응용약물학회지, The Journal of Applied Pharmacology, 8, 13-21(2000)

Influence of Quinidine on Catecholamine Secretion Evoked by Cholinergic Stimulation and Membrane Depolarization from the Perfused Rat Adrenal Gland

Dong-Yoon LIM*, Yong-Joon JEON, Won-Ho YANG, Geon-Han LIM, Il-Hwan KIM, Seung-Myeong LEE[1] and Soon-Pyo HONG[2]

Department of Pharmacology, [1]Neurosurgery and
[2]Internal Medicine, College of Medicine, Chosun University, Kwangju 501-759, KOREA

(Received February 23, 1999; accepted March 20, 2000)

Abstract–The present study was designed to investigate the effect of quinidine on catecholamine (CA) secretion evoked by ACh, high K^+, DMPP, McN-A-343, cyclopiazonic acid and Bay-K-8644 from the isolated perfused rat adrenal gland and to establish the mechanism of its action. The perfusion of quinidine (15-150 μM) into an adrenal vein for 60 min produced relatively dose- and time-dependent inhibition in CA secretion evoked by ACh (5.32×10^{-3} M), high K^+ (5.6×10^{-2} M), DMPP (10^{-4} M for 2 min), McN-A-343 (10^{-4} M for 2 min), cyclopiazonic acid (10^{-5} M for 4 min) and Bay-K-8644 (10^{-5} M for 4 min). Furthermore, in adrenal glands pre-loaded with quinine (5×10^{-5} M), CA secretory responses evoked by veratridine (10^{-4} M) was time-dependently inhibited. Also, in the presence of lidocaine (10^{-4} M), which is also known to be a sodium channel blocker, CA secretory responses evoked by ACh, high potassium, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid were also greatly reduced in similar fashion to that of quinidine-treatment. Taken together, these results suggest that quinidine causes greatly the inhibition of CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as by membrane depolarization, indicating strongly that this effect may be mediated by inhibiting influx of extracellular calcium and release in intracellular calcium in the rat adrenomedullary chromaffin cells. Furthermore, these findings indicate strongly that this inhibitory action of quinidine appears to be associated to the blocking action of sodium channels at least in CA secretion from the rat adrenal gland.

Key words Quinidine, Adrenal Gland, Catecholamine Secretion, Cholinergic Stimulation, Membrane Depolarization

It has been found that quinidine and its stereoisomer quinine are prototypes of a category of compounds that are well known because of their antiarrhythmic action on excitable cardiac cell. Class 1 antiarrhythmic agents, quinidine, disopyramide and procainamide modify myocardial refractory period, conduction velocity and excitability by blocking sodium channels (Roden, 1996); in addition they all possess atropine-like activity to varying degrees (Corr *et al.*, 1978; Mirro *et al.*, 1980; Roden, 1996). They include those drugs producing blockade of cardiac Na^+ channels, which results in a decrease in the maximum rate of depolarization of cardiac cells (Tamargo *et al.*, 1992). Most class 1 drugs inhibit Na^+ channels in a voltage- and rate-dependent manner. In addition, most of the currently available class 1 drugs can also inhibit Ca^{2+} and K^+ channels (Delgado *et al.*, 1993; Delpon *et al.*, 1991; 1995; Salata & Wasserstrom, 1988; Scamps *et al*; 1989; Slawsky & Castle, 1994; Tamargo *et al.*, 1992; 1995; Woosely, 1991).

In vascular smooth muscle cells, the blockade of Ca^{2+} entry through voltage-gated channels would be expected to decrease peripheral vascular resistance and arterial blood pressure. In fact, intravenous administration of most class 1 antiarrhythmic drugs can decrease mean arterial blood pressure and even result in severe hypotension (Mariano *et al.*, 1992). It was shown that quinidine inhibited high KCl-induced contraction in a time- and voltage-dependent manner in isolated rat aorta, an effect that was accompanied by a decrease in Ca^{2+} entry, whereas the inhibition produced by propafenone was not time or voltage dependent (Fernandez del Pozo *et al.*, 1996; Perez-Vizcaino *et al.*, 1994).

*To whom correspondence should be addressed.

Recently, Fernandez del Pozo and his coworkers (1997) found that most class 1 antiarrhythmic drugs induced a concentration-dependent relaxation in isolated endothelium-denuded rat aorta pre-contracted with either KCl or norepinephrine by inhibiting Ca^{2+} entry through voltage- and receptor-gated channels as well as Ca^{2+} release from intracellular stores. As a consequence, these drugs decrease the availability of intracellular free Ca^{2+} required for vascular smooth muscle contraction. However, it was reported that quinidine increased the release of norepinephrine from atria. It is also concluded that quinidine- and atropine-like agents exert atrium-specific positive inotropic effects by blocking muscarinic receptors (Deng *et al.*, 1997). More recently, Kim (1998) has shown that quinine, a stereoisomer of quinidine, inhibits the secretion of catecholamines (CA) evoked by either cholinergic stimulation or membrane depolarization from the isolated rat adrenal gland.

