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# Influence of Nicorandil on Catecholamine Release in the Perfused Rat Adrenal Medulla

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**The present study was attempted to investigate the effect of nicorandil, which is an ATP-sensitive potassium ($K_{ATP}$) channel opener, on secretion of catecholamines (CA) evoked by cholinergic stimulation and membrane depolarization from the isolated perfused rat adrenal glands. The perfusion of nicorandil (0.3~3.0 mM) into an adrenal vein for 90 min produced relatively dose-and time-dependent inhibition in CA secretion evoked by ACh (5.32 mM), high $K^+$ (a direct membrane depolarizer, 56 mM), DMPP (a selective neuronal nicotinic receptor agonist, 100 $\mu$M for 2 min), McN-A-343 (a selective muscarinic $M_1$ receptor agonist, 100 $\mu$M for 4 min), Bay-K-8644 (an activator of L-type dihydropyridine $Ca^{2+}$ channels, 10 $\mu$M for 4 min) and cyclopiazonic acid (an activator of cytoplasmic $Ca^{2+}$-ATPase, 10 $\mu$M for 4 min). In adrenal glands simultaneously preloaded with nicorandil (1.0 mM) and glibenclamide (a nonspecific $K_{ATP}$-channel blocker, 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 nicorandil-treatment only. Taken together, the present study demonstrates that nicorandil inhibits 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 inhibitory effect of nicorandil may be mediated by inhibiting both $Ca^{2+}$ influx and the $Ca^{2+}$ release from intracellular store through activation of $K_{ATP}$ channels in the rat adrenomedullary chromaffin cells. These results suggest that nicorandil-sensitive $K_{ATP}$ channels may play an inhibitory role in the regulation of the rat adrenomedullary CA secretion.**

**Key Words: Nicorandil, Glibenclamide, Catecholamine release, Adrenal medulla, ATP-sensitive $K^+$ ($K_{ATP}$) channels**

## INTRODUCTION

Nicorandil, *N*-(2-hydroxyethyl)-nicotinamide nitrate ester, is found to dose-dependently inhibit halothane-epinephrine arrhythmias in rats through mitochondrial ATP-sensitive $K^+$ channels and nitric oxide is required for the antiarrhythmic effect of nicorandil (Kawai et al, 2002). It has also been reported that the potency of nicorandil to cause coronary vasorelaxation is increased under conditions of metabolic inhibition. This effect appears to result from the $K^+$ channel opening action of the drug, and may have significant consequences for its therapeutic effectiveness (Davie et al, 1993). The vasodilator nicorandil has a combined chemical structure of an organic nitrate and a nicotinamide and is clinically an efficacious drug for treatment of angina pectoris (Frampton et al, 1992; Goldschmidt et al, 1996). 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 & 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). The contribution of these two pathways to vasorelaxation appears to vary according to the tissue under study and the concentration of nicorandil used, the relative importance of the $K^+$ channel opening mechanism being greater in small vessels and at lower concentrations of nicorandil (Holzmann et al, 1992; Kukovetz et al, 1991; Akai et al, 1995).

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**ABBREVIATIONS:** CA, catecholamine; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide; ($K_{ATP}$) channels, ATP-sensitive $K^+$ channels; $K_{Ca}$ channels, $Ca^{2+}$-activated $K^+$ channels; $SK_{Ca}$- and $BK_{Ca}$-conductance $K_{Ca}$ channels, small-and large-conductance $K_{Ca}$ channels, respectively.

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 membrane. Masuda and his coworkers (1994) found that in cultured bovine adrenal chormaffin 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.

$Ca^{2+}$-activated $K^+$ ($K_{Ca}$) channels, such as small ($SK_{Ca}$)- and large ($BK_{Ca}$)-conductance $K_{Ca}$ channels, are present on adrenal chromaffin cells (Marty & Neher., 1985), but the role of each type in the CA secretion is not fully understood. $SK_{Ca}$ channels are characterized by indirect regulation of $Ca^{2+}$ movement and CA secretion in bovine (Lara et al, 1995; Wada et al, 1995) and cat (Uceda et al, 1992; Uceda et al, 1994; Montiel et al, 1995) chromaffin cells. Nagayama and colleagues (Nagayama et al, 1997; Nagayama et al, 1998) suggested that $SK_{Ca}$ channels play an inhibitory role in adrenal CA secretion in the dog. On the other hand, blockade of $BK_{Ca}$ channels enhances the CA secretion induced by carbachol, a nicotinic agonist, in bovine chromaffin cells (Wada et al, 1995), but it does not affect the transmural electrical stimulation (ES)-induced CA secretion in adrenal gland of the cat (Montiel et al, 1995) and the dog (Nagayama et al, 1997). Rat chromaffin cells possess $SK_{Ca}$ and $BK_{Ca}$ channels (Neely & Lingle, 1992), but there has been no evidence for participation of these $K_{Ca}$ channels in CA secretion.

ATP-sensitive potassium ($K_{ATP}$)-channels have been identified in numerous different tissues, including central neurons (Ashford et al, 1988; Murphy & 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 & 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 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 nicorandil, an $K_{ATP}$-channel activator, on the CA secretion from the isolated perfused rat adrenal gland in response to the selective neuronal nicotinic receptor agonist DMPP, ACh, the selective muscarinic $M_1$-receptor agonist McN-A-343, and the direct membrane-depolarizer high $K^+$.

