate was collected for 8 minutes. *p<0.05, **p<0.01.

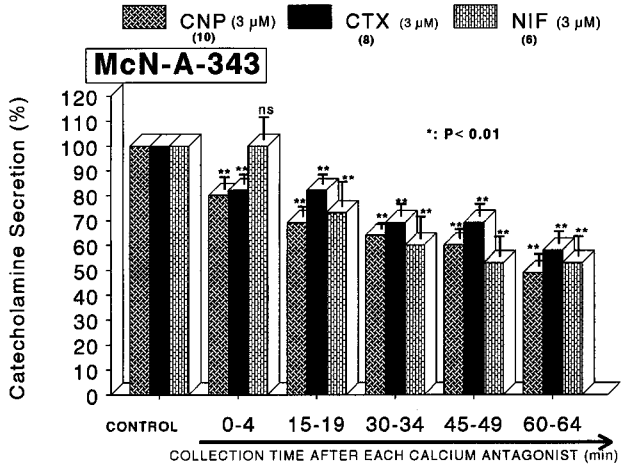

Fig. 4. Time course effect of cilnidipine (CNP), ω-contoxin GVIA (CTX) and nifedipine (NIF) on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. The CA secretion by a perfusion of McN-A-343 (10^{-4} M) for 2 min was evoked at 15 min intervals after preloading with CNP (3 μM), CTX (3 μM) and NIF (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 1. McN-A-343-induced perfusate was collected for 4 minutes. *p<0.05, **p<0.01. ns: statistically not significant.

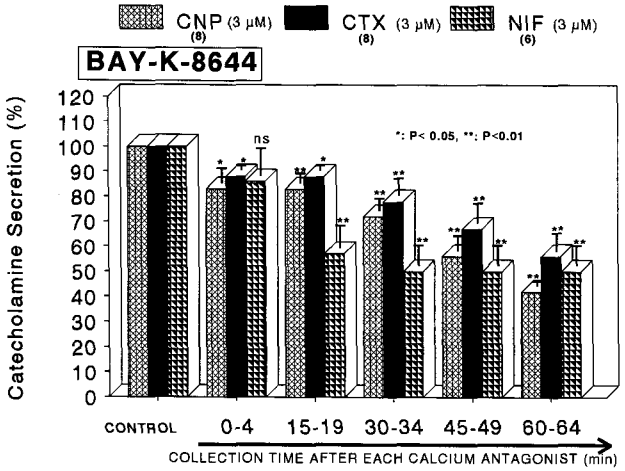

Fig. 5. Time course effect of cilnidipine (CNP), ω-contoxin GVIA (CTX) and nifedipine (NIF) on the secretory responses of catecholamines (CA) evoked by Bay-K-8644 from the isolated perfused rat adrenal glands. The CA secretion by a perfusion of Bay-K-8644 (10^{-5} M) for 4 min was evoked at 15 min intervals after preloading with CNP (3 μM), CTX (3 μM) and NIF (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 1. Bay-K-8644-induced perfusate was collected for 4 minutes. *p<0.05, **p<0.01. ns: statistically not significant.

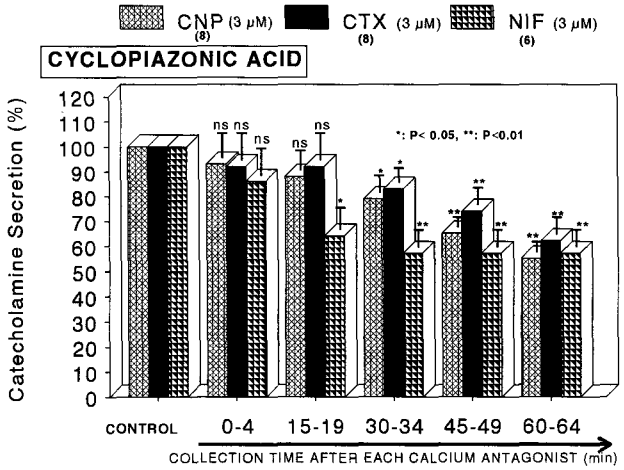

Fig. 6. Time course effect of cilnidipine (CNP), ω-contoxin GVIA (CTX) and nifedipine (NIF) on the secretory responses of catecholamines (CA) evoked by cyclopiazonic acid from the isolated perfused rat adrenal glands. The CA secretion by a perfusion of cyclopiazonic acid (10^{-5} M) for 4 min was evoked at 15 min intervals after preloading with CNP (3 μM), CTX (3 μM) and NIF (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 1. Cyclopiazonic acid-induced perfusate was collected for 4 minutes. *p<0.05, **p<0.01. ns: statistically not significant.

by McN-A-343 was diminished by 49% of the control release (100%) in a relative time-dependent fashion. Also, in the presence of ω-conotoxin GVIA (3×10^{-6} M) for 60 min, the CA secretion evoked by McN-A-343 was inhibited by 58% of the corresponding control release (100%), as shown in Fig. 4. Nifedipine (3×10^{-6} M), given in to the adrenal medulla, also inhibited time-dependently the CA secretion evoked by McN-A-343 by 53% of the corresponding control (Fig. 4).

