Influence of Glibenclamide on Catecholamine Secretion in the Isolated Rat Adrenal Gland

Hae-Jeong No^{1,3}, Seong-Chang Woo², and Dong-Yoon Lim^{1*}

¹Department of Pharmacology, College of Medicine, Chosun University,
Gwangju 501-759, KOREA; Department of ²Anesthesiology and

³Family Medicine, Eulji University Hospital, Daejeon 302-120, KOREA

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Abstract – The aim of the present study was to investigate the effect of glibenclamide, a hypoglycemic sulfonylurea, which selectively blocks ATP-sensitive K⁺ channels, on secretion of catecholamines (CA) evoked by cholinergic stimulation and membrane depolarization from the isolated perfused rat adrenal glands. The perfusion of glibenclamide (1.0 mM) into an adrenal vein for 90 min produced time-dependently enhanced the CA secretory responses evoked by ACh (5.32 mM), high K⁺ (a direct membrane depolarizer, 56 mM), DMPP (a selective neuronal nicotinic receptor agonist, 100 μM for 2 min), McN-A-343 (a selective muscarinic M₁ receptor agonist, 100 μM for 2 min), Bay-K-8644 (an activator of L-type dihydropyridine Ca²⁺ channels, 10 μM for 4 min) and cyclopiazonic acid (an activator of cytoplasmic Ca²⁺-ATPase, 10 μM for 4 min). In adrenal glands simultaneously preloaded with glibenclamide (1.0 mM) and nicorandil (a selective opener of ATP-sensitive K⁺ channels, 1.0 mM), the CA secretory responses evoked by ACh, high potassium, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid were recovered to the considerable extent of the control release in comparison with that of glibenclamide-treatment only. Taken together, the present study demonstrates that glibenclamide enhances the adrenal CA secretion in response to stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as by membrane depolarization from the isolated perfused rat adrenal glands. It seems that this facilitatory effect of glibenclamide may be mediated by enhancement of both Ca²⁺ influx and the Ca²⁺ release from intracellular store through the blockade of K_{ATP} channels in the rat adrenomedullary chromaffin cells. These results suggest that glibenclamide-sensitive K_{ATP} channels may play a regulatory role in the rat adrenomedullary CA secretion.

Keywords □ Glibenclamide, Nicorandil, Catecholamine secretion, Adrenal gland, ATP-sensitive K⁺ (K_{ATP}) channels

INTRODUCTION

Glibenclamide is known to inhibit competitively the vasorelaxant effects of UR-8225, which is a K_{ATP}-channel opener, in rat portal veins and aorta (Perez-Vizcaino *et al.*, 1993). Asano and his coworkers (1994) have found that cromakalim causes arterial relaxation via the opening of K_{ATP}-channels in both SHR and Wistar-Kyoto rats, which is blocked by glibenclamide. K_{ATP}-channels are now established as octomeric proteins, consisting of four inward rectifier K⁺ channel subunits associ-

ated with four sulfonylurea receptors (SURs) (Aguilar-Bryan *et al.*, 1998). SURs are the binding sites for known blockers (e.g., glibenclamide) and activators (e.g., pinacidil and cromakalim) of these channels, which have found valuable therapeutic uses. K_{ATP} channels are classically identified as being inhibited by intracellular ATP and were first described in cardiac myocytes (Noma, 1983) in which they were believed to act under conditions of metabolic stress to shorten action potential durations and so reduce the energy demands of myocytes when intracellular ATP levels are reduced (Benndorf *et al.*, 1997).

In general, it has been shown that membrane K⁺ channels in various cells are responsible for controlling the membrane potential and excitability of cells (Petersen & Maruyama, 1984; Cook, 1988; Watson & Abbott 1991). The opening (activation) of these channels causes hyperpolarization, and conversely, their closing (inhibition) causes depolarization of the cell mem-

*Corresponding author

Tel: +82-62-230-6335, Fax: +82-62-227-4693

E-mail: dylim@chosun.ac.kr

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brane. Masuda and his coworkers (1994) found that in cultured bovine adrenal chromaffin cells, the K^+ channel openers, cromakalim and pinacidil, selectively inhibit the secretory responses of catecholamines (CA) induced by moderate depolarization or by stimulation of nicotinic acetylcholine (ACh) receptors. Wada and his coworkers (1987) have shown that the cultured bovine adrenal medullary cells have, at least, three distinct types of K^+ permeability mechanisms: (1) basal K^+ efflux, (2) Ca^{2+} -dependent K^+ efflux, and (3) Na^+ -dependent K^+ efflux, and that nicotinic receptors mediate K^+ efflux by increasing Na^+ influx via nicotinic receptor-associated ionic channels rather than Ca^{2+} influx via voltage-dependent Ca^{2+} channels.