## 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 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 $37 \pm 1°C$.

### *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 $\mu$g/ml) and ascorbic acid (100 $\mu$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 ($5.32 \times 10^{-3}$ M) and KCl ($5.6 \times 10^{-2}$ 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 nicorandil or pinacidil on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing nicorandil 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 nicorandil or pinacidil, 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 & 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 difference between the control and pretreated groups was determined by the Student's *t* and ANOVA tests. A p-value of less than 0.05 was considered to represent statistically significant changes unless specifically noted in the text. Values given in the text refer to means and the standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computer program described by Tallarida & Murray (1987).

### *Drugs and their sources*

The following drugs were used: 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 acd, 3-(m-cholro-phenyl-carbamoyl-oxy)-2-butynyltrimethyl 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.

## RESULTS

### *Effect of nicorandil 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 22±3 ng for 2 min (n=6) in all groups. There were no significant differences in these basal values among the experimental groups. Both nitroprusside and nicorandil accelerate the decrease in free intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) without changing the peak values of the initial $[Ca^{2+}]_i$ increase of $Ca^{2+}$ transient. These drugs, however, do not affect carbachol-induced CA secretion (Shono et al, 1997). Therefore, it was attempted initially to examine the effects of nicorandil itself on CA secretion from the perfused model of the rat adrenal glands. In the present study, nicorandil (0.3~3.0 mM) itself did not produce any effect on basal CA output from perfused rat adrenal glands (data not shown). Thus, it was decided to investigate the effects of nicorandil on cholinergic receptor stimulation-as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15 to 20 min-intervals. Nicorandil 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 391±21 ng for 4 min. However, the pretreatment with nicorandil in the range of 0.3~3.0 mM for 90 min relatively concentration-and time-dependently inhibited ACh-stimulated CA secretion. As shown in Fig. 1 (Left), in the presence of nicorandil, CA releasing responses were inhibited to 68% of the corresponding control release. Also, it has been found that depolarizing agent like KCl stimulates markedly CA secretion (188±13 ng for 0~4 min). High $K^+$ (56 mM)-stimulated CA secretion after the pretreatment with 0.3 mM nicorandil was not affected for about 80 min as compared with its corresponding control secretion (Fig. 1-Right). However, following the pretreatment with higher concentrations of nicorandil (1.0 mM and 3.0 mM), excess $K^+$ (56 mM)-stimulated CA secretion was significantly inhibited to 56% of the control after 45 min period, although it was not initially affected by nicorandil. DMPP ($10^{-4}$ M), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (345±18 ng for 0~8 min). However, as shown in Fig. 2 (Left), DMPP-stimulated CA secretion after pretreatment with nicorandil was reduced to 75% of the control release. McN-A-343 ($10^{-4}$ M), which is a selective muscarinic $M_1$-agonist (Hammer & 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 nicorandil was markedly depressed to 60% of the corresponding control secretion as depicted in Fig. 2 (Right).

### *Effect of nicorandil 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,

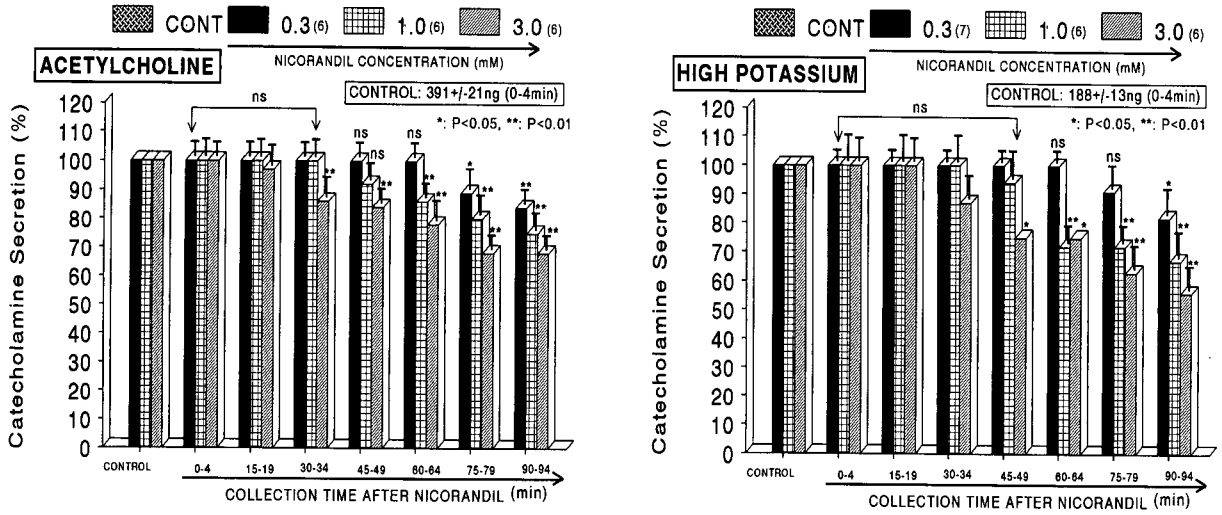


**Fig. 1.** Dose-dependent effects of nicorandil on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh, Left) and by high $K^+$ (Right) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32 mM) or $K^+$ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 0.3, 1.0, and 3.0 mM of nicorandil 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 nicorandil. Pefusates induced by ACh and high $K^+$ were collected for 4 minutes, respectively. *p<0.05, **p<0.01. ns: Statistically not significant.

