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Influence of Quinine on Catecholamine Release Evoked by Cholinergic Stimulation and Membrane Depolarization from the Rat Adrenal Gland

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The present study was attempted to investigate the effect of quinine on secretion of catecholamines (CA) evoked by cholinergic stimulation and membrane depolarization from the isolated perfused rat adrenal gland. The perfusion of quinine (15-150 μM) into an adrenal vein for 60 min produced 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). Also, under the presence of pinacidil (10^{-4} M), which is also known to be a selective potassium channel activator, CA secretory responses evoked by ACh, high potassium, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid were also greatly reduced. When preloaded along with quinine (5×10^{-5} M) and glibenclamide (10^{-6} M), a specific blocker of ATP-regulated potassium channels, CA secretory responses evoked by ACh, high potassium, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid were recovered as compared to those of quinine-treatment only. Taken together, these results demonstrate that quinine inhibits CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as by membrane depolarization through inhibiting influx of extracellular calcium and release in intracellular calcium in the rat adrenomedullary chromaffin cells. These findings suggest that activation of potassium channels may be involved at least in inhibitory action of quinine on CA secretion from the rat adrenal gland.

Key words: Quinine, Adrenal gland, Catecholamine secretion, Potassium channels

INTRODUCTION

Quinine is the chief alkaloid of cinchona derived from the bark of the south american cinchona tree. Quinine and its stereoisomer, quinidine, belong to a category of compounds known for their antiarrhythmic action on cardiac cells. It was originally reported to be the specific blocker of the Ca^{2+}-activated K^+ conductance in red blood cells (Armando-Hardy *et al.*, 1975; Lew and Ferreira, 1978). Tang and his coworkers (1990) have found that, in bovine chromaffin cells, quinine produces the inhibition of the K^+-evoked catecholamine (CA) release, and that this inhibitory effect is at least partly due to a decrease in Ca^{2+} influx. Moreover, there is a report that quinine also reduces the release of noradrenaline from cultured chromaffin cells induced by high potassium (Glavinovic, Dagher and Trifaro, 1985). Furthermore, quinine and quinidine are reported to affect delayed rectifier currents in *Aplysia* (Hermann and Gorman, 1984) and inward rectifier currents in insulin-secreting cells (Findlay *et al.*, 1985) as well as Na^+ conductance in frog auricular fibers (Ducouret, 1976). They also affect Ca^{2+} conductance in frog heart (Ducouret, 1976), and in molluscan neurons (Plant and Standen, 1976). It has been shown that quinine also reduces K^+ conductance as well as membrane potential in pancreatic β-cells (Atwater et al, 1979; Ribalet and Beigelman, 1980; Rosario *et al.*, 1985).

However, patch clamp studies on cultured bovine chromaffin cells have shown that quinine is far from being a specific blocker but is in fact a very inefficient flickery blocker of Ca^{2+}-activated K^+ channels (Findlay *et al.*, 1985; Glavinovic and Trifaro, 1988). There are so far a few reports related to the effect of quinine on CA

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secretion, especially from the perfused model of the adrenal gland. Therefore, the present study was attempted to investigate whether quinine affects the CA secretion evoked by cholinergic stimulation and membrane depolarization from the perfused model of the rat adrenal gland and to establish 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 midline 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 $37 \pm 1°C$.

Perfusion of adrenal gland

The adrenal glands were perfused by means of 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 mg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of catecholamines.

Drug administration

The perfusions of DMPP (10^{-4}M) and McN-A-343 (10^{-4} M) for 2 minutes and/or a single injection of ACh (5.32×10^{-3} M) and KCl (5.6×10^{-2} M) in a volume of 0.05 ml were made into perfusion stream via a three way stopcock, respectively. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were also perfused for 4 min, respectively.