Therefore, in this study, it was attempted to examine whether quinidine affects the CA release evoked by cholinergic stimulation or membrane depolarization from the isolated perfused rat adrenal gland and to clarify the mechanism of its action.

MATERIALS AND METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180 to 300 grams, 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 mid-line incision, and the left adrenal gland and surrounding area were exposed by placing three hook retractors. 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 ± 1°C.

Perfusion of adrenal gland

The adrenal glands were perfused by means of an ISCO pump (WIZ Co.) at a rate of 0.33 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.

Drug administration

The perfusions of DMPP (100 μM) for 1 min and McN-A-343 (100 μM) for 4 minutes, and the injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml were made into perfusion stream via a three way stopcock. Bay-K-8644 (10^{-5} M), cyclopiazonic acid (10^{-5} M) and veratridine (10^{-4} M) were also perfused for 4 min, respectively.

In the preliminary experiments it was found that the secretory responses to ACh, KCl, McN-A-343, Bay-K-8644, cyclopiazonic acid and veratridine returned to pre-njection 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 those secreted from the stimulated sample to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effect of quinidine on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing quinidine, then the perfusate was collected for a certain (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 and 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 secretagogues 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 and Murray (1987).

Drugs and their sources

The following drugs were used: quinidine hydrochloride, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), veratridine, norepinephrine bitartrate, methyl-1, 4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (BAY-K8644) (Sigma Chemical Co., U.S.A.), cyclopiazonic acd, (3-(m-cholro-phenyl -carbamoyl-oxy)-2-butynyl trimethyl ammonium chloride [McN-A-343] (RBI, U.S.A.), and lidocaine hydrochloride (The Vitarine Co., U.S.A.). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644 and veratridine, which were dissolved in 99.5% ethanol and diluted appropriately (final concentration of alcohol was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

Effect of quinidine on CA secretion evoked by ACh, high K^+, DMPP and McN-A-343 from the 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 22.5 ±2.2 ng/2 min (n=8). It has shown that most class 1 antiarrhythmic drugs including quinidine induced a concentration-dependent relaxation in isolated endothelium-denuded rat aorta pre-contracted with either KCl or norepinephrine by inhibiting Ca^{2+} entry through voltage- and receptor-gated channels as well as Ca^{2+} release from intracellular stores (Fernandez del Pozo *et al.*, 1997). Therefore, it was attempted initially to examine the effects of quinidine itself on CA secretion from perfused rat adrenal glands. However, in the present study, quinidine itself did not produce any effect on basal CA output from perfused rat adrenal glands (data not shown). Therefore, it was decided to investigate the effects of quinidine on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15 min-intervals. quinidine was present 20 min before initiation of stimulation.

When ACh (5.32×10^{-3} M) in a volume of 0.05 ml was

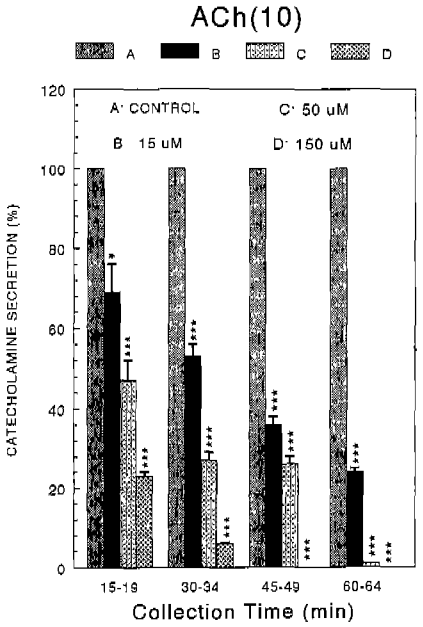

Fig. 1. Dose-dependent effect of quinidine on secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml was induced at 15 min intervals after preloading with 15 mM (B), 50 μM (C), 150 μM (D) of quinidine for 60 min, respectively. Numbers in the parenthesis indicate number of experimental 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 (A) with each concentration-pretreated group of quinidine (B, C, and D). ACh-induced perfusate was collected for 4 minutes. *P<0.05, ***P<0.01

