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Inhibitory Mechanism of Nicorandil on Catecholamine Secretion from the Rat Adrenal Medulla

조선대학교 대학원

의학과

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흰쥐 부신수질에서 니코란딜의 카테콜아민 분비작용에 대한 억제기전

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이 논문을 의학박사 학위 신청논문으로 제출함.

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CONTENTS

CONTENTS OF FIGURES

<국문 초록>

흰쥐 부신수질에서 니코란딜의 카테콜아민 분비작용에 대한 억제기전

이 은 숙

(지도교수: 임 동 윤)

조선대학교 대학원 의학과

본 연구에서는 흰쥐 적출관류 부신을 이용하여 ATP 민감성 칼륨 (K_{ATP}) 채널 개방약인 nicorandil이 콜린성 흥분 및 막탈분극에 의한 catecholamine (CA) 분비작용에 미치는 영향을 검색하고 그 작용의 본태를 규명코자 본 연구를 시행하여 다음과 같은 연구결과를 얻었다.

Nicorandil (0.3 ~ 3.0 mM)을 부신정맥 내로 90 분간 주입하였을 때, acetylcholine (ACh, 5.32 mM), 직접 막탈분극을 일으키는 고농도의 K^+ (56 mM), 선택적인 니코틴 수용체 작동제인 DMPP (100 μM), 선택적인 M_1-무스카린 수용체 작동제인 McN-A-343 (100 μM), 디히드로피리딘계 L형 칼슘통로 개방약인 Bay-K-8644 (10 μM) 및 세포질내 Ca^{2+}-ATPase를 억제하여

유리칼슘을 증가시키는 약물인 cyclopiazonic acid (10 μM)에 의한 CA 분비작용이 비교적 용량 및 시간 의존적으로 억제되었다. 그러나 비특이성 K_{ATP} 차단약인 glibenclamide (1.0 mM)은 위 약물의 CA 분비작용을 오히려 유의하게 증강시켰다. 흥미롭게도, nicorandil (1.0 mM) 과 glibenclamide (1.0 mM)을 90분간 동시 처치하였을 때 ACh, 고농도의 K^+, DMPP, McN-A-343, Bay-K-8644 및 cyclopiazonic acid 의 CA 분비효과가 nicorandil 단독처치 시 나타나는 효과에 비하여 대조치의 상당한 수준까지 회복되었다. 또 다른 K_{ATP} 통로 개방약인 pinacidil (1.0 mM) 존재 하에서도 ACh, 고농도의 K^+, DMPP, McN-A-343, Bay-K-8644 및 cyclopiazonic acid에 의한 CA 분비작용은 뚜렷이 억제되었다.

이와 같은 연구결과를 종합하여 보면, nicorandil은 흰쥐 적출 관류부신에서 콜린성 (니코틴 및 무스카린) 수용체 흥분작용과 막탈분극에 의한 CA 분비효과를 억제하는 반면, glibenclamide는 이들 분비작용을 오히려 증강시키며, 이러한 nicorandil의 억제작용은 흰쥐의 부신 수질 크롬친화세포에서 K_{ATP} 및 small conductance Ca^{2+}-activated K (SK_{ca}) 통로의 활성화를 통하여 세포외칼슘 유입 및 세포질내 칼슘 유리에 대한 직접적인

억제작용에 기인되는 것으로 사료된다. 또한 nicorandil 민감성 K_{ATP} 및 SK_{ca} 통로가 흰쥐 부신수질의 CA 분비작용을 조절하는데 억제 역할을 하는 것으로 생각된다.

I. 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., 1998). 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 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). 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).

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 and 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 (Montiel et al., 1995, Uceda et al., 1994, Uceda et al., 1992) 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 and 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 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 nicorandil, an K_{ATP}-channel activator, glbenclamide, a nonspecific K_{ATP}-channel blocker (Liu et al., 2001), and pinacidil, another K_{ATP}-channel opener, on the secretion of CAs 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^+.

II. 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 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 $\pm 1^{o}$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 μg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of CAs.