Effects of cilnidipine, ω-conotoxin GVIA and nifedipine on CA secretion evoked by Bay-K-8644 from th perfused rat adrenal glands

It has been found that Bay-K-8644 is a selective L-type calcium channel activator, which causes positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm et al, 1982; Wada et al, 1985) and enhances basal Ca^{2+} uptake (Garcia et al, 1984) and CA release (Lim et al, 1992). Therefore, it was of interest to determine the effects of cilnidipine, ω-conotoxin GVIA and nifedipine on the CA secretion evoked by Bay-K-8644 from the isolated perfused rat adrenal glands. In the absence of Ca^{2+} channel antagonists, Bay-K-8644 (10^{-5} M) given into the perfusion stream for 4 min produced CA secretion of 117±12 ng (0~4 min). However, in the presence of cilnidipine (3×10^{-6} M), the CA secretion evoked by Bay-K-8644 was time-dependently inhibited by 42% of the corresponding control release (Fig. 5). Also, ω-conotoxin GVIA (3×10^{-6} M) and nifedipine (3×10^{-6} M), given into the adrenal gland for 60 min, reduced the CA secretory responses evoked by Bay-K-8644 to 56% and 50% of the corresponding control responses (100%), respectively, which were produced relatively in time-dependent fashion (Fig. 5).

Effects of cilnidipine, ω-conotoxin GVIA and nifedipine on CA secretion evoked by cyclopiazonic acid from the perfused rat adrenal glands

Cyclopiazonic acid, a mycotoxin from Aspergillus and Penicillium, has been described as a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Georger & Riley, 1989; Seidler et al, 1989). It has been shown that the mobilization of Ca^{2+} from Ca^{2+} stores by pharmacological manipulation (e.g., inhibition of Ca^{2+}-ATPase in the stores by thapsigargin or cyclopiazonic acid) activates Ca^{2+} entry through store-operated Ca^{2+} channel (SOC) (Takemura et al, 1989; Fasolato et al, 1994; Berridge, 1995). It was excitable to test the effects of cilnidipine, ω-conotoxin GVIA and nifedipine on the CA secretion evoked by cyclopiazonic acid from the isolated perfused rat adrenal glands. As shown in Fig. 6, cyclopiazonic acid (10^{-5} M)-evoked CA secretion prior to the treatment with cilnidipine (3×10^{-6} M) was 93±10 ng for 0~4 min. However, in presence of cilnidipine (3×10^{-6} M), the cyclopiazonic acid (10^{-5} M)-evoked CA secretion was inhibited by 55% of the control response (100%), although it was not affected for early 0~19 min period. Also, the pretreatment with ω-conotoxin GVIA (3×10^{-6} M) for 60 min depressed the CA secretion evoked by cyclopiazonic acid by 62% of the corresponding control release (100%), as shown in Fig. 6. Nifedipine (3×10^{-6} M), given in to the adrenal medulla, also time-dependently inhibited the CA secretion evoked by cyclopiazonic acid by 57% of the corresponding control (Fig. 6).

DISCUSSION

These experimental results demonstrate that all VDCC

blockers of cilnidipine, ω-conotoxin GVIA, and nifedipine inhibit greatly the release of CA from the isolated perfused rat adrenal gland evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors and the membrane depolarization without affecting the basal release, respectively. It seems likely that the inhibitory effects of cilnidipine, ω-conotoxin GVIA, and nifedipine are mediated by the blockade of both L- and N-type, N-type only, and L-type only VDCCs located on the rat adrenomedullary chromaffin cells, respectively. It is also suggested that N-type VDCCs play an important role in the rat adrenomedullary CA secretion, in addition to L-type VDCCs.

Generally, it has been found that the adrenal medulla possesses characteristics of postganglionic sympathetic neurons, and both L- and N-type VDCCs have been identified in medullary chromaffin cells (Gandia et al, 1995). Adrenal CA secretion has been found to be mediated by muscarinic receptors as well as nicotinic receptors in various species (Harish et al, 1987; Nakazato et al, 1988), including the dog (Kimura et al, 1992). However, little is known about the involvement of VDCCs in contributing to the muscarinic receptor-mediated CA secretion (Uceda et al, 1994).

Nifedipine inhibited the increases in CA output induced by ACh and the nicotinic agonist DMPP as well as by McN-A-343 and high K^+. The inhibitory effects of nifedipine on the ACh-induced CA secretion can be explained by its blocking action on the nicotinic receptor-mediated pathway. L-type VDCCs may thus contribute to the secretion of CA mediated by nicotinic receptors. Nifedipine also inhibited the DMPP-induced increase in CA output, suggesting that L-type VDCCs surely contribute to nicotinic receptor-mediated CA secretion. In support of this idea, it has been reported that furnidipine, an L-type VDCC blocker, inhibits the K^+-induced norepinephrine secretion more than that of epinephrine in bovine chromaffin cells (Lomax et al, 1997). These findings, including the present results, suggest that the nifedipine's blocking effect of L-type VDCCs to K^+-induced CA secretion is greater than those to other VDCC blockers. This explains the weaker inhibitory effect of nifedipine on ACh-induced CA secretion in comparison with that of cilnidipine, although it was stronger than that of ω-conotoxin GVIA. Here, the question arises as to why nifedipine influences the nicotinic receptor-mediated CA secretion differently; a potent inhibition in the case of DMPP and a weak inhibition in the case of ACh-evoked CA secretion. This difference might be explained by assuming a differential distribution of L-type voltage-dependent Ca^{2+} channels on the CA-containing cell membrane in synaptic zones and extrasynaptic regions (Fig. 7).