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 preinjection 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, the 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 effect of quinine on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing quinine for 20 min, then the perfusate was collected for a certain period (background sample). Then the medium was changed to the one containing the stimulating agent or along with quinine, and the perfusates were collected for the same period as that for the background sample. 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 (Anton and Sayre, 1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981) using fluorospectrophotometer (Kontron Co., Milano, 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 the Student's *t* and ANOVA 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: quinine hydrochloride, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), glibenclamide, pinacidil, norepine-phrine 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.). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644, which was 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 quinine on CA secretion evoked by ACh, excess K+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

After the perfusion with oxygenated Krebs-bicarbonate solution for 1 h, basal CA release from the isolated perfused rat adrenal glands amounted to 21.3 ± 2.4 ng/2 min (n=6). Since in cultured bovine adrenal chromaffin cells, quinine produces a dose-related inhibition of CA secretion in response to depolarizing concentration of K^+ (Tang *et al.*, 1990), it was attempted initially to examine the effects of quinine itself on CA secretion from the perfused model of the rat adrenal glands. However, in the present study, quinine (15 μM) 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 quinine on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15 min-intervals. Quinine was present 15 min before initiation of stimulation. Quinine itself did not produce any effect on basal CA output (data not shown).

When ACh (5.32×10^{-3} M) in a volume of 0.05 ml was injected into the perfusion stream, the amounts of CA secreted was 558 ± 63 ng for 4 min. However, the pretreatment with quinine in the range of 1.5×10^{-5}~1.5×10^{-4} M for 20 min concentration- and time-depen-dently inhibited ACh-stimulated CA secretion from 6 adrenal glands, as shown in Fig. 1. Also, it has been found that depolarizing agent like KCl stimulates sharply CA secretion (241 ± 28 ng for 0-4 min). Excess K^+ (5.6×10^{-2} M)-stimulated CA secretion after the pretreatment with qui-nine was greatly inhibited as compared with its corres-ponding control secretion (100%) from 6 glands (Fig. 2). When perfused through the rat adrenal gland, DMPP (10^{-4} M

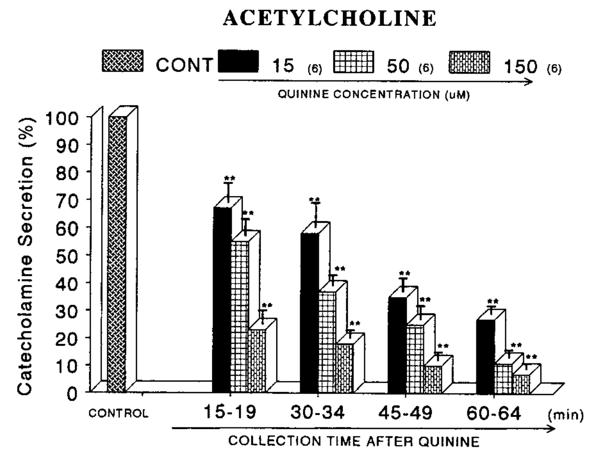

Fig. 1. Dose-dependent effect of quinine on secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 15, 50, 150 μM of quinine for 60 min as indicated at an arrow mark, respectively. 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 (CONT) with each concentration-pretreated group of quinine. ACh-induced perfusate was collected for 4 min. **: $P<0.01$

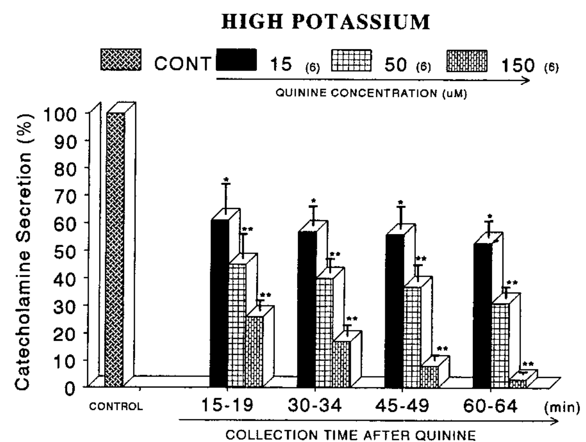

Fig. 2. Dose-dependent effect of quinine on secretory responses of catecholamines (CA) evoked by high K^+ from the isolated perfused rat adrenal glands. CA secretion by a single injection of K^+ (56 mM) was injected in a volume of 0.005 ml at 15 min intervals after preloading with 15, 50, 150 μM of quinine for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control (A) with each concentration-pretreated group of quinine. K^+-induced perfusate was collected for 4 min. *: $P<0.05$, **: $P<0.01$

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. 3, DMPP-stimulated CA secretion (643 ± 74 ng for 0-4 min; 159 ± 19 ng for 4-8 min) after pretreatment with quinine was greatly reduced in 6 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 (159 ± 18 ng for 0-4 min) from 8 glands. However, McN-A-343-stimulated CA secretion in the presence of quinine was markedly depressed as compared to the corresponding control secretion (100%) as depicted in Fig. 4.