Drug administration

The perfusions of DMPP (10^{-4} M) for 2 minutes and/or a single injection of ACh (5.32 x 10^{-3} M) and KCl (5.6 x 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 or pinacidil for 60 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 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 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 and Murray (1987).

Drugs and their sources

The following drugs were used: nicorandil (Choong Wae Pharma. Corp., Korea), glibenclamide, pinacidil, 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.

III. RESULTS

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

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 accelerated 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, did not affect carbachol-induced catecholamine 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 (3×10^{-5} ~ 3×10^{-4} M) 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 (5.32×10^{-3} M) 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 3×10^{-5} ~ 3×10^{-4} M for 90 min relatively concentration- and time-dependently inhibited ACh-stimulated CA secretion. As shown in Fig. 2, 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^+ (5.6×10^{-2} M)-stimulated CA secretion after the pretreatment with 3×10^{-5} M nicorandil was not affected about for 80 min as compared with its corresponding control secretion (100%) (Fig. 3). However, following the pretreatment with higher concentrations of nicorandil (10^{-4} M and 3×10^{-4} M), excess K^+ (5.6×10^{-2} M)-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. 4, DMPP-stimulated CA secretion after pretreatment with nicorandil was reduced to 75% of the control release (100%). McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-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 nicorandil was markedly depressed to 60% of the corresponding control secretion (100%) as depicted in Fig. 5.

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

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 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. 6.

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 nicorandil on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 7. However, in the presence of nicorandil in 7 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was also inhibited to 63% of the control response (174±12 ng for 0-4 min).

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

As shown in Fig. 2 ~ 7, 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 (Quast & Cook, 1989; Ashcroft, 1988), therefore, in order to confirm the effect of nicorandil on CA release, it was likely of interest to examine effect of glibenclamide on the CA secretion evoked by by cholinergic stimulation and membrane depolarization from the isolated perfused rat adrenal glands. In order to test the effect of

glibenclamde on the CA secretion evoked by these secretagogues, 10^{-3} M concentration of glibenclamide was loaded into the perfused rat adrenal medulla.

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 seretory responses evoked by ACh, high K^+, DMPP and McN-A-343 were examined. In the present experiment, ACh (5.32×10^{-3} M)-evoked CA release prior to the perfusion with glibenclamide was 384±13 ng (0-4 min). In the presence of glibenclamide (1.0 mM) for 90 min, it was significantly increased by 120% of the control response, but not affected at 0-34 min period (Fig. 8). High K^+ (56 mM KCl) stimulates CA secretion (166±10 ng, 0-4 min). In the present work, high K^+-evoked CA release in the presence of glibenclamide (1.0 mM) for 90 min was also enhanced by 138% of the corresponding control secretion, as shown in Fig. 9. DMPP (10^{-4} M)-stimulated CA secretion following the loading with glibenclamide (1.0 mM) was greatly potentiated by 122% compared to the corresponding control secretion (346±10 ng, 0-8 min), but not altered at 0-48 min period (Fig. 10). As illustrated in Fig. 11, in the presence of glibenclamide (1.0 mM), McN-A-343-evoked CA secretion was significantly increased by 136% of the corresponding control release (179±9 ng for 0-4 min) without any alteration at 0-49 min period.

Bay-K-8644 (10^{-5} M)-stimulated CA secretion in the presence of glibenclamide was greatly enhanced to 141% of the corresponding control release (154±10 ng for 0-4 min) from 10 rat adrenal glands, as shown in Fig. 12.

As depicted in Fig. 13, in the presence of glibenclamide from 10 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was potentiated to

138% of the control response (166±10 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

It has also been found that, in this study, nicorandil inhibits the CA secretory response evoked by cholinergic stimulation in the perfused rat adrenal gland. 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 membrane 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. 14. 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 60-64 min period in comparison to the control secretion (179±9 ng, 0-4 min) from 10 glands (Fig. 15).

As shown in Fig. 16, 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 2 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. 17.

As shown in Fig. 18-19, 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 and cyclopiazonic acid 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.