If L-type VDCCs are primarily concentrated in extrasynaptic regions but less so in synaptic zones, they could affect the depolarization during activation of extrasynaptic nicotinic receptors but hardly affect the depolarization during activation of intrasynaptic nicotinic receptors (see Fig. 7). It has also been reported that the L-type VDCC blocker isradipine partially inhibits electrical stimulation- and ACh-induced CA secretion, but potently inhibits nicotine- and K^+-induced secretion in the perfused rat adrenal gland (Lopez et al, 1992). This observation is consistent with the results in the present study.

Electrophysiological studies have suggested that in rat chromaffin cells L-type voltage-dependent Ca^{2+} channels predominate but other subtypes of voltage-dependent Ca^{2+} channels such as N-, P- and Q-type are also present (Gandia et al, 1995). N-type Ca^{2+} current has been shown to be recruited during exocytosis from rat chromaffin cells (Kim et al, 1995), but there has been no direct evidence for the participation of N-type VDCCs in CA secretion. In the present study, ω-conotoxin GVIA, an N-type VDCC blocker, inhibited the secretory responses of CA in response to ACh, DMPP or McN-A-343. Also, ω-conotoxin GVIA caused the weak inhibitory effect on ACh-induced CA secretion, compared to cilnidipine or nifedipine. The weak inhibitory effect of ω-conotoxin GVIA on the CA secretory responses evoked by ACh may be due to a poor tissue penetration ability or insufficient concentration, although a submicromolar concentration (10 nM) of ω-conotoxin GVIA has been reported to reduce nerve stimulation-induced CA secretion by 75% in perfused bovine adrenal gland under experimental conditions similar to those used here (O'Farrell et al, 1997). In the present study, a higher concentration (3,000 nM) of ω-conotoxin GVIA was used. Thus, the present results suggest that N-type VDCCs have at least minor role in the secretion of CA from the perfused rat adrenal gland, in comparison to L-type VDCCs.

Furthermore, in the present investigation, cilnidipine as well as ω-conotoxin GVIA infused into the rat adrenal gland significantly inhibited CA output evoked by DMPP as well as McN-A-343 and ACh without affecting the basal CA output. It has been reported that cilnidipine has potent inhibitory actions on N-type as well as L-type VDCCs in rat dorsal root ganglion neurons (Fujii et al, 1997). Previously, by using experimental conditions similar to those in the anesthetized dog, it has also been demonstrated that the SNS-induced CA secretion is inhibited by ω-conotoxin GVIA, an N-type VDCC blocker, but not by nifedipine and verapamil, L-type VDCC blockers (Kimura et al, 1994).

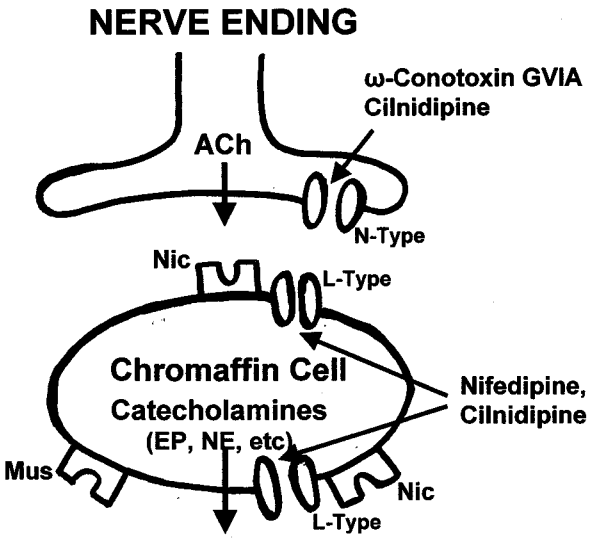

Fig. 7. Schematic diagram of possible action site of voltage-dependent Ca^{2+} channel blockers at the cholinergic nerve ending-chromaffin cell synapse in the rat adrenal gland. This diagram demonstrates possible localizations of voltage-dependent Ca^{2+} channels and cholinergic receptors mediating adrenal CA secretion. Catecholamine-containing cells possess synaptic nicotinic receptors, extrasynaptic nicotinic and muscarinic receptors, and L-type voltage-dependent Ca^{2+} channels close to the synaptic and extrasynaptic nicotinic receptors. N-type voltage-dependent Ca^{2+} channenls are located on cholinergic nerve endings.