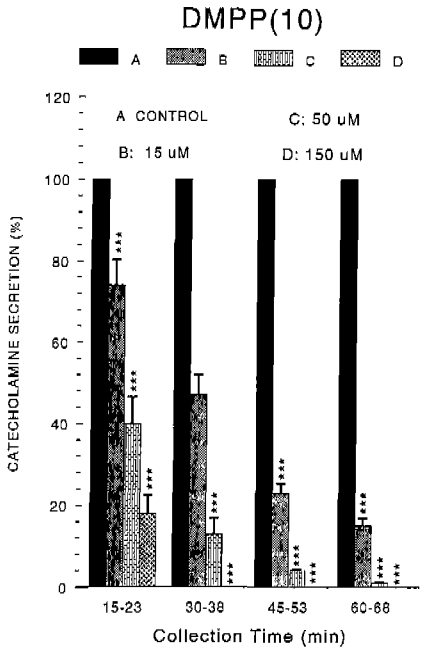

Fig. 2. Dose-dependent effect of quinidine on secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. CA secretion by a single injection of DPPP (10^{-4} M) was infused for 1 min at 15 min intervals after preloading with 15 μM (B), 50 μM (C), 150 μM (D) of quinidine for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control (A) with each concentration-pretreated group of quinidine (B, C, and D). DMPP-induced perfusate was collected for 8 minutes. ***P<0.01

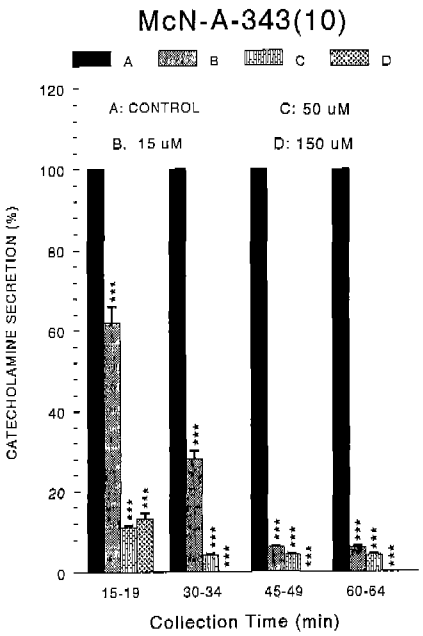

Fig. 3. Dose-dependent effect of quinidine on secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. CA secretion by a single injection of McN-A-343 (10^{-4} M) was infused for 4 min at 15 min intervals after preloading with 15 μM (B), 50 μM (C), 150 μM (D) of quinidine for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control (A) with each concentration-pretreated group of quinidine (B, C, and D). McN-A-343-induced perfusate was collected for 4 minutes. ***P<0.01

injected into the perfusion stream, the amounts of CA secreted was 375 ± 36 ng for 4 min. However, the treatment with quinidine in the range of 1.5×10^{-5}~1.5×10^{-4} M for 60 min inhibited significantly CA secretion evoked by administration of ACh at 15 min intervals in a concentration-dependent manner from 10 adrenal glands, as shown in Fig. 1. When perfused through the rat adrenal gland, DMPP (10^{-4} M for 1 min), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion. However, as shown in Fig. 2, DMPP-stimulated CA secretion after treatment with quinidine was greatly reduced in 10 rat adrenal glands. McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-agonist (Hammer and Giachetti, 1982), perfused into an adrenal gland for 2 min caused an increased CA secretion from 10 glands. However, McN-A-343-stimulated CA secretion in the presence of quinidine was markedly inhibited as compared to the corresponding control secretion (100 %) as depicted in Fig. 3.

Also, it has been found that depolarizing agent like KCl stimulates sharply CA secretion. In the present work, excess K^+ (5.6×10^{-2} M)-stimulated CA secretion after the pretreatment with quinidine was significantly inhibited as compared with its corresponding control secretion (100%) from 7 glands (Fig. 4).

Effect of quinidine on CA secretion evoked by Bay-K-8644, cyclopiazonic acid and veratridine from the perfused rat adrenal glands

Since Bay-K-8644 is known to be a calcium channel activator which enhances basal Ca^{2+} uptake (Garcia *et al.*, 1984) and CA release (Lim *et al.*, 1992), it was of interest to determine the effects of quinidine on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10^{-5} M)-stimulated CA secretion under the presence of quinidine (5×10^{-4} M) was strikingly depressed to 19% as compared to the corresponding control release from 6 glands as shown in Fig. 5.

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

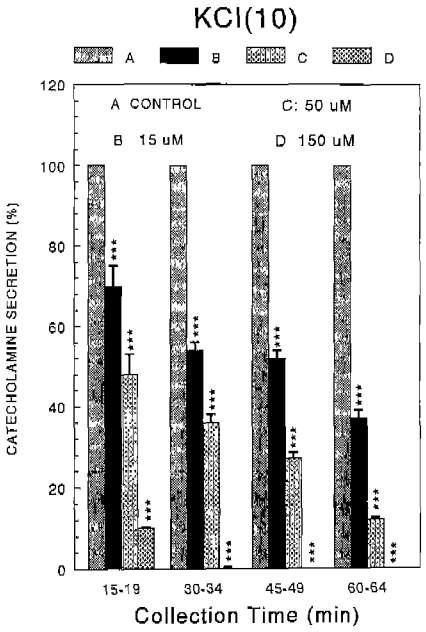

Fig. 4. Dose-dependent effect of quinidine on secretory responses of catecholamines (CA) evoked by high K^+ from the isolated perfused rat adrenal glands. CA secretion by a single injection of K^+ (56 mM) was injected in a volume of 0.005 ml at 15 min intervals after preloading with 15 μM (B), 50 μM (C), 150 μM (D) of quinidine for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control (A) with each concentration-pretreated group of quinidine (B, C, and D). K^+-induced perfusate was collected for 4 minutes. ***P<0.01