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

In the previous experimental results as shown in Fig. 2-7, it was found that nicorandil showed relatively a dose-dependent inhibition in CA secretory responses evoked by cholinergic stimulation and membrane depolarization. Since it has been reported that, in cultured bovine adrenal chormaffin cells, the K^+ channel openers, cromakalim and pinacidil, selectively inhibit CA secretory responses induced by moderate depolarization or by stimulation of nicotinic ACh receptors (Masuda et al., 1994), therefore, it is likely of very interest to examine

the effect of pinacidil on CA secretion evoked by various secretagogues.

CA release evoked by ACh (5.32 mM) and excess K^+ (56 mM) after preloading with 1.0 mM pinacidil for 90 min amounted to 76% and 60% of each corresponding control secretion (411±27 ng for 0-4 min and 160±10 ng for 4 min), respectively as shown in Fig. 20 and 21.

DMPP (100 μM)- and McN-A-343 (100 μM)-stimulated CA releases prior to loading pinacidil were 384±13 ng (0-8 min) and 171±9 ng (0-4 min), respectively. However, after preloading with 1.0 mM pinacidil for 90 min, they were significantly reduced to 77% and 63% of each corresponding control release, respectively, as shown in Fig. 22 and 23. In the presence of 0.1 mM pinacidil, the secretory responses evoked by Bay-K-8644 (10 μM) and cyclopiazonic acid (10 μM) given into the adrenal gland for 4 min were greatly depressed to 65% and 61% of their the corresponding control responses (181±7 ng for 4 min and 166±10 ng for 4 min), respectively (Fig. 24 and 25).

IV. 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, whereas glibenclamide facilitates the CA secretion in response to these secretagogues. It is thought that this inhibitory effect of nicorandil may be mediated by inhibiting influx of extracellular calcium and the release of intracellular calcium through activation of K_{ATP} channels or SK_{Ca} channels in the rat adrenomedullary chromaffin cells. These results also suggest that nicorandil-sensitive K_{ATP} channels or SK_{Ca} 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-innervating 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 and pinacidil 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 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 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, nicorandil-induced inhibitory effect of CA secretion was recovered to the considerable extent of the corresponding control release in comparison with that by nicorandil only.

Subtypes of K^+ channels have been identified in various tissues (Rudy, 1988), including adrenal medullary chromaffin cells (Marty and Neher, 1985). It has been reported that K_A channels, one type of voltage-dependent K^+ channel, are located in sympathetic neurons in the bullfrog (Adams et al., 1982) and the rat (Belluzzi et al., 1985; Galvan and Sedlmeir, 1984). These experiments suggest that K_A channels play an important role in the regulation of neuronal excitability. However, the physiological role of K_A channels in the regulation of adrenal CA secretion has

not been understood. On the other hand, small-conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels, one type of Ca^{2+}-activated K^+ channel, have been characterized by indirect regulation of Ca^{2+} movement and CA secretion in chromaffin cells in the bovine (Lara et al., 1995; Wada et al., 1995) and the cat (Montiel et al., 1995; Uceda et al., 1994; Uceda et al., 1992). It has also been suggested that apamin-sensitive SK_{Ca} channels may play an inhibitory role in the regulation of adrenal CA secretion in the dog (Nagayama et a., 1997; Nagayama et a., 1998). In the present study, nicorandil infused into the adrenal gland inhibited the ACh-and DMPP-induced increase in CA output in a dose-dependent manner without affecting the basal CA output. This indicates that nicorandil influences the secretion process induced by ACh and DMPP but does not stimulate the secretion process by itself. Therefore, the inhibitory effect of nicorandil on the ACh- or DMPP-induced secretion of CA is explained by its inhibitory action on the nicotinic receptor-mediated pathway. Previously, it was demonstrated under the same experimental conditions as in this study that the SNS (splanchnic nerve stimulation)-induced CA secretion is mainly mediated by nicotinic receptors (Kimura et al., 1992; Shimamura et al., 1991). It has also been demonstrated that exogenous ACh causes the secretion of CA by activating both nicotinic and muscarinic receptors (Kimura et al., 1992). Here, the question arises as to why nicorandil influences the nicotinic receptor-mediated CA secretion differently, causing an inhibition in the case of ACh although its effect on SNS-induced CA secretory response was not examined.