Therefore, in terms of these findings, it is probable that cilnidipine, like ω-conotoxin GVIA inhibits the secretion of CA in response to DMPP as well as McN-A-343 and ACh through blockade of N-type VDCCs on the rat adrenomedullary chromaffin cells, in addition to the blocking action of L-type VDCCs. The present result that ω-conotoxin GVIA also inhibited CA secretion by ACh as well as DMPP and McN-A343 given into the rat adrenal gland indicates that it can depress the secretion of CA evoked by those agents through blockade of N-type VDCCs. Previously, it was also demonstrated that the exogenous ACh-induced CA secretion is inhibited by ω-conotoxin GVIA, nifedipine and verapamil (Kimura et al, 1994). In support of this result, it has been found that ω-conotoxins, ω-conotoxin MVIIA and ω-conotoxin GVIA potently block N-type Ca^{2+} channels (Kasai et al, 1987; McCleskey et al, 1987; Plummer et al, 1989; Regan et al, 1991; Miljanich & Ramachandran, 1995). From these results, it is suggested that cilnidipine inhibits the ACh-induced CA secretion through blockade of both L- and N-type VDCCs. Therefore it seems likely that the inhibition by cilnidipine of the ACh-induced CA secretion is attributed at least partly to its blocking action in N-type VDCCs. The possible explanation for the differential effects of nifedipine on CA secretion between endogenous and exogenous ACh was described previously (Kimura et al, 1994).

Nagayama and his coworkers (1998) have reported that cilnidipine significantly inhibited both EP and NE output induced by DMPP, as well as SNS and ACh in the anesthetized dog. Previously, it was demonstrated, under the same experimental conditions, that the SNS-induced secretion of CA is mediated mainly by nicotinic receptors (Shimamura et al, 1991; Kimura et al, 1992). Therefore the inhibitory effect of cilnidipine and nifedipine on the DMPP-induced CA secretion indicates that cilnidipine as well as nifedipine inhibit the CA secretion by affecting the process mediated by nicotinic receptors. It has also been shown that ACh simulates the CA secretion by activating both nicotinic and muscarinic receptors (Kimura et al, 1992). Also, like nifedipine, the inhibition by cilnidipine of the ACh-induced secretion of CA can be explained at least in part by its inhibitory action on nicotinic receptor-mediated pathway. Moreover, PC12 cells are derived from a rat pheochromocytoma cell line that is very popular for investigation neuronal differentiation. A typical L-type Ca^{2+} channel blocker such as nifedipine has been reported to possess a small effect on the Ca^{2+} channel-operated functions (Uneyama et al, 1998). These characteristics are consistent with those of differentiated PC12 cells, which have characteristics similar to those of peripheral sympathetic neurons, including the development of N-type Ca^{2+} channel (Greene & Tischler, 1982; Takahashi et al, 1985; Usowicz et al, 1990; Mullikin-Kilpatrick & Treistman, 1995). Moreover, Uneyama and his coworkers (1998) have clearly shown that a DHP type of antithyperthensive drug, cilnidipine, strongly inhibited the high K^+-evoked CA secretion, $[Ca^{2+}]_i$ elevation, and high voltage-activated Ca^{2+} channel currents in differentiated rat PC12 cells. In contrast to cilnidipine, the selective L-type Ca^{2+} channel antagonist nifedipine showed a weak inhibition on all three parameters in the PC12 cells after differentiation by nerve growth factor. Cilnidipine directly blocked the isolated N-type channel current, but had no effect on the L-type channel current in the differentiated rat PC12 cells. In the present work, the results that cilnidipine inhibited CA secretory responses evoked by cholinergic stimulation as well as by membrane depolarization suggest that the blockade of N-type Ca^{2+} channels by cilnidipine results in reduced Ca^{2+} influx through these channels and thereby reduces CA secretions closely linked with $[Ca^{2+}]_i$ elevation, which is evoked by various depolarizing stimulation.

N-type Ca^{2+} channels play a key role in the regulation of sympathetic nerve activity. It is well characterized that Ca^{2+} influx through N-type Ca^{2+} channels is closely related to the CA secretion at the nerve endings of the sympathetic neurons as well (Hirning et al, 1988), and a peptide antagonist selective for N-type Ca^{2+} channel, ω-conotoxin-GVIA, blocks NE release from vessels (Nakashima et al, 1991). It has also been found that, in spontaneously hypertensive rats, cilnidipine reduced blood pressure without elevating heart rate and plasma NE concentrations (Hosono et al, 1995a). In this model, other DHPs (amlodipine, nifedipine, manidipine, nicardipine, benidipine) induced baroreflex tachycardia combined with plasma NE elevation. Therefore, the blockade by cilnidipine of CA secretion from the isolated perfused model of the adrenal gland might well explain why cilnidipine failed to induce elevation of plasma CA concentration by hypotension-evoked baroreflexes in vivo (Hosono et al, 1995b) or why the drug blocked the NE release from isolated vessels (Nakashima et al, 1991). The present results are in agreement with the results in differentiated PC12 cells (Uneyama et al, 1998) and in anesthetized dog (Nagayama et al, 1998). In support of this idea, it has been well known that sources of plasma CA are mainly sympathetic nerve endings and adrenal chromaffin cells, at which N-type Ca^{2+} channels are predominantly distributed. It has also been reported that the blockade of peripherally distributed N-type Ca^{2+} channels by ω-conotoxin GVIA led to reduction of plasma CA concentration by inhibition of its secretion from the vascular beds (Friedman & Duckles, 1994) and the anesthetized dog adrenal glands (Kimura et al, 1994). Especially, it is well known that CA secretion from sympathetic neurons is insensitive to L-type VDCC antagonist, but sensitive to ω-conotoxin GVIA (Hirning et al, 1988).