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 quinine 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 quinine was perfectly blocked as compared to the corresponding control release (158 ± 19 ng for 0-4 min) 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 (Goeger and Riley, 1989; Seidler *et al.*, 1989). The inhibitory action of quinine on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 5. Under the presence of quinine in 6 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was almost completely blocked as compared to the control response (125 ± 14 ng for 0-4 min).

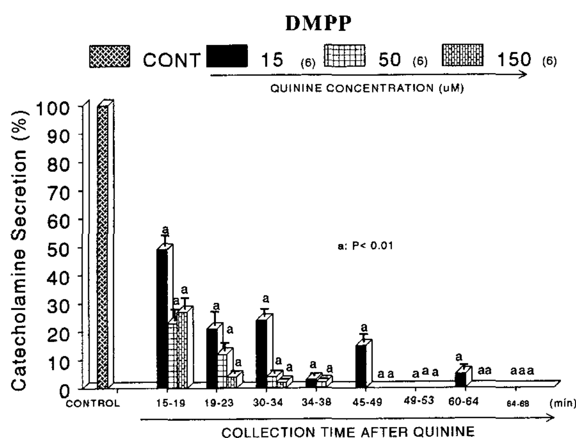

Fig. 3. Dose-dependent effect of quinine on secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. CA secretion by a single injection of DPPP (10^{-4} M) was infused for 2 min at 15 min intervals after preloading with 15, 50, 150 μM of quinine for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control (CONT) with each concentration-pretreated group of quinine. DMPP-induced per-fusate was collected for 8 min at 4 min interval. a: P<0.01

The effects of quinine plus glibenclamide on CA release evoked by ACh, excess K+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

Since it has been found that glibenclamide, a hypoglycemic sulfonylurea which selectively blocks ATP-sensitive K^+ channels (Ashcroft, 1988), inhibits competitvely the

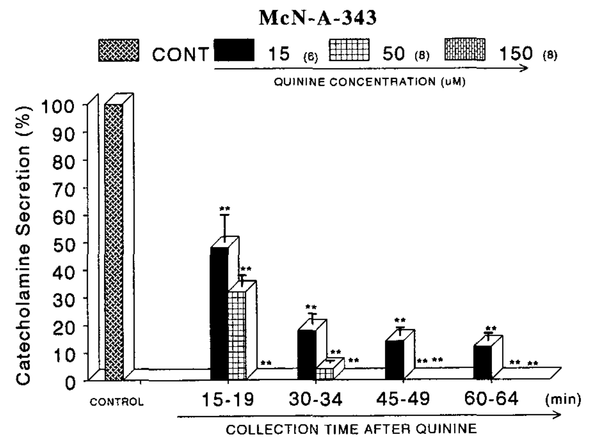

Fig. 4. Dose-dependent effect of quinine on secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. CA secretion by a single injection of McN-A-343 (10^{-4} M) was infused for 4 min at 15 min intervals after preloading with 15, 50, 150 μM of quinine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

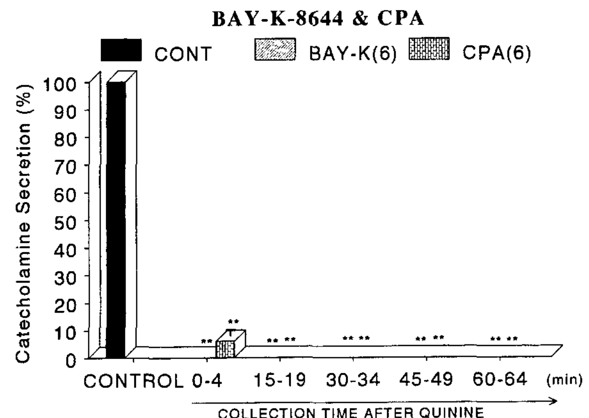

Fig. 5. Effects of quinine on CA release evoked by Bay-K-8644 and cyclopiazonic acid from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of quinine (5×10^{-4} M) for 60 min, respectively.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

vasorelaxant effects of UR-8225, which is an ATP-sensitive channel opener, in rat portal veins and aorta (Perez-Vizcaino *et al.*, 1993), it was tried to determine the effect of quinine in the presence of glibenclamide on CA secretion evoked by various secretagogues from the isolated rat adrenal glands.