ATP-sensitive potassium (K_{ATP})-channels have been identified in numerous different tissues, including central neurons (Ashford *et al.*, 1988; Murphy and Greenfield, 1992; Finta *et al.*, 1993; Pierrefiche *et al.*, 1996). Their role in the normal functioning of neuronal activity is not well established, but they have been shown to alter electrical excitability (primarily by causing membrane hyperpolarization when open) under hypoxic or ischemic conditions (Murphy and Greenfield, 1992; Wu *et al.*, 1996). Evidence has emerged that K_{ATP} channels may be active under normoxic conditions when intracellular ATP levels would not be expected to be depleted (Pierrefiche *et al.*, 1996). There is so far a little evidence about the influence of K_{ATP} -channels on the CA secretion from the perfused model of the isolated rat adrenal gland.

The aim of this study is to elucidate the functional role of K_{ATP} -channels in controlling the adrenal CA secretion. To this end, the present study was undertaken to examine the effects of glibenclamide, a selective K_{ATP} -channel blocker, on the CA secretion from the isolated perfused rat adrenal gland in response to the selective neuronal nicotinic receptor agonist 1,1-dimethyl-4-phenyl-piperazinium (DMPP), ACh, the selective muscarinic M_1 -receptor agonist McN-A-343, and the direct membrane-depolarizer high K^+ .

MATERIALS AND METHODS

Experimental procedure

Male Sprague-Dawley rats (supplied by Chosun University Center for Experimental Animals), weighing 180 to 300 grams, were anesthetized with thiopental sodium (40 mg/kg) intraperitoneally. The adrenal gland was isolated by some modification of 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 the placement of 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 $37 \pm 1^\circ\text{C}$.

Perfusion of adrenal gland

The adrenal glands were perfused by means of peristaltic pump (ISCO, WIZ Co., U.S.A.) 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 $\mu\text{g/ml}$) and ascorbic acid (100 $\mu\text{g/ml}$) to prevent oxidation of CA.

Drug administration

The perfusions of DMPP (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. McN-A-343 (10^{-4} M), 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 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,

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 glibenclamide on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing glibenclamide for 90 min, and 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 glibenclamide, 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.

Drugs and their sources

The following drugs were used: nicorandil (Choong Wae Pharma. Corp., Korea), glibenclamide, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, methyl-1, 4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate (Bay-K-8644) (Sigma Chemical Co., U.S.A.), and cyclopiazonic acid, (3-(m-chloro-phenyl-carbamoyl-oxy)-2-butylnyltrimethyl 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 and nicorandil, which

were dissolved in 99.5% ethanol and 99.5% dimethyl sulfoxide (DMSO), respectively, and diluted appropriately with Krebs-bicarbonate solution (final concentration of alcohol or DMSO was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base.

Statistical analysis

The statistical difference between the control and pretreated groups was determined by the Student's paired *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).

RESULTS

Effect of glibenclamide on CA release evoked by ACh, high K⁺, DMPP and McN-A-343 from the perfused rat adrenal medulla

After the perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 21 ± 2 ng for 2 min ($n=6$) in all groups. There were no significant differences in these basal values among the experimental groups. glibenclamide is known to inhibit competitively the vasorelaxant effects of UR-8225, which is a K_{ATP}-channel opener, in rat portal veins and aorta (Perez-Vizcaino *et al.*, 1993). Therefore, it was attempted initially to examine the effects of glibenclamide itself on CA secretion from the perfused model of the rat adrenal glands. In the present study, In 6 rat adrenal glands, glibenclamide-evoked CA secretory response was maximally about 80 ng (0–60 min), which seemed to be a very weak secretagogue. Therefore, in the subsequent experiments, the time course effects of glibenclamide on the CA secretory responses evoked by ACh, high K⁺, DMPP and McN-A-343 were examined. Secretagogues were given at 15 to 20 min-intervals. Glibenclamide was present for 90 minutes after the establishment of the control release to secretagogues.