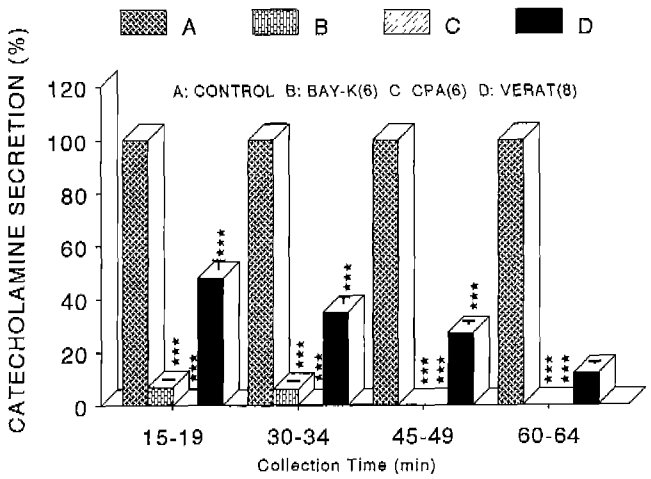

Fig. 5. Effects of quinidine on CA release evoked by Bay-K-8644, cyclopiazonic acid and veratridine from the rat adrenal glands. Bay-K-8644 (10^{-5} M), cyclopiazonic acid (10^{-5} M) and veratridine (10^{-4} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of quinidine (5×10^{-4} M) for 60 min, respectively. Other legends are the same as in Fig. 1. BAY-K: Bay-K-8644, CPA: cyclopiazonic acid, VERAT: veratridine. ***P<0.01

(Goeger and Riley, 1989; Seidler *et al.*, 1989). The inhibitory action of quinidine on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 5. Under the effect of quinidine (5×10^{-4} M) in 6 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was greatly reduced as compared to the control response.

It has been found that veratridine promotes CA release from the adrenal gland by activation of the tetrodotoxin-sensitive Na^+ channel; this increases the intracellular positive charge which leads to depolarizaton and subsequently opens the voltage-sensitive slow calcium channels (Kirpekar & Prat, 1979). Therefore, it was tried to determine the CA secretory effect evoked by veratridine in the presence of quinidine from the isolated rat adrenal glands. As depicted in Fig. 5, veratridine (100 μM)-induced CA secretion under the existence of quinidine (5×10^{-4} M) effect was greatly inhibited to 48 ±4% (15-19 min, P<0.01), 35 3 ng (30~34 min, P<0.01), 27 ± 1.5 % (45-49 min, P<0.01) and 12 ± 1.0% (60-64 min, P<0.01), respectively from the 8 rat adrenal glands as compared to the corresponding control response (100%).

Effect of lidocaine on CA secretion evoked by ACh, excess K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

In the previous experimental results as shown in Fig. 1-4, it was found that quinidine showed a dose-dependent inhibition in CA secretory responses. It has been shown that local anesthetics block conduction by decreasing or preventing the large transient increase in the permeability of excitable membranes to Na^+ that normally is produced by a slight depolarization of the membrane (Strichartz and Ritchie, 1987). Therefore, it is likely of very interest to examine the effect of lidocaine on CA secretion evoked by various secretagogues. CA release evoked by ACh (5.32×10^{-3} M) after preloading with lidocaine (5×10^{-5} M) for 20 min amounted to 34 ±7% (P<0.01, n=6) as compared to each corresponding control secretion (100%) as shown in Fig. 6. DMPP (10^{-4} M)- and McN-A-343 (10^{-4} M)-stimulated CA releases after preloading with lidocaine were also significantly reduced to 39 ± 7% (P< 0.01, n=5) and 45 ± 4% (P<0.01, n=5), respectively as compared to each corresponding control secretion (100%) (Fig. 6). Excess K^+ (5.6 ± 10^{-2} M)-evoked CA release after preloading with lidocaine (5×10^{-5} M) was also inhibited to 52 ± 8 % (P<0.05, n=5) as compared to each corresponding control secretion (100%) as shown in Fig. 6.