ACh (5.32×10^{-3} M)- and excess K^+ (5.6×10^{-2} M)-evoked CA releases in the presence of quinine (10^{-4} M) along with glibenclamide (10^{-6} M) were recovered to $71 \pm 4\%$ ($P<0.01$, $n=6$) and $98 \pm 5\%$ ($P<0.01$, $n=6$) of their control secretion (100%), respectively as compared to their secretory responses of 37 4 % and 45 ± 6 % of their controls in the presence of quinine (10^{-4} M) only (Fig. 6). On the other hand, quinine (10^{-4} M) along with glibenclamide (10^{-6} M) treatment did fail to affect the basal CA secretory response (data not shown). As depicted in Fig. 6 and 7, CA secretions under the presence of quinine (10^{-4} M) along with glibenclamide (10^{-6} M) were also greatly recovered to $54 \pm 3\%$ (0-4 min, $P<0.05$, $n=6$) and $51 \pm 7\%$ (4-8 min, $P<0.05$, $n=6$) in response to DMPP and $56 \pm 6\%$ (0-4 min, $P<0.01$, $n=8$) in response to McN-A-343 of their corresponding control responses, respectively as compared to the secretory responses of 2 ± 0.2 (0-4 min) and $2 \pm 0.2\%$ (4-8 min) for DMPP, and $4 \pm 1\%$ (0-4 min) for McN-A-343 of the control in the presence of quinine (10^{-4} M) only.

CPA (10^{-5} M)- and Bay-K-8644 (10^{-5} M)-induced CA secretory responses after preloading with Krebs solution containing quinine (10^{-4} M) along with glibenclamide (10^{-6} M) were reduced to $103 \pm 13\%$ ($P<0.01$, $n=6$) and $116 \pm 12\%$ ($P<0.01$, $n=6$) of each corresponding control (100 %), respectively as compared to the secretory responses of $0 \pm 0\%$ and $0 \pm 0\%$ of the controls in the presence of quinine only as shown in Fig. 7.

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

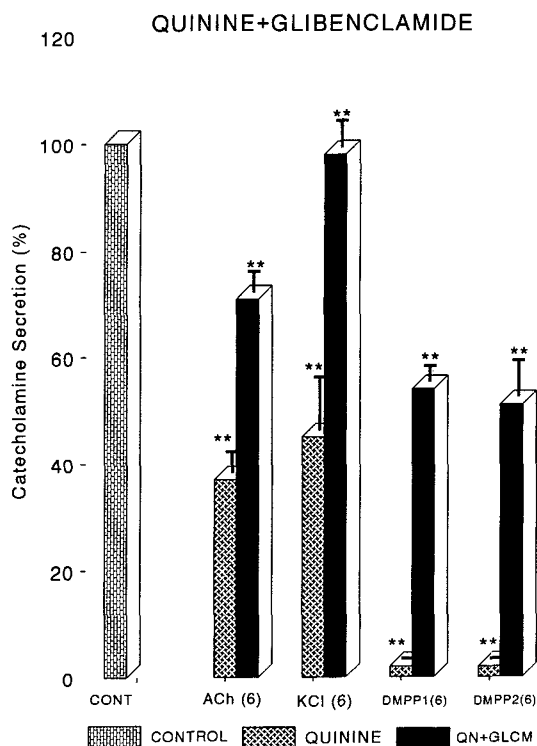

Fig. 6. Effect of quinine plus glibenclamide on CA release evoked by ACh, excess K^+, and DMPP. A single injection of ACh (5.32×10^{-3} M) and excess K^+ (5.6×10^{-2} M), and perfusion of DMPP (10^{-4} M) for 2min were induced before and after preloading with 10^{-4} M quinine only or 5×10^{-5} M QN plus 10^{-6} M GLCM for 20 min, respectively. Statistical difference was obtained by comparing CONT with quinine-treatment only, and by comparing quinine-treatment only with QN+GLCM, respectively. Perfusate for DMPP were collected for 8 min at 4 min interval: DMPP1 (0-4 min), DMPP2 (4-8 min). Other legends are the same as in Fig. 1. QN: quinine, GLCM: glibenclamide. **: P<0.01.