When ACh (5.32 mM) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 384 ± 13 ng for 4 min. However, the pretreatment with 1.0 mM glibenclamide for 90 min relatively time-dependently enhanced ACh-stimulated CA secretion. As shown in Fig. 1 (Upper), in the presence of glibenclamide, CA-releasing response was

enhanced to 120% of the corresponding control release, but not affected at 0-34 min period. Also, it has been found that depolarizing agent like KCl stimulates markedly CA secretion (166 ± 10 ng for 0-4 min). High K^+ (56 mM)-stimulated CA secretion after the pretreatment with 1.0 mM glibenclamide was not affected for about 50 min as compared with its corresponding control secretion (100%) (Fig. 1-Lower). However, in 60-94

min period, it was greatly enhanced to 138% of the control release. DMPP (100 μ M), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (346 ± 10 ng for 0-8 min). However, as shown in Fig. 2 (Upper), DMPP-stimulated CA secretion after pretreatment with glibenclamide was increased to 122% of the control release (100%). McN-A-343 (100 μ M), which is a selective muscarinic M1-agonist (Hammer and Giachetti, 1982), perfused into an adrenal gland for 4 min caused an increased CA secretion (172 ± 9 ng for 0-4 min). However, McN-A-343-stimulated CA secretion in the presence of glibenclamide was markedly enhanced to 136% of the correspond-

Fig. 1. Time-course effect of glibenclamide on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh, **Upper**) and by high K^+ (**Lower**) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32 mM) or K^+ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 1.0 mM glibenclamide for 90 min 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 (CONT) with each concentration-pretreated group of glibenclamide. Perfusates induced by ACh and high K^+ were collected for 4 minutes, respectively. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

Fig. 2. Time-course effect of glibenclamide on the secretory responses of catecholamines (CA) evoked by DMPP (**Upper**) and McN-A-343 (**Lower**) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DMPP (100 μ M) and McN-A-343 (100 μ M) for 2 min at 20 and 15 min intervals were induced after preloading with 1.0 mM glibenclamide for 90 min, respectively. Perfusates induced by DMPP and McN-A-343 were collected for 8 and 4 minutes, respectively. Other legends are the same as in Fig. 1. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

ing control secretion (100%), although it was not affected at 0-49 min period, as depicted in Fig. 2 (Lower)

Effect of glibenclamide on CA release evoked by Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal medulla

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 glibenclamide on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10 μM)-stimulated CA secretion in the presence of glibenclamide was greatly elevated to 141% of the control except for

Fig. 3. Time-course effect of glibenclamide on CA release evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower) from the rat adrenal glands. Bay-K-8644 (10 μM) and cyclopiazonic acid (10 μM) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of glibenclamide (1.0 mM) for 90 min, respectively. Other legends are the same as in Fig. 1. **: P < 0.01. ns: Statistically not significant.

early 45 min as compared to the corresponding control release (154 $\pm$ 10 ng for 0-4 min) from 10 rat adrenal glands, as shown in Fig. 3 (Upper).

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 glibenclamide on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 3 (Lower). However, in the presence of glibenclamide in 10 rat adrenal glands, cyclopiazonic acid (10 μM)-evoked CA secretion was also elevated to 138% of the control response (166 $\pm$ 10 ng for 0-4 min).

Effect of glibenclamide plus nicorandil on CA release evoked by ACh, high K^+ , DMPP, McN-A-343, BAY-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

As shown in Fig. 1~2, glibenclamide significantly enhanced the CA secretory responses evoked by cholinergic stimulation and membrane depolarization from the perfused rat adrenal glands. Nicorandil has at least two mechanisms of action; This drug relaxes vascular smooth muscle by stimulating soluble guanylate cyclase leading to increased cGMP levels (Endo and Taira, 1983; Holzmann, 1983; Meisheri *et al.*, 1991) and also opening of ATP-sensitive K^+ (K_{ATP}) channels to hyperpolarize the plasma membrane (Furukawa *et al.*, 1981; Kukovetz *et al.*, 1991; Holzmann *et al.*, 1992). Therefore, in the presence of glibenclamide (1.0 mM), the effect of nicorandil on the CA secretion evoked by cholinergic stimulation and membrane-depolarization in the perfused rat adrenal medulla was examined.