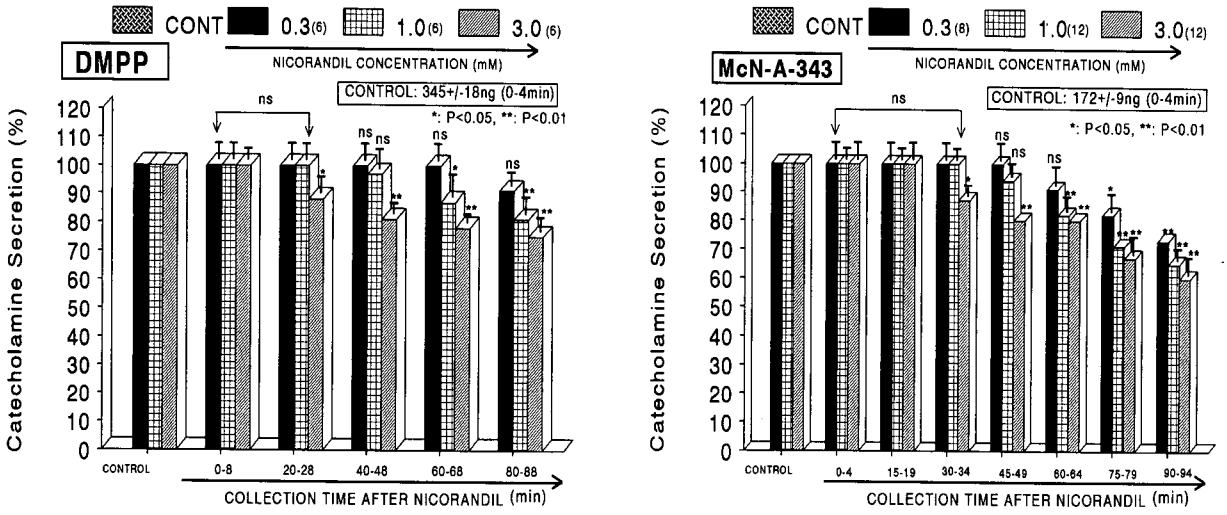


**Fig. 2.** Dose-dependent effects of nicorandil on the secretory responses of catecholamines (CA) evoked by DMPP (Left) and McN-A-343 (Right) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DMPP for 2 min (100 $\mu$M) and McN-A-343 (100 $\mu$M) for 4 min at 20 and 15 min intervals were induced after preloading with 0.3, 1.0, and 3.0 mM of nicorandil for 90 min, respectively. Pefusates 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.

1984) and CA release (Lim et al, 1992), it was of interest to determine the effects of nicorandil on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 ($10^{-5}$ M)-stimulated CA secretion in the presence of nicorandil was greatly blocked to 63% of the control except for early 45 min as compared to the corresponding control release (171±9 ng for 0~4 min) from 12 rat adrenal glands as shown in Fig. 3 (Left).

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 & Riley, 1989; Seidler et al, 1989). The inhibitory action of nicorandil on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 3 (Right). However, in the presence of nicorandil in 7 rat adrenal glands, cyclopiazonic acid ($10^{-5}$ M)-evoked CA