In the presence of lidocaine (5×10^{-5} M), the CA secretory

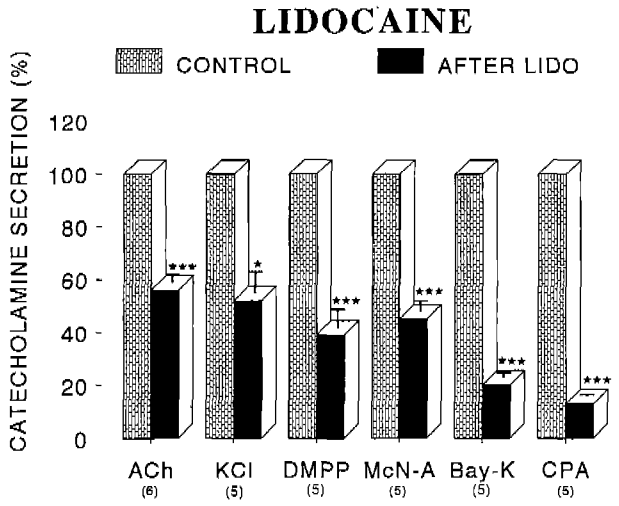

Fig. 6. Effect of lidocaine on CA releasing responses evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the rat adrenal glands. CA secretory responses evoked by the injection of ACh (5.32×10^{-3} M) and perfusion of DMPP (10^{-4} M for 1 min), McN-A-343 (10^{-4} M for 4 min), Bay-K-8644 (10^{-5} M for 4min) and cyclopiazonic acid (10^{-5} M for 4 min) were induced before and after preloading with lidocaine (5×10^{-5} M) for 20 min, respectively. Other legends are the same as in Fig. 1. McN-A: McN-A-343, Bay-K: Bay-K-8644, CPA: cyclopiazonic acid. *P<0.05, ***P<0.01

response by cyclopiazonic acid (10^{-5} M) given into the adrenal gland was reduced to $13 \pm 0.7\%$ (P<0.01) as compared to the corresponding control response (100%) from 5 experiments as shown in Fig. 6. Bay-K-8644-evoked CA secretion under the presence of lidocaine was strikingly depressed as compared to the corresponding control release; thus, the release was reduced to $20 \pm 1.9\%$ (P<0.01, n=5) of the control secretion (Fig. 6).

DISCUSSION

The present experimental results demonstrate that quinidine inhibits CA secretory responses evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as by membrane depolarization in dose- and time-dependent fashions, indicating that this effect may be exerted by inhibiting influx of extracellular calcium and release in intracellular calcium from the rat adrenomedullary chromaffin cells. Furthermore, these findings indicate strongly that this inhibitory action of quinidine is relevant to blocking action of sodium channels at least in CA secretion from the rat adrenal gland.

Generally, chromaffin cells of the adrenal medulla fire action potentials at rest, and the spike frequency is enhanced by ACh (Biales *et al.*, 1976; Brandt *et al.*, 1976). The action potentials result from voltage-dependent Na^+, Ca^{2+} and K^+ currents similar to those of neurones and muscle fibres (Biales *et al.*, 1976; Brandt *et al.*, 1976; Kidokoro & Ritchie, 1980). In the present study, the finding that quinidine has inhibited CA secretory responses evoked by veratridine seems that the inhibitory effect of quinidine may be exerted by the blockade of the Na^+ channels in the chromaffin cells. In support of this finding, veratridine (0.1 mM) was found to be effective in producing an increase in CA secretion from perfused guinea pig adrenal glands by an increase in the tetrodotoxin (TTX)-sensitive Na^+ permeability of chromaffin cell membrane (Ito *et al.*, 1978). Moreover, veratridine failed to increase the CA secretion in the absence of extracellular Ca^{2+} or Na^+ from the guinea-pig adrenal glands and this veratridine-evoked CA secretion was inhibited by the pretreatment with tetrodotoxin and excess Mg^{2+} while potentiated by ouabain (Ito *et al.*, 1979). These results demonstrated surely that Na^+-dependent Ca^{2+} influx as well as voltage-dependent Ca^{2+} influx mechanisms may be involved in the CA secretion evoked by veratridine. Based on these findings, in the present study, the fact that quinidine inhibited veratridine-induced CA release indicates that the quinidine's inhibitory mechanism is relevant to the blockade of Na^+ channels in the perfused rat adrenal glands.

It is well-known that Ca^{2+} is indispensable for stimulus-secretion coupling in adrenal chromaffin cells (Douglas, 1968; 1975). Furthermore, Ca^{2+} movement across chromaffin cell membranes was found to be affected by the Na^+ gradient (Aguirre *et al.*, 1977; Rink, 1977). The removal of extracellular Na^+ and the addition of TTX were also found to be effective in inhibiting veratridine-induced CA release. Ohta and his coworkers (1973) reported that, in the squid giant axons, veratridine causes a depolarization resulting from a selective increase in the resting Na^+ permeability and that the effect was inhibited by the addition of TTX and the removal of extracellular Na^+ ions. Moreover, it was also found that an increase in the Na^+ permeability of chromaffin cell membranes plays a critical role in the release of CA induced by veratridine from perfuse guinea-pig adrenal glands (Ito *et al.*, 1979).