Furthermore, cilnidipine had a therapeutic effect even in the mental stress-induced hypertension model (cold stress model) whereas other DHPs were ineffective (Hosono et al, 1995b). Thus, it is suggested that dual N- and L-type Ca^{2+} channel antagonists such as cilnidipine, may be antihypertensive Ca^{2+} channel antagonists to minimize the unfavorable effects of antihypertensive Ca^{2+} channel antagonism.

In the present investigation, the results that cilnidipine as well as ω-conotoxin GVIA inhibits CA secretion evoked by stimulation of muscarinic receptors with McN-A-343, a selective muscarinic M_1-receptor agonist, suggest strongly that muscarinic M_1-receptors are involved in the regulation of the secretory responses in the rat adrenal medulla. In support of this hypothesis, the muscarinic receptor-mediated secretion of adrenal CA has been thought to be caused by Ca^{2+} mobilized from intracellular storage sites (Cheek & Burgoyne, 1985; Misbahuddin & Oka, 1988; Nakazato et al, 1988; Yamada et al, 1988). Furthermore, it has been shown that muscarinic stimulation generates a depolarizing signal, which triggers the firing of action potentials, resulting in the increased CA release in the rat chromaffin cells (Akaike et al, 1990), guinea pigs (Inoue & Kuriyama, 1991) and the perfused rat adrenal gland (Lim & Hwang, 1991). These observations are in line with a previous report showing that Bay-K-8644 almost trippled

the peak secretory response to muscarine in perfused cat adrenal glands (Ladona et al, 1987; Uceda et al, 1992). In the present experiment, cilnidipine also depressed greatly CA secretion induced by Bay-K-8644, which is found to potentiate the release of CA by increasing Ca^{2+} influx through L-type Ca^{2+} channels in chromaffin cells (Garcia et al, 1984). Also, cilnidipine attenuated CA secretions evoked by high potassium, a direct membrane-depolarizing agent. These findings that cilnidipine, like nifedipine inhibited the CA secretion evoked by Bay-K-8644 as well as by high K^+ suggest that cilnidipine directly inhibits the VDCCs. In the bovine chromaffin cells, stimulation of nicotinic, but not muscarinic ACh receptors is known to cause CA secretion by increasing Ca^{2+} influx largely through VDCCs (Oka et al, 1979; Burgoyne, 1984). Therefore, it seems that cilnidipine inhibits DMPP-evoked CA secretion by inhibiting Ca^{2+} influx through VDCCs activated by nicotinic ACh receptors.

The present study has also shown that cilnidipine inhibits the CA secretion evoked by cyclopiazonic acid. Cyclopiazonic acid is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Geoger & Riley, 1989; Seidler et al, 1989) and a valuable pharmacological tool for investigating intracellular Ca^{2+} mobilization and ionic currents regulated by intracellular Ca^{2+} (Suzuki et al, 1992). Therefore, it is felt that the inhibitory effect of cilnidipine and ω-conotoxin GVIA on CA secretion evoked by cholinergic stimulation as well as by membrane-depolarization may be associated with the mobilization of intracellular Ca^{2+} from the cytoplasmic calcium store. This indicates that the cilnidipine as well as ω-conotoxin GVIA has an inhibitory effect on the release of Ca^{2+} from the intracellular pools induced by stimulation of muscarinic ACh receptors, which is weakly responsible for the secretion of CA. It has been shown that Ca^{2+}-uptake into intracellular storage sites susceptible to caffeine (Ilno, 1989) is almost completely abolished by treatment with cyclopiazonic acid during the proceeding of Ca^{2+} load (Suzuki et al, 1992). This is consistent with the findings obtained in skinned smooth muscle fibers of the longitudinal layer of the guinea-pig ileum, where Ca^{2+} -uptake was also inhibited by cylopiazonic acid (Uyama et al, 1992). Suzuki and his coworkers (1992) have shown that cyclopiazonic acid easily penetrates into the cytoplasm through the plasma membrane and reduces Ca^{2+}-ATPase activity in sarcoplasmic/endoplasmic reticulum, resulting in increase in the subsequent Ca^{2+} release from those storage sites. Moreover, in bovine adrenal chromaffin cells, stimulation of muscarinic ACh receptors is also proposed to cause activation of phosphoinositide metabolism, resulting in the formation of inositol 1, 4, 5-trisphosphate, which induces the mobilization of Ca^{2+} from the intracellular pools (Cheek et al, 1989; Challis et al, 1991). However, in the present study, it is uncertain whether the inhibitory effect of cilnidipine and ω-conotoxin GVIA on Ca^{2+} movement from intracellular pools is due to its direct effect on the PI response or the indirect effects.

Collectively, these results obtained from the present study demonstrate that all VDCC blockers of cilnidipine, nifedipine, and ω-conotoxin GVIA inhibit the release of CA evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors and the membrane depolarization from the isolated perfused rat adrenal gland, respectively. It seems that the inhibitory effects of cilnidipine, nifedipine, and ω-conotoxin GVIA are mediated by the blockade of both L- and N-type, L-type only and N-type only VDCCs located on the rat adrenomedullary chromaffin cells, respectively. They look likely to block the Ca^{2+} influx into the medullary chromaffin cells as well as Ca^{2+} release from the cytoplasmic calcium store. It is also suggested that N-type VDCCs play an important role in the rat adrenomedullary CA secretion, in addition to L-type VDCCs.