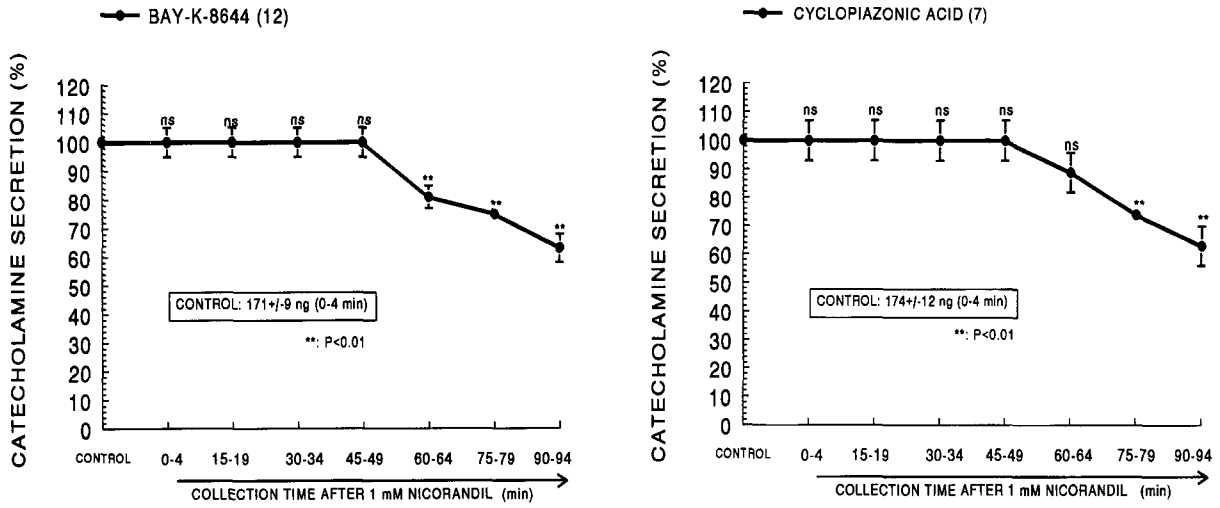


**Fig. 3.** Effects of nicorandil on CA release evoked by Bay-K-8644 (Left) and cyclopiazonic acid (Right) from the rat adrenal glands. Bay-K-8644 (10 $\mu$M) and cyclopiazonic acid (10 $\mu$M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of nicorandil (1.0 mM) for 90 min, respectively. Other legends are the same as in Fig. 1. **p<0.01. ns: Statistically not significant.

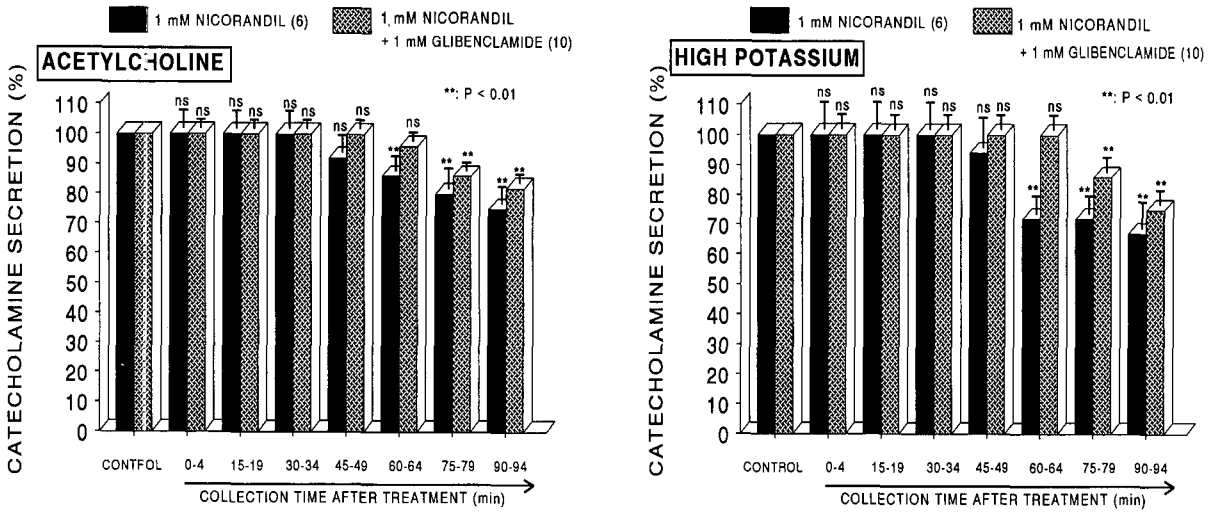


**Fig. 4.** Effects of nicorandil plus glibenclamide on catecholamine release evoked by acetylcholine (Left) and high $K^+$ (Right) from the isolated perfused rat adrenal glands. CA secretion by a single injection of Ach (5.32 mM) or high $K^+$ (56 mM) was induced "BEFORE (CONTROL)" and "AFTER" preloading 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.

secretion was also inhibited to 63% of the control response (174±12 ng for 0~4 min).

### *Effect of nicorandil plus glibenclamide 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 and 2, nicorandil significantly inhibited the CA secretory responses evoked by cholinergic stimulation and membrane depolarization form the perfused rat adrenal glands. Since glibenclamide, a hypoglycemic sulfonylurea, is found to selectively block ATP-sensitive $K^+$ channels (Ashcroft, 1988; Quast & Cook, 1989), therefore, in the presence of glibenclamide (1.0 mM), the effect of nicornadil on the CA secretion evoked by these secretagogues in the perfused rat adrenal medulla was studied.

In 6 rat adrenal glands, the CA secretory response evoked by glibenclamide itself was maximally about 80 ng (0~90 min), which seemed to be a very weak secretagogue. Therefore, to study the relationship between $K_{ATP}$-channel opener and $K_{ATP}$-channel blocker in the CA release from the rat adrenal glands, the effect of glibenclamide on nicorandil-induced inhibitory responses of CA secretion evoked by cholinergic receptor-stimulation as well as mem-