The explanation is that intrasynaptic K_A channels in the adrenal medullary cells may play an inhibitory role in the secretion of CA. In support of this idea, it has been reported that K_A channels, one type of voltage-dependent K^+ channel, are

located in sympathetic neurons in the bullfrog (Adams et al., 1982) and the rat (Belluzzi et al., 1985; Galvan and Sedlmeir, 1984). These experiments suggest that K_A channels play an important role in the regulation of neuronal excitability. Activation of nicotinic receptors promotes Na^+ and Ca^{2+} influx through receptor-linked ion channels, and the resulting depolarization produces Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Cena et al., 1983; Garcia et al., 1984). Simultaneously, the depolarization may activate K_A channels, and activated K_A channels increase K^+ efflux, and the resulting facilitation of repolarization may lead to inhibition of further Ca^{2+} influx. As a result, the secretion of CA may be inhibited. Therefore, it is probable that nicorandil inhibits the CA secretion mediated by nicotinic receptors by activating the K_A channel-mediated inhibition of Ca^{2+} influx. Endogenous ACh released from the splanchnic nerves would predominantly activate nicotinic receptors located intrasynaptically. Like this endogenous ACh, exogenous ACh and DMPP given into an adrenal vein could diffuse into extrasynaptic regions and would predominantly activate nicotinic receptors located extrasynaptically. If K_A channels are primarily concentrated in intrasynaptic zones but not in extrasynaptic regions, they could affect the depolarization due to the activation of intrasynaptic nicotinic receptors but could not affect the depolarization due to the activation of extrasynaptic nicotinic receptors. Previously, it was reported that K_A channels are particularly concentrated at the site of synaptic contacts on postsynaptic membranes in rat supraoptic nucleus neurons (Alonso and Widmer, 1997). This finding might support this hypothesis, although there is no available report suggesting synaptic localization of K_A channels in chromaffin cells. However, the CA secretion under physiological conditions is caused by activation of the splanchnic nerves.

Therefore, the physiological role of extrasynaptic K_A channels remains to be resolved.

Nicorandil infused into the adrenal gland inhibited the adrenal CA secretion in response to ACh, DMPP, and McN-A-343 in a dose-dependent manner without affecting the basal CA output. 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 secretion of CA by affecting pathways mediated by both nicotinic and muscarinic receptors but that it does not inhibit the secretion process by itself. In support of this idea, it has been shown that both apamin (Nagayama et a., 1997) and scyllatoxin (Nagayama et a., 1998), SK_{Ca} channel blockers in the anesthetized dog, enhance the CA secretory responses evoked by ACh, DMPP, and muscarine in a dose-dependent manner without affecting the basal CA output. Base on these results, it is thought that nicorandil inhibits the CA secretion mediated by both nicotinic and muscarinic receptors through activation of K_{ATP} channels as well as SK_{Ca} channels, as suggested in the perfused cat adrenal gland (Montiel et al., 1995; Uceda et al., 1994; Uceda et al., 1992) and in bovine adrenal chromaffin cells (Lara et al., 1995). It seems likely that K_{ATP} channels as well as SK_{Ca} channels play an inhibitory role in the adrenal CA secretion mediated by cholinergic receptor stimulation. The elevation of intracellular Ca^{2+} resulting from the activation of nicotinic receptors triggers the CA secretion and simultaneously may activate K_{ATP} channels or SK_{Ca} channels. Increases of K^+ efflux caused by the activation of K_{ATP} channels or SK_{Ca} channels results in hyperpolarization, which leads to inhibition of further Ca^{2+} influx, and the secretion of CA may be inhibited. Therefore, it seems probable that nicorandil inhibits the CA secretion mediated by