Furthermore, in terms of the fact that lidocaine depressed the CA release evoked by ACh, DMPP, McN-A-343, and high K^+, it appears that quinidine may inhibit CA release through the blockade of sodium channels. In fact, local anesthetics block conduction by decreasing or preventing the large

transient increase in the permeability of excitable membranes to Na^+ that normally is produced by a slight depolarization of the membrane (Strichartz and Ritchie, 1987). This action of local anesthetics is due to their direct interaction with voltage-gated Na^+ channels. In terms of this fact, quinidine also seems to possess the blocking activity of Na^+ channels in the rat adrenal gland. Class 1 antiarrhythmic drugs block transmembrane Na^+ transport thereby reducing the amount of Na^+ ions available for Na^+-Ca^{2+} exchange and decreasing the concentration of intracellular Ca^{2+}. Therefore, it was proposed that the vascular relaxation of class 1 antiarrhythmic drugs could be explained by the inhibition of Ca^{2+} entry through the depolarized cell membrane in rat aorta (Carron *et al.*, 1991: Dohi *et al.*, 1994; Fernandez del Pozo *et al.*, 1996; Perez Vizcaino *et al.*, 1991; 1994). As previously described (Perez-Vizcaino *et al.*, 1994), the inhibitory effects of quinidine on CA secretion evoked by high K^+ increased as the time of exposure to quinidine was prolonged.

In the present study, the findings that quinidine inhibits CA secretory responses evoked by DMPP (a nicotinic receptor agonist), McN-A-343 (a muscarinic M_1-receptor agonist) and high potassium (membrane depolarization) in a concentration-dependent fashion from the isolated perfused rat adrenal gland suggest that this inhibitory effect of quinidine may be associated to the blockade of sodium channels. The result that quinidine inhibits CA secretion evoked by stimulation of muscarinic receptors with McN-A-343 suggests strongly that Na^+ channels are involved in the regulation of the overall secretory responses evoked by muscarinic stimulation. In support of this hypothesis, 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; Lim & Hwang, 1991). These observations are in line with a previous report (Ladona *et al*, 1987; Uceda *et al*, 1992) showing that Bay-K-8644 almost trippled the peak secretory response to muscarine in perfused cat adrenal glands.

In this experiment, quinidine also depresses 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). These findings that quinidine inhibited CA secretion evoked by high K^+ as well as by Bay-K-8644 suggest that quinidine inhibits directly the voltage-dependent Ca^{2+} channels through the blockade of Na^+ channels, just like Ca^{2+} channel blockers (Cena *et al.*, 1983), which have direct actions on voltage-dependent Ca^{2+} channels. 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 voltage-dependent Ca^{2+} channels (Burgoyne, 1984; Oka *et al.*, 1979). Therefore, it seems that the quinidine inhibits CA secretion evoked by DMPP by inhibiting Ca^{2+} influx through voltage-dependent Ca^{2+} channels activated by stimulation of nicotinic ACh receptors.

In the present work, it has been also shown that quinidine inhibits the increase in CA secretion evoked by cyclopiazonic acid, which is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Geoger & Riley, 1989; Seidler *et al.*, 1989). Therefore, based on these findings, it is felt that the inhibitory effect of quinidine on CA secretion evoked by cholinergic stimulation as well as by membrane-depolarization may be associated at least partly with the inhibition of mobilization of intracellular Ca^{2+} in the chromaffin cells. This indicates that quinidine also 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 Ca^{2+} load (Suzuki *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 and thereby increase of Ca^{2+}-dependent K^+-current. Moreover, in bovine adrenal chromaffin cells, stimulation of muscarinic ACh receptors is also proposed to cause activation of phosphoinositide (PI) 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 quinidine on Ca^{2+} movement from intracellular pools is due to its direct effect on the PI response or, on the contrary, an indirect effect as a result induced by the blockade of Na^+ channels.

In conclusion, the results of the present study have demonstrated that quinidine inhibits CA secretory responses evoked by cholinergic (both nicotinic and muscarinic) stimulation as well as by membrane depolarization, resulting in inhibition of Ca^{2+} influx through the L-type voltage-dependent calcium

channels and also in inhibition of Ca^{2+} mobilization from intracellular pools in the isolated perfused rat adrenal glands. Furthermore, these findings indicate strongly that this inhibitory action of quinidine is associated to blocking action of sodium channels at least in CA secretion from the rat adrenal gland.

REFERENCES

Aguirre, J., Pinto, J.E.B. and Trifaro, J.M. (1977). Calcium movements during the release of catecholamines from the adrenal medulla: effects of methoxyverapamil and external cations. *J. Physiol.*, **269**, 371-394.

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

Anton, A.H. and Sayre, D.F. (1976). A study of the factors affecting the aluminum oxidetrihydroxy indole procedure for the analysis of catecholamines. *J. Pharmacol. Exp. Ther.*, **138**, 360-375.