In the present study, ACh (5.32 mM)-evoked CA release before perfusion with nicorandil plus glibenclamide was 358 $\pm$ 10 ng (0-4 min) from 10 rat adrenal glands. In the simultaneous presence of nicorandil (1.0 mM) and glibenclamide (1.0 mM) for 90 min, it was initially not affected at 0-64 min, but later rather inhibited by 82% of the corresponding control release at 90-94 min period as illustrated in Fig. 4 (Upper). High K^+ (56 mM)-evoked CA release in the presence of nicorandil (1.0 mM) and glibenclamide (1.0 mM) for 90 min was also not changed for 0-64 min, but later inhibited to 74% of the corresponding control release only at the last period of 90-94 min period in comparison to the control secretion (179 $\pm$ 9 ng, 0-4 min) from 10 glands (Fig. 4-Lower).

As shown in Fig. 5 (Upper), DMPP-evoked CA release prior

Fig. 4. Effects of glibenclamide plus nicorandil on catecholamine release evoked by acetylcholine (upper) and high K^+ (lower) from the isolated perfused rat adrenal glands. CA secretion by a single injection of Ach (5.32 mM) or high K^+ (56 mM) was induced "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 1.0 mM nicorandil+1.0 mM glibenclamide for 90 min, respectively. Other legends are the same as in Fig. 1. **: $P < 0.01$. ns: Statistically not significant.

to simultaneous perfusion with nicorandil and glibenclamide was 371 ± 16 ng (0-8 min). The simultaneous perfusion of nicorandil and glibenclamide for 90 min no longer inhibited DMPP-evoked CA release for the period of 0-68 min from 10 experiments while later rather depressed to 85% of the control release at 80-88 min period. Moreover, in the presence of both nicorandil (1.0 mM) and glibenclamide (1.0 mM), the CA secretory response evoked by McN-A-343 (10^{-4} M for 4 min) was also not affected for 0-64 min, but later rather inhibited to 75% of the corresponding control release (179 ± 9 ng, 0-4 min) at the last period of 90-94 min period from 10 glands, as shown in Fig. 5 (Lower).

As shown in Fig. 6, the simultaneous perfusion of nicorandil (1.0 mM) and glibenclamide (1.0 mM) for 90 min did not

Fig. 5. Effects of glibenclamide plus nicorandil on catecholamine release evoked by DMPP (Upper) and McN-A-343 (Lower) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DMPP (100 μ M) and McN-A-343 (100 μ M) for 2 min and 4 min at 20 and 15 min intervals were induced "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 1.0 mM nicorandil+1.0 mM glibenclamide for 90 min, respectively. Other legends are the same as in Fig. 1. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

inhibit the CA release evoked by Bay-K-644 (Upper) and cyclopiazonic acid (Lower) for the period of 0-79 min from 10 experiments, but later rather depressed to 72% and 79% of each control release only at 90-94 min period in comparison to their corresponding control responses (179 ± 9 ng, 0-4 min and 181 ± 7 ng, 0-4 min), respectively.

DISCUSSION

The present experimental results demonstrate that glibenclamide enhances the adrenal CA secretion in response to stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as by membrane depolarization from the isolated perfused rat adrenal glands.

Fig. 6. Effects of glibenclamide plus nicorandil on catecholamine release evoked by Bay-K-8644 (**Upper**) and cyclopiazonic acid (**Lower**) from the rat adrenal glands. Bay-K-8644 (10 μ M) and cyclopiazonic acid (10 μ M) were perfused into an adrenal vein for 4 min at 15 min intervals "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 1.0 mM nicorandil+1.0 mM glibenclamide for 90 min, respectively. Other legends are the same as in Fig. 1. **: $P < 0.01$. ns: Statistically not significant.