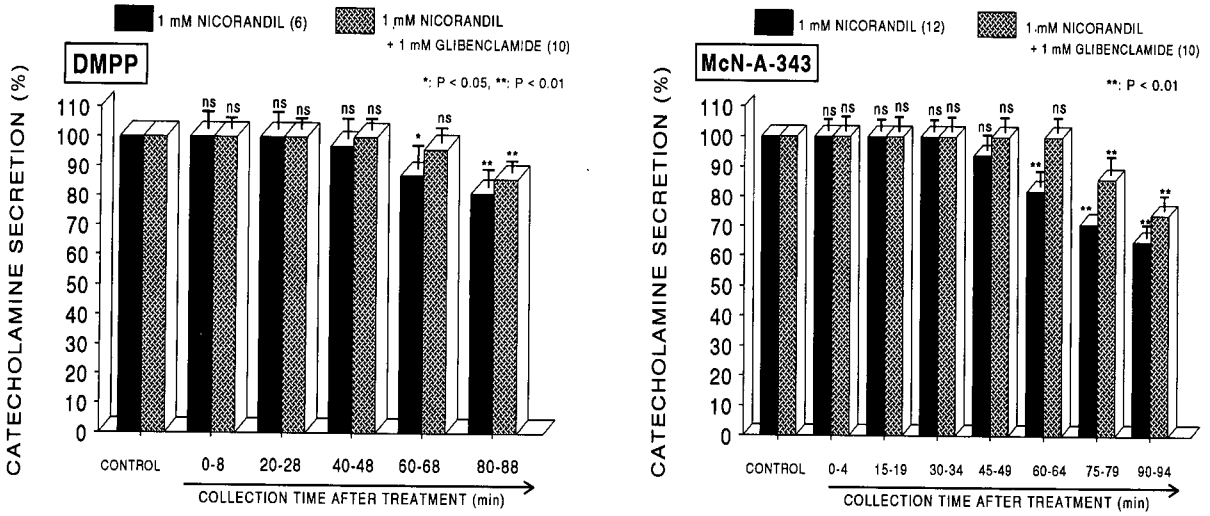


**Fig. 5.** Effects of nicorandil plus glibenclamide on catecholamine release evoked by DMPP (Left) and McN-A-343 (Right) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DMPP for 2 min (100 $\mu$M) and McN-A-343 (100 $\mu$M) for 4 min and 4 min at 20 and 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.

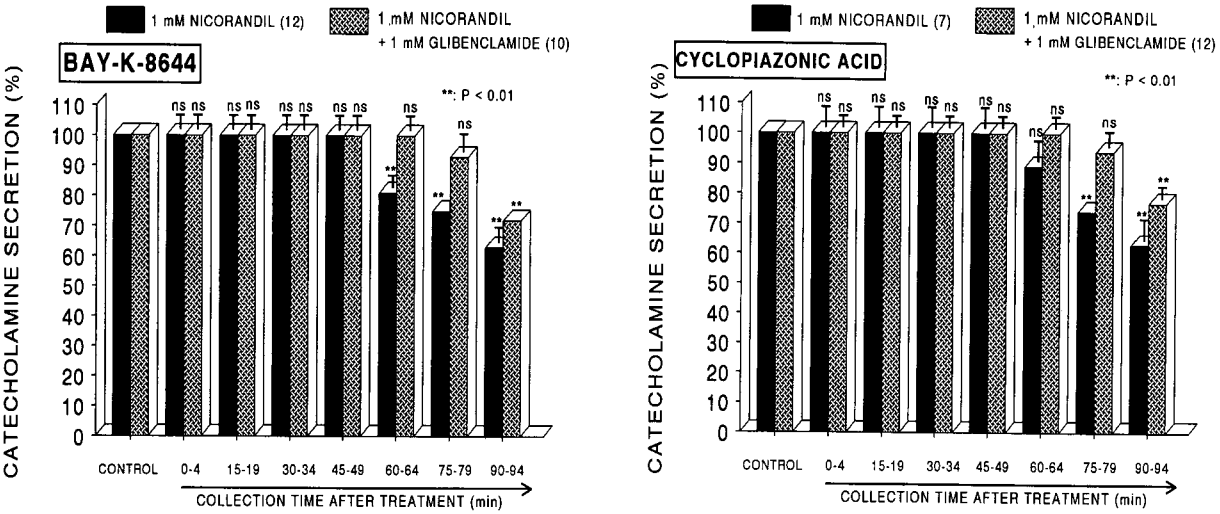


**Fig. 6.** Effects of nicorandil plus glibenclamide on catecholamine release evoked by Bay-K-8644 (Left) and cyclopiazonic acid (Right) from the rat adrenal glands. Bay-K-8644 (10 $\mu$M) and cyclopiazonic acid (10 $\mu$M) were perfused into an adrenal vein for 4 min at 15 min intervals "BEFORE (CONTROL)" 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.

brane depolarization was examined. In the present study, ACh (5.32 mM)-evoked CA release before perfusion with nicorandil plus glibenclamide was 358±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 (Left). 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±9 ng, 0~4 min) from 10 glands (Fig. 4) (Right).

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

As shown in Fig. 6, the simultaneous perfusion of nicorandil (1.0 mM) and glibenclamide (1.0 mM) for 90 min did not inhibit the CA release evoked by Bay-K-644 (Left) and cyclopiazonic acid (Right) 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 nicorandil inhibits 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 is thought that this inhibitory effect of nicorandil may be mediated by inhibiting both $Ca^{2+}$ influx and the $Ca^{2+}$ release from intracellular store through activation of $K_{ATP}$ channels in the rat adrenomedullary chromaffin cells. These results suggest that nicorandil-sensitive $K_{ATP}$ channels may play an inhibitory role in the regulation of the rat adrenomedullary CA secretion.