Biales, B., Dichter, M. and Tischler, A. (1976). Electrical excitability of cultured adrenal chromaffin cells. *J. Physiol.*, **262**, 743-753.

Brandt, B.L., Jagiwara, S., Kidokoro, Y. and Miyazaki, S. (1976). Action potentials in the rat chromaffin cell and effects of acetylcholine. *J. Physiol.*, **263**, 417-439.

Burgoyne, R.D. (1984). Mechanism of secretion from adrenal chromaffin cells. *Biochem. Biophys. Acta.*, **779**, 201-216.

Cena, V., Nicolas, G.P., Sanchez-Garcia, P., Kirpekar, S.M. and Garcia, A.G. (1983). Pharmacological dissection of receptor-associated and voltage-sensitive ionic channels involved in catecholamine release. *Neuroscience.*, **10**, 1455-1462.

Challiss, R.A.J., Jones, J.A., Owen, P.J. and Boarder, M.R. (1991). 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.

Cheek, T.R., O'Sullivan, A.J., Moreton, R.B., Berridge, M.J. and Burgoyne, R.D. (1989). Spatial localizaion of the stimulus-induced rise in cytosolic Ca^{2+} in bovine adrenal chromaffin cells: Distinct nicotinic and muscarinic patterns. *F.E.B.S. Lett.*, **247**, 429-434.

Corr, P.B., Helke, C.J. and Gillis, R.A. (1978). Exacerbation of coronary occlusion-induced ventricular arrhythmias by the vagolytic effect of procainamide. *Cardiovasc. Res.*, **12**, 486-492.

Delgado, C., Tamargo, J., Henzel, D. and Lorente, P. (1993). Effects of propafenone on caclium currents in guinea-pig ventricular myocytes. *Br. J. Pharmac.*, **108**, 721-727.

Delpon, E., Valenzuela, C., Perez, O., Casis, O. and Tamargo, J. (1995). Propafenone preferentially blocks the rapidly activating component of delayed rectifier K^+ current in guinea-pig ventricular myocytes: voltage-independent and time-dependent block of the slowly activating component. *Cir. Res.*, **76**, 223-235.

Deng, X.F., Mulay, S., Chemtob, S. and Varma, D.R. (1997). Mechanisms of the atrium-specific positive inotropic activities of quinidine and atropine-like agents in rats. *J. Pharmacol. Expt. Ther.*, **281**, 322-329.

Dohi, Y., Masayoshi, K. and Sato, K. (1994). Vasorelaxant effect of mexiletine in mesenteric resistance arteries of rats. *Br. J. Pharmac.*, **111**, 673-680.

Douglas, W.W. (1968). Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. The First Gaddum Memorial Lecture, Cambridge 1967. *Br. J. Pharmac.*, **34**, 451-474.

Douglas, W.W. (1975). Secretomotor control of adrenal medullary secretion: synaptic, membrane and ionic events in stimulus-secretion coupling, In *Handbook of Physiology*, section 7, Endocrinology, Vol. VI, *The Adrenal Gland* (Blaschko, H., Sayers, G. and Smith, A.D.). pp. 367-388, Am. Physiol. Soc., Washington D.C.

Fernandez del Pozo, B., Perez-Vizcaino, F., Fernandez, C., Zaragoza, F. and Tamargo, J. (1997). Effects of several Class 1 antiarrhythmic drugs on isolated rat aortic vascular smooth muscle. *Gen. Pharmac.*, **29**(4), 539-543.

Garcia, A.G., Sala, F., Reig, J.A., Viniegra, S., Frias, J., Fonteriz, R. and Gandia, L. (1984). Dihydropyridine Bay-K-8644 activates chromaffin cell calcium channels. *Nature*, **309**, 89-71.

Goeger, D.E. and Riley, R.T. (1989). 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.

Hammer, R. and Giachetti, A. (1982). Muscarinic receptor subtypes: M_1 and M_2 biochemical and functional characterization. *Life. Sci.*, **31**, 2992-2998.

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

Ito, S., Nakazato, Y. and Ohga, A. (1978). Pharmacological evidence for the involvement of Na channels in the release of catecholamines from perfused adrenal glands. *Br. J. Pharmac.*, **62**, 359-361.

Ito, S., Nakazato, Y. and Ohga, A. (1979). The effect of veratridine on the release of catecholamines from the perfused adrenal glad. *Br. J. Pharmac.*, **65**, 319-330.

Kidokoro, Y. and Ritchie, A.K. (1980). Chromaffin cell action potentials and their possible role in adrenaline secretion from rat adrenal medulla. *J. Physiol.*, **307**, 199-216.

Kim, J.I. (1998). Influence of quinine on the release of catecholamines evoked by Ach, DMPP, McN-A-343 and high K^+ From the rat adrenal gland. *Ph. D. Thesis. Chosun University.*

Kirpekar, S.M. and Prat, J.C. (1979). Release of catecholamines from perfused rat adrenal gland by veratridine. *Proc. Natl. Acad. Sci. USA.*, **76**, 2081-2083.