nicotinic receptors by activating the SK_{Ca} channel-mediated inhibition of Ca^{2+} influx. On the other hand, the elevation of intracellular Ca^{2+} mobilized from intracellular storage sites is thought to contribute to the muscarinic receptor-mediated secretion of adrenal CA (Harish et al., 1987; Misbahuddin et al., 1985; Nakazato et al., 1988). Furthermore, it has been shown that muscarinic receptor activation depolarizes the adrenal chromaffin cells of chickens (Knight and Baker, 1986), rats (Akaike et al., 1990), and guinea pigs (Inoue and Kuriyama, 1991) and that the secretion of CA induced by methacholine, a pure muscarinic agonist, is potentiated by apamin (Nagayama et a., 1997) and scyllatoxin (Nagayama et a., 1998), but, in the presence of furnidipine, an L-type Ca^{2+} channel blocker, its potentiation disappears (Uceda et al., 1994). In light of these findings, in this study, the inhibitory effect of nicorandil on the muscarinic receptor-mediated secretion of CA can be explained in the same manner as for the nicotinic receptor-mediated secretion.

It has been shown that muscarinic stimulation generates a depolarizing signal which triggers the firing of action potentials, resulting in the increased CA release in the rat chromaffin cells (Akaike et al, 1990; Lim & Hwang, 1991). These observations are in line with a previous report (Ladona et al, 1987; Uceda et al, 1992) showing that Bay-K-8644 almost trippled the peak secretory response to muscarine in perfused Ca^{2+} cat adrenal glands. In this experiment, both nicorandil and pinacidil 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 (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 secretion of CA 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 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 proceding 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 the present work, glibenclamide, a hypoglycemic sulfonylurea which selectively blocks ATP-sensitive K^+ channels (Quast & Cook, 1993; Ashcroft, 1988), 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; 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 nicorandil may inhibit the CA secretory responses at least through activation 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).

On the other hand, glibenclamide is known to exert effects other than inhibition of K_{ATP} channels in other systems. For example, it is a well known inhibitor of Cl^- channels (Liu et al., 1998) and can also block Ca^{2+} channels in

smooth muscle (Sadraei and Beech, 1995), as well as voltage-gated K^+ channels in a human neuroblastoma (Reeve et al., 1992). Additionally, glibenclamide has been suggested to bind to thromboxane A_2 receptors in a species-dependent manner (Kemp and McPherson, 1998). However, such nonspecific effects of glibenclamide are usually observed using high micromolar concentrations, and in no case have such effects been observed to be reduced or reversed by activators of K_{ATP} channels. Furthermore, Eliasson et al. (1996) have reported that sulfonylureas can potentiate secretion of insulin from pancreatic β cells (mediated by Ca^{2+} influx through voltage-gated Ca^{2+} channels) via a mechanism that does not involve K_{ATP} channel inhibition. These authors found these potentiating effects of sulfonylureas to be dependent on protein kinase C (PKC). A subsequent study failed to reproduce these findings (Garcia-Barrado et al., 1996). However, Tian et al. (1998) have indicated a potentiating effect on insulin secretion, but direct PKC activation was not involved. Although Eliasson et al. (1996) did not attempt to reverse the potentiating effects of sulfonylureas with K_{ATP} activators, they speculated that SURs constituted a functional part of a regulatory exocytotic protein.

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 nicorandil inhibits 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,

kromakalim 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.

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, whereas glibenclamide facilitates the CA secretion in response to these secretagogues. It is thought that this inhibitory effect of nicorandil may be mediated by inhibiting influx of extracellular calcium and the release of intracellular calcium through activation of K_{ATP} channels and SK_{Ca} channels in the rat adrenomedullary chromaffin cells. These results suggest that nicorandil-sensitive K_{ATP} channels and SK_{Ca} channels may play an inhibitory role in the regulation of the rat adrenomedullary CA secretion.

V. SUMMARY

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 μM for 2 min), McN-A-343 (a selective muscarinic M_1 receptor agonist, 100 μM for 2 min), Bay-K-8644 (an activator of L-type dihydropyridine Ca^{2+} channels, 10 μM for 4 min) and cyclopiazonic acid (an activator of cytoplasmic Ca^{2+}-ATPase, 10 μM for 4 min). However, glibenclamide (1.0 mM), a nonspecific K_{ATP}- channel blocker, rather significantly enhanced the secretory responses of CA evoked by these secretagogues. In adrenal glands simultaneously preloaded with nicorandil (1.0 mM) and glibenclamide (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 only. In the presence of pinacidil (1.0 mM), which is also known to be a potassium channel activator, CA secretory responses evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid were also significantly depressed.