In the present work, glibenclamide, which selectively blocks ATP-sensitive K^+ channels (Quast & Cook, 1989; Ashcroft, 1988), restored the inhibitory responses by nicorandil on the CA secretion evoked by ACh, DMPP, McN-A-343 and high potassium to the considerable extent of the corresponding control level. These findings suggest strongly that ATP-sensitive K^+ channels are involved in regulating the CA secretion in the rat adrenal medullary chromaffin cells. In support of this idea, it has been shown that K_{ATP} -channel openers, such as cromakalim, pinacidil, and nicorandil, produce vasorelaxation by preventing the opening of voltage-activated Ca^{2+} channels through the opening of K_{ATP} -channels and the resulting membrane hyperpolarization (Cook, 1988; Hamilton & Weston,

1989; Weston, 1988; Standen *et al.*, 1989; Quast & Cook, 1989; Weston *et al.*, 1990; Edwards *et al.*, 1992). Moreover, glibenclamide is known to inhibit competitively the vasorelaxant effects of UR-8225, which is a K_{ATP} -channel opener, in rat portal veins and aorta (Perez-Vizcaino *et al.*, 1993). Asano and his coworkers (1994) have found that cromakalim causes arterial relaxation via the opening of K_{ATP} -channels in both SHR and Wistar-Kyoto rats, which is blocked by glibenclamide. In terms of these findings, the present results indicate that glibenclamide may enhance the CA secretory responses at least through blockade of K_{ATP} -channels located on the rat adrenomedullary chromaffin cells. K_{ATP} -channels are now established as octomeric proteins, consisting of four inward rectifier K^+ channel subunits associated with four sulfonylurea receptors (SURs) (Aguilar-Bryan *et al.*, 1998). SURs are the binding sites for known blockers (e.g., glibenclamide) and activators (e.g., pinacidil and cromakalim) of these channels, which have found valuable therapeutic uses. K_{ATP} channels are classically identified as being inhibited by intracellular ATP and were first described in cardiac myocytes (Noma, 1983) in which they were believed to act under conditions of metabolic stress to shorten action potential durations and so reduce the energy demands of myocytes when intracellular ATP levels are reduced (Benndorf *et al.*, 1997).

In the present work, in the presence of glibenclamide, the CA exocytosis evoked by both high K^+ and Bay-K-8644 was clearly enhanced and this facilitatory effect is prevented in the simultaneous presence of glibenclamide and nicorandil. Based on these results, it seems that glibenclamide enhances the CA secretion partly through SUR-mediated action. This action of glibenclamide is very similar with previous result as shown by pinacidil (Lim *et al.*, 2000), although two agents are structurally distinct activators of K_{ATP} . In neuronal tissue, cromakalim and pinacidil agents are also known to reverse the effects of glibenclamide (Schmid-Antomarchi *et al.*, 1990). This evidence leads to conclusion that a novel role for SUR in modulating exocytosis in a neuronal tissue is identified. Furthermore, these findings would suggest that this role is functionally downstream of Ca^{2+} entry or mobilization. However, more detailed mechanism of action on the CA secretory effects of nicorandil and glibenclamide remains to be resolved in future.

Generally, the CA secretion from the adrenal medulla is controlled by splanchnic nerve-innervated chromaffin cells. Activation of the splanchnic nerve causes the release of acetylcholine (ACh) from its terminal into the intrasynaptic cleft, which subsequently activates nicotinic receptors of the

adrenal medullary chromaffin cells. Stimulation of nicotinic receptors depolarizes the chromaffin cell membrane, and the resulting depolarization causes Ca^{2+} influx through the opening of voltage-dependent Ca^{2+} channels (Cena *et al.*, 1983; Corcoran and Kirshner, 1983). The elevation of intracellular Ca^{2+} triggers the exocytotic secretion of adrenal CA (Garcia *et al.*, 1984). The membrane depolarization may activate voltage-dependent K^{+} channels, leading to the facilitation of repolarization, and the elevation of intracellular Ca^{2+} may activate Ca^{2+} -activated K^{+} channels, leading to hyperpolarization. The facilitation of repolarization or hyperpolarization may cause the inhibition of further influx of Ca^{2+} . Therefore, blockade of K^{+} channels is thought to facilitate the depolarizing phase and results in the enhancement of adrenal CA secretion through the increase in Ca^{2+} influx.