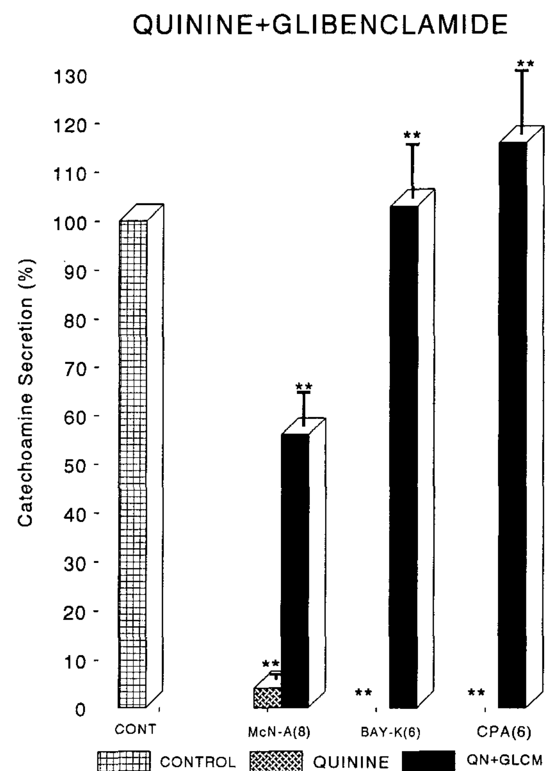

Fig. 7. Effect of quinine plus glibenclamide on CA release evoked by McN-A-343, Bay-K-8644 and cyclopiazonic acid. Perfusion of McN-A-343 (10^{-4} M) and Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) for 2 min were induced before and after preloading with 10^{-4} M quinine only or 5×10^{-5} M QN plus 10^{-6} M GLCM for 20 min, respectively. Statistical difference was obtained by comparing CONT with quinine-treatment only, and by comparing quinine-treatment only with QN+GLCM, respectively. Other legends are the same as in Fig. 1 and 6. QN: quinine, GLCM: glibenclamide, McN-A: McN-A-343, BAY-K: Bay-K-8644, CPA: cyclopiazonic acid **: P<0.01.

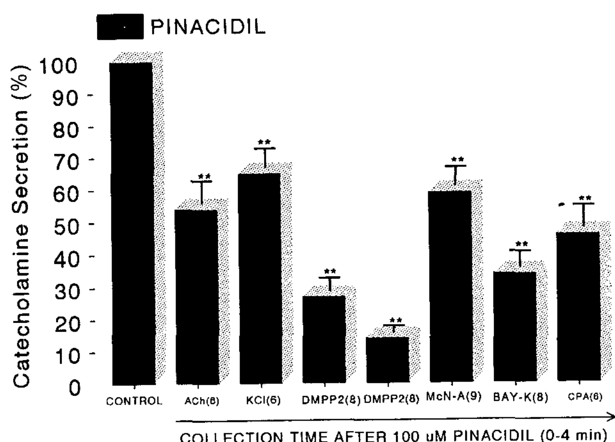

Fig. 8. Effect of pinacidil on CA release evoked by ACh, excess K^+, DMPP-, McN-A-343, Bay-K-8644 and cyclopiazonic acid. CA secretion evoked by ACh (5.32×10^{-3} M), excess K^+ (5.6×10^{-2} M), DMPP (10^{-4} M), McN-A-343 (10^{-4} M), Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were induced before and after preloading with pinacidil (10^{-4} M) for 20 min, respectively. Other legends are the same as in Fig. 1, 4 and 5. **: P<0.01.

As shown In Fig. 1-5, it was found that quinine showed dose- and time-dependent inhibition in CA secretory responses. It has been shown that in cultured bovine adrenal chromaffin cells, the K^+ channel openers, cromakalim and pinacidil, selectively inhibit CA secretory responses induced by membrane depolarization and by stimulation of nicotinic ACh receptors (Masuda *et al.*, 1994). Therefore, it was interesting to examine the effect of pin-acidil on CA secretion evoked by various secreta-gogues.