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REFERENCES

Akaike A, Mine Y, Sasa M, Takaori S. Voltage and current clamp studies of muscarinic and nicotinic excitation of the rat adrenal chromaffin cells. *J Pharmacol Expt Ther* 255: 333−339, 1990

Albillos A, Artalejo AR, Lopez MG, Gandia L, Garcia AG, Carbone E. Calcium channel subtypes in cat chromaffin cells. *J Physio* 477: 197−213, 1994

Anton AH, Sayre DF. A study of the factors affecting the aluminum oxide trihydroxy indole procedure for the analysis of catecholamines. *J Pharmacol Exp Ther* 138: 360−375, 1962

Artalejo CR, Dahmer MK, Perlman RL, Fox AP. Two types of Ca^{2+} currents are found in bovine chromaffin cells: facilitation is due to the recruitment of one type. *J Physio* 432: 681−707, 1991

Artalejo CR, Perlman RL, Fox AP. -Conotoxin GVIA blocks a Ca^{2+} current in bovine chromaffin cells that is not of the 'classic' N type. *Neuron* 8: 85−95, 1992

Berridge MJ. Capacitative calcium entry. *Biochem J* 312: 1−11, 1995

Burgoyne RD. Mechanism of secretion from adrenal chromaffin cells. *Biochem Biophys Acta* 779: 201−216, 1984

Challis RAJ, Jones JA, Owen PJ, Boarder MR. Changes in inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate mass accumulations in cultured adrenal chromaffin cells in response to bradykinin and histamine. *J Neurochem* 56: 1083−1086, 1991

Cheek TR, Burgoyne RD. Effects of activation of muscarinic receptors on intracellular free calcium and secretion in bovine chromaffin cells. *Biochim Biophys Acta* 846: 167−173, 1985

Cheek TR, O'Sullivan AJ, Moreton RB, Berridge MJ, Burgoyne RD. Spatial localization of the stimulus-induced rise in cytosolic Ca^{2+} in bovine adrenal chromaffin cells: Distinct nicotinic and muscarinic patterns. *FEBS Lett* 247: 429−434, 1989

Douglas WW, Poisner AM. Preferential release of adrenaline from the adrenal medulla by muscarine and pilocarpine. *Nature* 208: 1102−1103, 1965

Fasolato C, Innocenti B, Pozzan T. Receptor-activated Ca^{2+} influx: how many mechanisms for how many channels. *Trends Pharmacol Sci* 15: 77−83, 1994

Friedman DJ, Duckles SP. Influence of age on control of norepinephrine release: Ca^{2+} channels and dopamine D2 receptors. *Eur J Pharmacol* 252: 1−9, 1994

Fujii S, Kameyama K, Hosono M, Hayashi Y, Kitamura K. 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: 1184−1191, 1997

Gandia L, Albillos A, Garcia AG. Bovine chromaffin cells possess FTX-sensitive calcium channels. *Biochem Biophys Res Commun* 194: 671−676, 1994

Gandia L, Borges R, Albillos A, Garcia AG. Multiple calcium channel subtypes in isolated rat chromaffin cells. *Pflugers Arch* 430: 55−63, 1995

Garcia AG, Sala F, Reig JA, Viniegra S, Frias J, Fonteriz R, Gandia L. Dihydropyridine Bay-K-8644 activates chromaffin cell calcium channels. *Nature* 309: 69−71, 1984

Goeger DE, Riley RT. Interaction of cyclopiazonic acid with rat skeletal muscle sarcoplasmic reticulum vesicles. Effect on Ca^{2+} binding and Ca^{2+} permeability. *Biochem Pharmacol* 38: 3995–4003, 1989

Greene LA, Tischler AS. PC12 pheochromocytoma cultures in neurobiological research. *Adv Cell Neurobiol* 3: 373-414, 1982

Hammer R, Giachetti A. Muscarinic receptor subtypes: M1 and M2 biochemical and functional characterization. *Life Sci* 31: 2992–2998, 1982

Hans M, Illes P, Takeda K. The blocking effects of -conotoxin on Ca current in bovine chromaffin cells. *Neurosci Lett* 114: 63–68, 1990

Harish OE, Kao LS, Raffaniello R, Wakade AR, Shneider AS. Calcium dependence of muscarinic receptor-mediated catecholamine secretion from the perfused adrenal medulla. *J Neurochem* 48: 1730–1735, 1987

Hirning LD, Fox AP, McCleskey EW, Olivera BM, Thayer SA, Miller RJ, Tsien RW. Dominant role of N-type Ca^{2+} channels in evoked release of norepinephrine from sympathetic neurons. *Science* 239: 57–61, 1988

Hosono M, Fujii S, Himura T, Watanabe K, Hayashi Y, Ohnishi H, Takata Y, Kato H. Inhibitory effect of cilnidipine on vascular sympathetic neurotransmission and subsequent vasoconstriction in spontaneously hypertensive rats. *Jpn J Pharmacol* 69: 127–134, 1995b

Hosono M, Himura T, Watanabe K, Hayashi Y, Ohnishi H, Takata Y, Kato H. Inhibitory effect of cilnidipine on pressor response to acute cold stress in spontaneously hypertensive rats. *Jpn J Pharmacol* 69: 119–125, 1995a