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 & 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, $^{45}Ca^{2+}$ 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.

In the present work, glibenclamide, a hypoglycemic sulfonylurea which selectively blocks ATP-sensitive $K^+$ channels (Ashcroft, 1988; Quast & Cook, 1989), restored the inhibitory responses by nicorandil of CA secretions evoked by cholinergic stimulation and membrane depolarization to the considerable extent of the corresponding control level. These findings suggest strongly that ATP-sensitive $K^+$ channels are involved in regulating 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; Weston, 1988; Hamilton & Weston, 1989; Quast & Cook, 1989; Standen et al, 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 nicorandil may inhibit the CA secretory responses at least through activation of $K_{ATP}$-channels located on the rat adrenomedullary chromaffin cells.

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 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 CA secretion by increasing $Ca^{2+}$ influx largely through voltage-dependent $Ca^{2+}$ channels (Oka et al, 1979; Burgoyne, 1984). 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 secretion of CA from the

cultured bovine chromaffin cells induced by Bay-K-8644 (Garcia et al, 1984) or $Ba^{2+}$ (Heldman et al, 1989; Terbush & Holz, 1992), 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 CA secretion.

In this study, nicorandil inhibited 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 (Goeger & 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, these results suggest that the inhibitory effect of nicorandil on 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 (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 logitudinal layer of the guinea-pig ileum, where $Ca^{2+}$-uptake was also inhibited by cylopiazonic acid (Uyama et al, 1992). Suzuki and his coworkers (1992) have shown that cyclopiazonic acid easily penetrates into the cytoplasm through the plasma membrane and reduces $Ca^{2+}$-ATPase activity in sarcoplasmic/endoplasmic reticulum, resulting in increase in the subsequent $Ca^{2+}$ release from those storage sites 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; Challis 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 nicorandil inhibits 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 inhibitory effect of nicorandil may be mediated by inhibiting both $Ca^{2+}$ influx and the $Ca^{2+}$ release from intracellular store through activation of $K_{ATP}$ channels in the rat adrenomedullary chromaffin cells. These results suggest that nicorandil-sensitive $K_{ATP}$ channels may play an inhibitory role in the regulation of the rat adrenomedullary CA secretion.

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## REFERENCES

Akai K, Wang Y, Sato K, Sekiguchi N, Sugimura A, Kumagai T, Komaru T, Kanatsuka H, Shirato K. Vasodilatory effect of nicorandil on coronary arterial microvessels: its dependency on vessel size and the involvement of the ATP-sensitive potassium channels. *J Cardiovasc Pharmacol* 26: 541–547, 1995

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

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

Asano M, Masuzawa-Ito K, Matsuda T. Vasodilating actions of cromakalim in resting and contracting states of carotid arteries from spontaneously hypertensive rats. *European J Pharmacol* 263: 121–131, 1994

Ashcroft FM. Adenosine 5'-triphosphate-sensitive potassium channels. *Annu Rev Neurosci* 11: 763–770, 1988

Ashford MLJ, Sturgess NC, Trout NJ, Gardner NJ, Hales CN. Adenosine 5'-triphosphate-sensitive ion channels in neonatal rat cultured central neurones. *Pflugers Arch* 412: 297–304, 1988

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

Cena V, Nicolas GP, Sanchez-Garcia P, Kirpekar SM, Garcia AG. Pharmacological dissection of receptor-associated and voltage-sensitive ionic channels involved in catecholamine release. *Neuroscience* 10: 1455–1462, 1983

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

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

Cook NS. The pharmacology of potassium channel and their therapeutic potential. *Trends Pharmacol Sci* 9: 21–28, 1988

Corcoran JJ, Kirshner N. Inhibition of calcium uptake, sodium uptake, and catecholamine secretion by methoxyverapamil (D600) in primary cultures of adrenal medulla cells. *J Neurochem* 40: 1106–1109, 1983

Davie CS, Kubo M, Standen NB. Potassium channel activation and relaxation by nicorandil in rat small mesenteric arteries. *Br J Pharmacol* 125: 1715–1725, 1998

Edwards G, Duty S, Trezise DJ, Weston AH. Effects of potassium-channel modulators on the cardiovascular system. In: Weston AH, Hamilton TC ed, *Potassium Channel Modulators: Phamacological, Molecular and Clinical Aspects.* Oxford Blackwell Science, p 369–463, 1992

Endoh M, Taira N. Relationship between relaxation and cyclic GMP formation caused by nicorandil in canine mesenteric arteries. *Naunyn-Schmiedeberg's Arch Pharmacol* 322: 319, 1983

Finta EP, Harms L, Sevick J, Fischer HD, Illes P. Effects of potassium channel openers and their antagonists on rat locus coerulus neurones. *Br J Pharmacol* 109: 308–315, 1993

Frampton J, Buckley MM, Fitton A. Nicorandil. A review of its pharmacology and therapeutic effects in angina pectoris. *Drugs* 44: 625–655, 1992

Furukawa K, Itoh I, Kajiwara M, Kitamura K, Suzuki H, Ito Y, Kuriyama H. Effects of 2-nicotinamidoethyl nitrate on smooth muscle cells and on adrenergic transmission in guinea-pig and porcine mesenteric arteries. *J Pharmacol Exp Ther* 218: 260, 1981