CA release evoked by ACh (5.32×10^{-3} M) after preloading with pinacidil (10^{-4} M) for 20 min amounted to 54 ± 7% (P<0.05, n=6) as compared to each correspond-ing control secretion (100%) as shown in Fig. 8. DMPP (10^{-4} M)- and McN-A-343 (10^{-4} M)-stimulated CA releases after preloading with pinacidil were also significantly reduced (Fig. 8). Excess K^+ (5.6×10^{-2} M)-evoked CA release after preloading with pinacidil (10^{-4} M) was also inhibited to 65 ± 6% (P<0.01, n=8) as compared to each corresponding control secretion (100%) as shown in Fig. 8.

In the presence of pinacidil (10^{-4} M), the CA secretory response by cyclopiazonic acid (10^{-5} M) given into the adrenal gland was reduced to 46 ± 6% (P<0.01, n=6) as compared to the corresponding control response (100%) as shown in Fig. 8. Bay-K-8644-evoked CA secretion under the presence of pinacidil was strikingly depressed as compared to the corresponding control release; thus, the release was reduced to 34 ± 5% (P<0.01, n=8) of the control secretion (Fig. 8).

DISCUSSION

The present experimental results demonstrate that quinine dose- and time-dependently inhibits CA secretory responses evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors and by membrane depolarization from the perfused rat adrenal gland. It is suggested strongly that this effect is mediated by inhibiting both influx of extracellular calcium and release in intracellular calcium from the rat adrenomedullary chromaffin cells. Furthermore, these findings indicate that this inhibitory action of quinine may be exerted at least by activation of potassium channels in the rat adrenal gland.

In general, the adrenomedullary chromaffin cells are derived embriologically from the neural crest sharing a common origin and similar functional and morphological features with neurons (Trifaro, 1982). One such feature is the storage of secretory products in membrane-bound organelles, the chromaffin granules. Upon stimulation by acetylcholine, the chromaffin cell depolarizes and Ca^{2+} channels open up, resulting in an increase in Ca^{2+} influx (Douglas, 1975; Trifaro, 1977). When the intracellular concentration of Ca^{2+} reaches a critical level, the release mechanism is triggered and the chromaffin granules release their contents to the cell exterior by exocytosis (Douglas, 1975; Trifaro, 1977). Therefore, Ca^{2+} plays a key role in the secretory process in the chromaffin cell and in other such systems that store their secretory products in subcellular granules (Trifaro, 1977). The increase in intracellular Ca^{2+} brought about by cell stimulation also activates Ca^{2+}-dependent K^+ channels leading to hyperpolarization and inhibition of further Ca^{2+} influx. Therefore, the K^+ channel which acts as a link between intracellular Ca^{2+} and the membrane potential might play an important role in the secretory process. Ca^{2+}-dependent K^+ channels have been demonstrated and characterized in chromaffin cells as well as in other cell types (Neel & Lingle, 1992; Artalejo *et al.*, 1993). Based on these facts, in the present study, the finding that quinine inhibited CA secretory responses evoked by high potassium is very similar to that obtained in cultured bovine adrenal chromaffin cells (Glavinovic, Dagher & Trifaro, 1985; Tang *et al.*, 1990). These results that quinine inhibits CA secretory responses evoked by DMPP (a nicotinic N_n receptor agonist), McN-A-343 (a muscarinic M_1-receptor agonist) and high potassium (a direct membrane-depolarizer) in a concentration-dependent fashion from the isolated perfused rat adrenal galnd suggest strongly that this inhibitory effect of quinine may be at least due to the activation of potassium channels. In support of this idea, in the experiment using cultured bovine chromaffin cells, it has been shown that quinine (25-400 μM) produces a dose-related inhibition of CA release in responses to depolarizing concentrations (12.5-50 mM) of K^+ (Glavinovic, Dagher and Trifaro, 1985; Tang *et al.*, 1990). Furthermore, quinine, even at high concentrations (>1 mM), is found to be a rather inefficient blocker of Ca^{2+}-activated large conduc-

tance K^+ channels from the cultured bovine chromaffin cells (Glavinovic and Trifaro, 1988). Although previous reports suggested that quinine is a specific blocker of the Ca^{2+}-activated K^+ conductance in red blood cells (Armando-Hardy *et al.*, 1975; Lew & Ferreira, 1978), in the present investigation, quinine inhibited the CA secretion evoked by cholinergic stimulation as well as by membrane depolarization. Moreover, this inhibitory effect of quinine is greatly attenuated by concurrent treatment with glibenclamide. Therefore, it is felt that quinine is no longer a blocker of potassium channels in the perfused rat adrenal gland.