Hosono M, Iida H, Ikeda K, Hayashi Y, Dohmoto H, Hashiguchi Y, Yamamoto H, Watanabe N, Yoshimoto R. In vivo and ex vivo Ca-antagonistic effect of 2-methoxyethyl(E)-3-phenyl-2-propen-1-yl(+/−)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) pyridine-3,5-dicarboxylate (FRC-8653), a new dihydropyridine derivative. *J Pharmacobiodyn* 15: 547–553, 1992a

Ilno M. Calcium-induced calcium release mechanism in guinea pig taenia caeci. *J Gen Physiol* 94: 363–383, 1989

Inoue M, Kuriyama H. Muscarinic receptor is coupled with a cation channel through a GTP-binding protein in guinea-pig chromaffin cells. *J Physiol (Lond)* 436: 511–29, 1991

Kasai H, Aosaki H, Fukuda J. Presynaptic Ca^{2+}-antagonist omega-conotoxin irreversibly blocks N-type Ca^{2+} channels in chick sensory neurons. *Neurosci Res* 4: 228–235, 1987

Kim SJ, Lim W, Kim J. Contribution of L- and N-type calcium currents to exocytosis in rat adrenal medullary chromaffin cells. *Brain Res* 675: 289–296, 1995

Kimura T, Shimamura T, Satoh S. Effects of pirenzepine and hexamethonium on adrenal catecholamine release in responses to endogenous and exogenous acetylcholine in anesthetized dogs. *J Cardiovasc Pharmacol* 20: 870–874, 1992

Kimura T, Takeuchi A, Satoh S. Inhibition by omega-conotoxin GVIA of adrenal catecholamine release in response to endogenous and exogenous acetylcholine. *Eur J Pharmacol* 264: 169–175, 1994

Ladona MG, Aunis D, Gandia AG, Garcia AG. Dihydropyridine modulation of the chromaffin cell secretory response. *J Neurochem* 48: 483–490, 1987

Lim DY, Hwang DH. Studies on secretion of catecholamines evoked by DMPP and McN-A-343 in the rat adrenal gland. *Korean J Pharmacol* 27: 53–67, 1991

Lim DY, Kim CD, Ahn KW. Influence of TMB-8 on secretion of catecholamines from the perfused rat adrenal glands. *Arch Pharm Res* 15: 115–125, 1992

Lomax RB, Michelrna P, Nunez L, Garcia-Sancho J, Garcia AG, Montiel C. Different contributions of L- and Q-type Ca^{2+} channels to Ca^{2+} signals and secretion in chromaffin cell subtypes. *Am J Physiol* 272: C476–C484, 1997

Lopez MG, Albillos A, de la Fuente MT, Borges R, Gandia L, Carbone E, Garcia AG, Artalejo AR. Localized L-type calcium channels control exocytosis in cat chromaffin cells. *Pflugers Archiv* 427: 348–354, 1994a

Lopez MG, Shukla A, Garcia AG, Wakade AR. A dihydropyridine-resistant component in the rat adrenal secretory response to splanchnic nerve stimulation. *J Neurochem* 58: 2139–2144, 1992

Lopez MG, Villarroya M, Lara B, Sierra RM, Albillos A, Garcia AG, Gandia L. Q- and L-type Ca^{2+} channels dominate the control of secretion in bovine chromaffin cells. *FEBS Letters* 349: 331–337, 1994b

Martin JR. Pressor effect of the putative M1 muscarinic receptor agonist McN-A-343 in the conscious rat. *Life Sci* 59: 1839–1852, 1996

McCleskey EW, Fox AP, Feldman D, Cruz LJ, Olivera BM, Tsien RW, Yoshikami D. ω-Conotoxin: direct and persistent block of specific types of Ca^{2+} channels in neurons but not muscle. *Proc Natl Acad Sci USA* 84: 4327–4331, 1987

Miljanich GP, Ramachandran J. Antagonists of neuronal calcium channels: structure, function and therapeutic implications. *Annu Rev Pharmacol Toxicol* 35: 707–734, 1995

Mintz IM, Venema VJ, Swiderek KM, Lee TD, Bean BP, Adams ME. P-type calcium channels blocked by the spider toxin -agatoxin-IVA. *Nature* 355: 827–829, 1992

Misbahuddin M, Oka M. Muscarinic stimulation of guinea pig adrenal chromaffin cells stimulates catecholamine secretion without significant increase in Ca^{2+} uptake. *Neurosci Lett* 87: 266–370, 1988

Mullikin-Kilpatrick D, Treistman SN. Inhibition of dihydropyridine-sensitive Ca^{2+} channels by ethanol in undifferentiated and nerve growth factor-treated PC12 cells: interaction with the inactivated state. *J Pharmacol Exp Ther* 272: 489–497, 1995

Nagayama T, Yoshida M, Suzuki-kusaba M, Hisa H, Kimura T, Satoh S. Effect of cilnidipine, a novel dihydropyridine Ca^{2+} channel blocker, on adrenal catecholamine secretion in anesthetized dogs. *J Cardiovasc Pharmacol* 32: 479–484, 1998

Nakashima M, Kou M, Hashitani H, Chen G, Ono H, Kuriyama H, Suzuki H. Action of FRC-8653 on smooth muscle cells of the rabbit mesenteric artery. *Jpn J Pharmacol* 57: 51–61, 1991