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

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

4003, 1989
Goldschmidt M, Landzberg BR, Frishman WH. Nicorandil. A potassium channel opening drug for treatment of ischemic heart disease. *J Clin Pharmacol* 36: 559–572, 1996
Habermann E. Bee and wasp venoms. *Science* 177: 314–322, 1972
Hamilton T, Weston A. Cromakalim, nicorandil and pinacidil: novel drugs which open potassium channels in smooth muscle. *Gen Pharmacol* 20: 1–9, 1989
Hammer R, Giachetti A. Muscarinic receptor subtypes: M1 and M2 biochemical and functional characterization. *Life Sci* 31: 2992–2998, 1982
Heldman E, Levine M, Rabeh L, Pollard HB. Barium ions enter chromaffin cells via voltage-dependent calcium channels and induce secretion by a mechanism independent of calcium. *J Biol Chem* 264: 7914–7920, 1989
Holzmann S. cGMP as a possible mediator of coronary arterial relaxation by nicorandil (SG-75). *J Cardiovasc Pharmacol* 5: 364–370, 1983
Holzmann S, Kukovetz WR, Braida C, Poch G. Pharmacological interaction experiments differentiate between glibenclamide-sensitive potassium channels and cyclic GMP as components of vasodilation by nicorandil. *Eur J Pharmacol* 215: 1–7, 1992
Ilno M. Calcium-induced calcium release mechanism in guinea pig taenia caeci. *J Gen Physiol* 94: 363–383, 1989
Kawai Y, Hayashi Y, Ito I, Kamibayashi T, Takada K, Kagawa K, Yamatodani A, Mashimo T. Nicorandil prevents epinephrine-induced arrhythmias in halothane-anesthetized rats by nitric oxide-dependent mechanism. *Naunyn Schmiedebergs Arch Pharmacol* 366: 522–527, 2002
Kukovetz WR, Holzmann S, Braida C, Poch G. Dual mechanism of the relaxing effect of nicorandil by stimulation of cGMP formation and by hyperpolarisation. *J Cardiovasc Pharmacol* 17: 627–633, 1991
Ladona MG, Aunis D, Gandia AG, Garcia AG. Dihydropyridine modulation of the chromaffin cell secretory response. *J Neurochemstry* 48: 483–490, 1987
Lara B, Zapater P, Montiel C, Teresa de la Fuente M, Martinez-Sierra R, Ballesta JJ, Gandia L, Garcia AG. Density of apamin-sensitive $Ca^{2+}$-dependent $K^+$ channels in bovine chromaffin cells: Relevance to secretion. *Biochem Pharmacol* 49: 1459–1468, 1995
Lim DY, Hwang DH. Studies on secretion of catecholamines evoked by DMPP and McN-A-343 in the rat adrenal gland. *Korean J Pharmacol* 27: 53–67, 1991
Lim DY, Kim CD, Ahn KW. Influence of TMB-8 on secretion of catecholamines from the perfused rat adrenal glands. *Arch Pharm Res* 15: 115–125, 1992
Lim DY, Park GH, Park SH. Inhibitory mechanism of pinacidil on catecholamine secretion from the rat perfused adrenal gland evoked by cholinergic stimulation and membrane depoialrization. *J Auton Pharmacol* 20: 123–132, 2000
Marty A, Neher E. Potassium channels in cultured bovine adrenal chromaffin cells. *J Physiol (Lond.)* 367: 117–141, 1985
Masuda Y, Yoshizumi M, Ishimura Y, Katoh I, Oka M. Effects of the potassium channel openers cromakalim and pinacidil on catecholamine secretion and calcium mobilization in cultured bovine adrenal chromaffin cells. *Biochem Pharmacol* 47: 1751–1758, 1994
Meisheri KD, Cipkus-Dubray LA, Hosner JM, Khan S. Nicorandil-induced vasorelaxation: functional evidence for $K^+$ channel-dependent and cyclic GMP-dependent components in a single vascular preparation. *J Cardiovasc Pharmacol* 17: 903, 1991
Montiel C, Lopez MG, Sanchez-Garcia P, Maroto R, Zapater P, Garcia AG. Contribution of SK and BK channels in the control of catecholamine release by electrical stimulation of the cat adrenal gland. *J Physiol (Lond.)* 486: 427–437, 1995
Murphy KP, Greenfield SA. Neuronal selectivity of ATP-sensitive potassium channels in guinea-pig substantia nigra revealed by responses to anoxia. *J Physiol (Lond)* 453: 167–183, 1992
Nagayama T, Koshika T, Hisa H, Kimura T, Satoh S. Apamin-sensitive SKCa channels modulate adrenal catecholamine release in anesthetized dogs. *Eur J Pharmacol* 327: 135–141, 1997
Nagayama T, Masada K, Yoshida M, Suzuki-Kusaba M, Hisa H, Kimura T, Satoh S. Role of $K^+$ channels in adrenal catecholamine secretion in anesthetized dogs. *Am J Physiol Regulatory Integrative Comp Physiol* 274: R1125–R1130, 1998