In the present work, glibenclamide, a hypoglycemic sulfonylurea which selectively blocks ATP-sensitive K^+ channels (Quast & Cook, 1993; Ashcroft, 1988), restored the inhibitory responses by quinine of CA secretions evoked by cholinergic stimulation and membrane depolarization to the state of the corresponding control (non-treated) level. These findings suggest that ATP-sensitive K^+ channels may be involved in the inhibitory activity of quinine in the rat adrenal medullary chromaffin cells. Moreover, the present finding that pinacidil also inhibited the CA secretion evoked by cholinergic stimulation as well as by membrane depolarization in a similar fashion to that of quinine indicates that quinine has the possibility to act as an opener of K^+ channels. In previous studies, it has been found that the K^+ channel opener, pinacidil, selectively inhibits CA secretory responses induced by moderate depolarization or by stimulation of nicotinic ACh receptors both in cultured bovine adrenal chormaffin cells (Masuda *et al.*, 1994) and the perfused rat adrenal gland (Lim *et al.*, 2000). Pinacidil is known to be a cyanoguanidine derivative and a novel antihypertensive agent that markedly reduces peripheral vascular resistance (Ahnfelt-Ronne, 1988). The vasodilator effect of pinacidil results from direct relaxation of vascular smooth muscle cells (Weston *et al.*, 1988), apparently by opening of potassium cahnnels (Hermsmeyer, 1988; Weston *et al.*, 1988). Moreover, Soares-da-Silva and Fernandes (1990) showed that pinacidil impairs transmitter release from the sympathetic innervation of the rat vas defferens, probably as a consequence of the opening of K^+ channels.

The result that quinine inhibited CA secretion evoked by stimulation of muscarinic receptors with McN-A-343 suggests that K^+ 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 previous reports (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, quinine 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). These findings that quinine inhibited CA secretion evoked by high K^+ as well as by Bay-K-8644 suggest that quinine inhibits directly the voltage-dependent Ca^{2+} channels through opening of K^+ 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 quinine inhibits DMPP-evoked CA secretion by inhibiting Ca^{2+} influx through voltage-dependent Ca^{2+} channels activated by stimulation of nicotinic ACh receptors. However, in contrast to the present results, Masuda and his co-workers (1994) found that cromakalim and pinacidil did not affect the secretion of CA induced by Bay-K-8644 (Garcia *et al.*, 1984) or Ba^{2+} (Terbush & Holz, 1992; Heldman *et al.*, 1989) from the cultured bovine chromaffin cells. This fact suggests that they do not inhibit influx of Ca^{2+} induced by an opener of L-type voltage-sensitive Ca^{2+} channels such as Bay-K-8644, or influx of Ba^{2+}, which is thought to pass through voltage-sensitive Ca^{2+} channels and to stimulate CA secretion. Based on these findings, it seems that there is difference between both species in CA secretion.

It was found that quinine also inhibits the CA secretion evoked by cyclopiazonic acid. It is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Geoger & Riley, 1989; Siedler *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, based on these findings, it is felt that the inhibitory effect of quinine 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 quinine 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). This is consistent with the findings obtained in skinned smooth muscle fibers of the longitudinal layer of the guinea-pig ileum, in which 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 and thereby increase of Ca^{2+}-dependent K^+-current.

Uceda and his coworkers (1992) have reported that intracellular Ca^{2+}-dependent K^+ channels, the small-conductance type (SK), seem to be involved in the modulation of muscarinic stimulation-evoked CA release responses in cat adrenal chromaffin cells. However, in the present study, the fact that McN-A-343-evoked CA secretion was depressed by pretreatment with quinine appears to be consistent with these previous results.

In conclusion, these results demonstrate that quinine inhibits CA secretory responses evoked by cholinergic (both nicotinic and muscarinic) stimulation as well as by membrane depolarization in the isolated perfused model of rat adrenal glands by inhibiting Ca^{2+} influx through the L-type voltage-dependent calcium channels and Ca^{2+} mobilization from intracellular pools. Furthermore, these findings suggest that this inhibitory action of quinine may be at least due to the activation of potassium channels in CA secretion from the rat adrenal gland.

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