Ladona, M.G., Aunis, D., Gandia, A.G. and Garcia, A.G. (1987). Dihydropyridine modulation of the chromaffin cell

secretory response. *J. Neurochem.*, **48**, 483-490.

Lim, D.Y. and Hwang, D.H. (1991). Studies on secretion of catecholamines evoked by DMPP and McN-A-343 in the rat adrenal gland. *Korean J. Pharmacol.*, **27**(1), 53-67.

Lim, D.Y., Kim, C.D. and Ahn, K.W. (1992). Influence of TMB-8 on secretion of catecholamines from the perfused rat adrenal glands. *Arch. Pharm. Res.*, **15**(2), 115-125.

Mariano, D.J., Schomer, S.J. and Rea, R.F. (1992). Effects of quinidine on vascular resistance and sympathetic nerve activity in humans. *J. Am. Coll. Cardiol.*, **20**, 1411-1416.

Mirro, M.J., Manalan, A.S., Bailey, J.C. and Watanabe, A.M. (1980). Anticholinergic effects of disopyramide and quinidine on guinea pig myocardium. *Circ. Res.*, **47**, 855-865.

Ohta, M., Narahashi, T. and Keeler, R.F. (1973). Effects of veratrum alkaloids on membrane potential and conductance of squid and crayfish giant axons. *J. Pharmac. Exp. Ther.*, **184**, 143-154.

Oka, M., Isosaki, M. and Yanagihara, N. (1979). Isolated bovine adrenal medullary cells: studies on regulation of catecholamine synthesis and release. In: *Catecholamines, Basic and Clinical frontiers* (Usdin, E., Kopin, I.J. and Brachas, J.), pp. 70-72, Pergamon Press, Oxford.

Perez-Vizcaino, F., Fernandez del Pozo, B., Zaragoza, F. and Tamargo, J. (1994). Voltage-and time-dependent inhibitory effects on rat aortic and porcine coronary artery contraction induced by propafenone and quinidine. *Br. J. Pharmac.*, **113**, 1281-1288.

Rink, T.J. (1977). The influence of sodium on calcium movements and catecholamine release in thin slices of bovine adrenal medulla. *J. Physiol.*, **266**, 297-325.

Roden, D.M. (1996). Antiarrhythmic drugs. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th ed. (Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. and Gilman, A.G., pp. 839-874, McGraw-Hill. New York.

Salata, J.J. and Wasserstrom, J.A. (1988). Effects of quinine on action potentials and ionic currents in isolated canine ventricular myocytes. *Circ. Res.*, **62**, 324-337.

Scamps, F., Undrovinas, A. and Vassort, G. (1989). Inhibition of Ica in single frog cardiac cells by quinidine, flecainide, ethmozin and ethacizin. *Am. J. Physiol.*, **256**, C549-C559.

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

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

Slawsky, M.T. and Castle, N.A. (1994). K^+ channel blocking actions of flecainide compared to those of propafenone and quinidine in adult rat ventricular myocytes. *J. Pharmac. Exp. Ther.*, **269**, 66-72.

Strichartz, G.R. and Ritchie, J.M. (1987). The action of local anesthetics on ion channels of excitable tissues. In, *Local Anesthetics.* (Strichartz, G.R., ed.) *Handbook of Experimental Pharmacology*, Vol, 18, pp. 21-53, Springer-Verlag, Berlin.

Suzuki, M., Muraki, K., Imaizumi, Y. and Watanabe, M. (1992). 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.

Tallarida, R.J. and Murray, R.B. (1987). Manual of pharmacologic calculation with computer programs. 2nd. Ed. pp. 132, New York Speringer-Verlag.

Tamargo, J., Valenzuela, C. and Delpon, E. (1992). New insights into the pharmacology of sodium channel blockers. *Eur. Heart. J.*, **13**(Suppl F), 2-13.

Tamargo, J., Valenzuela, C. and Delpon, E. (1995). Molecular pharmacology of electrical alterations of the heart. *In Molecular Cell Biology of Cardiovascular Diseases* (Diez, J., Dzau, V., Ferrari, R. and Frolich, E.H., ed.). pp. 433-454. Mosby/Doyma, Barcelona.

Uceda, G., Artalejo, A.R., Lopez, M.G., Abad, F., Neher, E. and Garcia, A.G. (1992). Ca^{2+}-activated K^+ channels modulated muscarinic secretion in ca chromaffin cells. *J. Physiol.*, **454**, 213-230.

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

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

Wakade, A.R. (1981). Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. *J. Physiol.*, **313**, 463-480.

Woosley, R.L. (1991). Antiarrhythmic drugs. *Annu. Res. Pharmac. Toxicol.*, **31**, 427-455.