In the present study, the finding that both nicorandil inhibited the CA secretory responses evoked by stimulation of nicotinic ACh receptors with DMPP and membrane depolarization with high K^{+} seems to be very similar to that obtained in cultured bovine adrenal chromaffin cells (Masuda *et al.*, 1994). In cultured bovine chromaffin cells, Masuda and his coworkers (1994) found that cromakalim and pinacidil inhibit the CA release, $^{45}\text{Ca}^{2+}$ influx and increase in intracellular Ca^{2+} induced by moderate depolarization by potassium as well as by stimulation of nicotinic receptors with carbamylcholine. Based on this finding, the present data indicate that the K_{ATP} -channel opener like nicorandil affects membrane potassium channels, resulting in an increase in K^{+} efflux and then a decrease in the CA secretion from rat adrenal chromaffin cells. In the present work, nicorandil inhibited the CA secretion evoked by ACh, high K^{+} , DMPP and McN-A-343. However, in the presence of glibenclamide, nicorandil-induced inhibitory effect of the CA secretion was recovered to the considerable extent of the corresponding control release in comparison with that by nicorandil-treatment only. These results are consistent with the observation with pinacidil, another K_{ATP} channel activator in the perfused rat adrenal medulla (Lim *et al.*, 2000), indicating that nicorandil suppresses the CA secretion by affecting pathways mediated by both nicotinic and muscarinic receptors but that it does not inhibit the secretion process by itself.

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 tripled the

peak secretory response to muscarine in perfused Ca^{2+} cat adrenal glands. In this experiment, both nicorandil also depress greatly the CA secretion induced by Bay-K-8644, which is found to enhance the CA release by increasing Ca^{2+} influx through L-type Ca^{2+} channels in chromaffin cells (Garcia *et al.*, 1984). These findings that nicorandil inhibited the CA secretion evoked by high K^{+} and also by Bay-K-8644 suggest that nicorandil 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 the CA secretion by increasing Ca^{2+} influx largely through voltage-dependent Ca^{2+} channels (Burgoyne, 1984; Oka *et al.*, 1979). Therefore, it seems that nicorandil inhibits DMPP-evoked CA secretion by inhibiting Ca^{2+} influx through voltage-dependent Ca^{2+} channels activated by nicotinic ACh receptors with DMPP. However, Masuda and his coworkers (1994) found that cromakalim and pinacidil did not affect the CA secretion from the cultured bovine chromaffin cells induced by Bay-K-8644 (Garcia *et al.*, 1984) or Ba^{2+} (Terbush & Holz, 1992; Heldman *et al.*, 1989), suggesting that they did 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 the CA secretion.

In this study, nicorandil inhibited the increase in the CA secretion evoked by cyclopiazonic acid, which is known to be a highly selective inhibitor of Ca^{2+} -ATPase in skeletal muscle sarcoplasmic reticulum (Goeger & 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, these results suggest that the inhibitory effect of nicorandil on the CA secretion evoked by cholinergic muscarinic stimulation might be associated with the mobilization of intracellular Ca^{2+} in the chromaffin cells. This indicates that the K_{ATP} channel opener 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 CA secretion. It has been shown that Ca^{2+} -uptake into intracellular storage sites susceptible to caffeine (Iino, 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, where Ca^{2+} -uptake was also

inhibited by cyclopiazonic 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. 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; Challiss *et al.*, 1991). However, in the present study, it is uncertain whether the inhibitory effect of the K_{ATP} channel opener on Ca^{2+} movement from intracellular pools is due to their direct effect on the PI response or an indirect effect as a result of the membrane hyperpolarization induced by opening of K_{ATP} channels.

In conclusion, the present study demonstrates that glibenclamide enhances the adrenal CA secretion in response to stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as by membrane depolarization from the isolated perfused rat adrenal glands. It seems that this facilitatory effect of glibenclamide may be mediated by enhancement of both Ca^{2+} influx and the Ca^{2+} release from intracellular store through the blockade of K_{ATP} channels in the rat adrenomedullary chromaffin cells. These results suggest that glibenclamide-sensitive K_{ATP} channels may play a regulatory role in the rat adrenomedullary CA secretion.

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