Neely A, Lingle CJ. Effects of muscarine on single rat adrenal chromaffin cells. *J Physiol (Lond)* 453: 133–166, 1992
Oka M, Isosaki M, Yanagihara N. Isolated bovine adrenal medullary cells: studies on regulation of catecholamine synthesis and release. In: Usdin E, Kopin IJ, Brachas J ed, *Catecholamines: Basic and Clinical frontiers.* Pergamon Press, Oxford, p 70–72, 1979
Perez-Vizcaino F, Casis O, Rodriguez R, Comez LA, Garcia Rafanell J, Tamargo J. Effect of the novel potassium channel opener, UR-8225, on contractile responses in rat isolated smooth muscle. *Br J Pharmacol* 110: 1165–1171, 1993
Petersen OH, Maruyama Y. Calcium-activated potassium channels and their role in secretion. *Nature* 307: 693–696, 1984
Pierrefiche O, Bischoff AM, Richter DW. ATP-sensitive $K^+$ channels are functional in expiratory neurones of normoxic cats. *J Physiol (Lond)* 494: 399–409, 1996
Quast U, Cook NS. Moving together: $K^+$ channel openers and ATP-sensitive $K^+$ channels. *Trends Pharmacol Sci* 10: 431–435, 1989
Seidler NW, Jona I, Vegh N, Martonosi A. Cyclopiazonic acid is a specific inhibitor of the $Ca^{2+}$-ATPase of sarcoplasimc reticulum. *J Biol Chem* 264: 17816–17823, 1989
Shono M, Houchi H, Oka M, Nakaya Y. Effects of Nitroprusside and Nicorandil on Catecholamine Secretion and Calcium Mobilization in Cultured Bovine Adrenal Chromaffin Cells. *J Cardivasc Pharmacol* 30: 419–423, 1997
Standen NB, Quatly JM, Davies NW, Brayden JE, Huang Y, Nelson MT. Hyperpolarizing vasodilators activate ATP-sensitive $K^+$ channels in arterial smooth muscle. *Science* 245: 177–1780, 1989
Suzuki M, Muraki K, Imaizumi Y, Watanabe M. Cyclopiazonic acid, an inhibitor of the sarcoplasmic reticulum $Ca^{2+}$-pump, reduces $Ca^{2+}$-dependent $K^+$ currents in guinea-pig smooth muscle cells. *Br J Pharmacol* 107: 134–140, 1992
Tallarida RJ, Murray RB. *Manual of Pharmacologic Calculation with Computer Programs.* 2nd ed. Speringer-Verlag, New York, p 132, 1987
Terbush DR, Holz RW. Barium and calcium stimulate secretion from digitonin-permeabilized bovine adrenal chromaffin cells by similar pathways. *J Neurochem* 58: 680–687, 1992
Uceda G, Artalejo AR, de la Fuente MT, Lopez MG, Albillos A, Michelena P, Garcia AG, Montiel C. Modulation by L-type $Ca^{2+}$ channels and apamin-sensitive $K^+$ channels of muscarinic responses in cat chromaffin cells. *Am J Physiol* 266 (Cell Physiol 35): C1432–C1439, 1994
Uceda G, Artalejo AR, Lopez MG, Abad F, Neher E, Garcia AG. $Ca^{2+}$-activated $K^+$ channels modulate muscarinic secretion in cat chromaffin cells. *J Physiol* 454: 213–230, 1992
Uyama Y, Imaizumi Y, Watanabe M. Effects of cyclopiazonic acid, a novel $Ca^{2+}$-ATPase inhibitor on contractile responses in skinned ileal smooth muscle. *Br J Pharmacol* 106: 208–214, 1992
Wada A, Kobayashi H, Arita M, Yanagihara N, Izumi F. Potassium channels in cultured bovine adrenal medullary cells: effects of high K, veratridine and carbachol on 86rubidium efflux. *Neuroscience* 22: 1085–1092, 1987
Wada A, Urabe M, Yuhi T, Yamamoto R, Yanagita T, Niina H, Kobayashi H. Large- and small-conductance $Ca^{2+}$-activated $K^+$ channels: their role in the nicotinic receptor-mediated catecholamine secretion in bovine adrenal medulla. *Naunyn Schmiedebergs Arch Pharmacol* 352: 545–549, 1995
Wakade AR. Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. *J Physiol* 313: 463–480, 1981
Watson S, Abbott A. Receptor Nomenclature Supplement. *Trends Pharmacol Sci* (Suppl):31–33, 1991

Weston AH, Longmore J, Newgreen DT, Edwards G, Bray KM, Duty S. The potassium channel openers: a new class of vasorelaxants. *Blood Vessels* 27: 306–313, 1990

Weston AH, Southerton JS, Bray KM, Newgreen DT, Taylor SG. The mode of action of pinacidil and its analogs P1060 and P1368: Results of studies in rat blood vessels. *J Cardiovasc Pharmacol* 12 (Suppl): S10–S16, 1988

Wu CW, Leung CK, Yung WH. Sulphonylureas reverse hypoxia induced $K^+$ conductance increase in substantia nigra pars reticulata neurones. *Neuro Report* 7: 2513–2517, 1996