Nakazato Y, Oleshanskly M, Tomita U, Yamada Y. Voltage-independent catecholamine release mediated by the activation of muscarinic receptors in guinea-pig adrenal glands. *Br J Pharmacol* 93: 101–109, 1988

O'Farrell M, Ziogas J, Marley PD. Effects of N- and L-type calcium channel antagonists and (?-Bay K8644 on nerve-induced catecholamine secretion from bovine perfused adrenal glands. *Br J Pharmacol* 121: 381–388, 1997

Oike M, Inoue Y, Kitamura K, Kuriyama H. Dual action of FRC8653, a novel dihydropyridine derivative, on the Ca^{2+} current recorded from the rabbit basilar artery. *Circ Res* 67: 993–1006, 1990

Oka M, Isosaki M, Yanagihara N. Isolated bovine adrenal medullary cells: studies on regulation of catecholamine synthesis and release. In: Usdin E, Kopin IJ, Brachas J ed, *Catecholamines: Basic and Clinical Frontiers*. Pergamon Press, Oxford, p 70–72, 1979

Plummer MR, Logothetis DE, Hess P. Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. *Neuron* 2: 1453–1463, 1989

Regan LJ, Sah DW, Bean BP. Ca^{2+} channels in rat central and peripheral neurons: high threshold current resistant to dihydropyridine blockers and ω-conotoxin. *Neuron* 6: 269–280, 1991

Schramm M, Thomas G, Towart R, Franckowiak G. Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. *Nature* 303: 535–537, 1982

Seidler NW, Jona I, Vegh N, Martonosi A. Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasimc reticulum. *J Biol Chem* 264: 17816–17823, 1989

Shimamura T, Kimura T, Satoh S. Effects of pirenzepine, AF-DX 116 and gallamine on the release of catecholamines from the dog adrenal gland in response to splanchnic nerve stimulation: interaction of M1 and M2 receptors with nicotinic receptors. *J Pharmacol Exp Ther* 257: 369–373, 1991

Suzuki M, Muraki K, Imaizumi Y, Watanabe M. Cyclopiazonic acid, an inhibitor of the sarcoplasmic reticulum Ca^{2+}-pump, reduces Ca^{2+}-dependent K^+ currents in guinea-pig smooth muscle cells.

Br J Pharmacol 107: 134−140, 1992

Takahashi M, Tsukui H, Hamada H. Neuronal differentiation of Ca^{2+} channel by nerve growth factor. *Brain Res* 341: 381−384, 1985

Takemura H, Hughes AR, Thastrup O, Putney JW Jr. Activation of calcium entry by the tumor promoter thapsigargin in parotid acinar cells. Evidence that an intracellular calcium pool and not an inositol phosphate regulates calcium fluxes at the plasma membrane. *J Biol Chem* 264: 12266−12272, 1989

Tallarida RJ, Murray RB. Manual of pharmacologic calculation with computer programs. 2nd ed. Speringer-Verlag, New York, p 132, 1987

Uceda G, Artalejo AR, de la Fuente MT, Lopez MG, Albillos A, Michelena P, Garcia AG, Montiel C. Modulation by L-type Ca^{2+} channels and apamin-sensitive K^+ channels of muscarinic responses in cat chromaffin cells. *Am J Physiol* 266(5 Pt 1): C1432−C1439, 1994

Uceda G, Artalejo AR, Lopez MG, Abad F, Neher E, Garcia AG. Ca^{2+}-activated K^+ channels modulate muscarinic secretion in cat chromaffin cells. *J Physiol* 454: 213−230, 1992

Uneyama H, Takahara A, Dohmoto H, Yoshimoto R, Inoue K, Akaike N. Blockade of N-type Ca^{2+} current by cilnidipine (FRC-8653) in acutely dissociated rat sympathetic neurones. *Br J Pharmacol* 122: 37−42, 1997

Uneyama H, Uchida H, Yoshimoto R, Ueno S, Inoue K, Akaike N. Effects of a novel antihypertensive drug, cilnidipine, on cate-cholamine secretion from differentiated PC12 cells. *Hypertension* 31: 1195−1199, 1998

Usowicz M, Porzig M, Becker C, Reuter H. Differential expression by nerve growth factor by two types of Ca^{2+} channels in rat phaeochromocytoma cell line. *J Physiol (Lond)* 426: 95−116, 1990

Uyama Y, Imaizumi Y, Watanabe M. Effects of cyclopiazonic acid, a novel Ca^{2+}-ATPase inhibitor on contractile responses in skinned ileal smooth muscle. *Br J Pharmacol* 106: 208−214, 1992

Wada Y, Satoh K, Taira N. Cardiovascular profile of Bay-K-8644, a presumed calcium channel activator in the dog. *Naunyn-Schmiedebergs Arch Pharmacol* 328: 382−387, 1985

Wakade AR. Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. *J Physiol* 313: 463−480, 1981

Yamada Y, Teraoka H, Nakazato Y, Ohga A. Intracellular Ca^{2+} antagonist TMB-8 blocks catecholamine secretion evoked by caffeine and acetylcholine from perfused cat adrenal glands in the absence of extracellular Ca^{2+}. *Neurosci Lett* 90: 338−342, 1988