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, whereas glibenclamide facilitates the CA secretion in response to these secretagogues. It is thought that this inhibitory effect of nicorandil may be mediated by inhibiting influx of extracellular calcium and the release of intracellular calcium through activation of K_{ATP} channels and SK_{Ca} channels in the rat adrenomedullary chromaffin cells. These results suggest that nicorandil-sensitive K_{ATP} channels and SK_{Ca} channels may play an inhibitory role in the regulation of the rat adrenomedullary CA secretion.

REFERENCES

Adams, P.R., Brown, D.A., Constanti, A.. “M-currents and other potassium currents in bullfrog sympathetic neurones.” *J Physiol (Lond.)* ***330:***537-572, 1982.

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.

Alonso, G., Widmer, H.. “Clustering of KV4.2 potassium channels in postsynaptic membrane of rat supraoptic neurons: an ultrastructural study.” *Neuroscience* ***77:***617-621, 1997.

Anton, A.H., Sayre, D.F.. “A study of the factors affecting the aluminum oxidetrihydroxy indole procedure for the analysis of catecholamines.” *J Pharmacol Exp Ther* ***138*****:**360-375, 1962.

Arguilar-Bryan, L., Clement, J.P., Gonzalez, G., Kunjilwar, K., Babenko, A., Bryan, J.. “Toward understanding the assembly and structure of K_{ATP} channels.” *Physiol Rev* ***78*****:**227-245, 1998.

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, F.M.. "Adenosine 5'-triphosphate-sensitive potassium channels." *Annu Rev Neurosci* ***11*****:**763-770, 1988.

Ashford, M.L.J., Sturgess, N.C., Trout, N.J., Gardner, N.J., Hales, C.N.. "Adenosine 5'-triphosphate-sensitive ion channels in neonatal rat cultured central neurones." *Pflügers Arch* ***412*****:**297-304, 1988.

Belluzzi, O., Sacchi, O., Wanke, E.. "A fast transient outward current in the rat sympathetic neuron studied under voltage-clamp conditions." *J Physiol (Lond.)* ***358:***91-108, 1985.

Benndorf, K., Thierfelder, S., Doepner, B., Gebhardt, C., Hirche, H.. "Role of cardiac K-ATP channels during anoxia and ischemia." *News Physiol Sci* ***12:***78-83, 1997.

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

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

Challiss, R.A.J., Jones, J.A., Owen, P.J., Boarder, M.R.. "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, T.R., O'Sullivan, A.J., Moreton, R.B., Berridge, M.J., Burgoyne, R.D.. "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, N.S.. “The pharmacology of potassium channel and their therapeutic potential.” *Trends Pharmacol Sci* ***9*****:**21–28, 1988.

Corcoran, J. J., 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, C.S., Kubo, M., Standen, N.B.. “Potassium channel activation and relaxation by nicorandil in rat small mesenteric arteries.” *Br J Pharmacol* ***125(8):***1715-1725, 1998.

Edwards, G., Duty, S., Trezise, D.J., Weston, A.H.. “Effects of potassium-channel modulators on the cardiovascular system.” In: Weston AH, Hamilton TC, eds. Potassium channel modulators: Phamacological, molecular and clinical aspects. *Oxford Blackwell Science* 369-463, 1992.

Eliasson, L., Renstrom, E., Ammala, C., Berggren, P.O., Bertorello, A.M., Bokvist, K., Chibalin, A., Deeney, J.T., Flatt, P.R., Gabel, J., Gromada, J., Larsson, O., Lindstrom, P., Rhodes, C.J., Rorsman, P.. “PKC-dependent stimulation of exocytosis by sulfonylureas in pancreatic β cells.” *Science* ***271:***813-815, 1996.

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, E.P., Harms, L., Sevick, J., Fischer, H.D., 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, M.M., 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.

Galvan, M., Sedlmeir, C.. "Outward currents in voltage-clamped rat sympathetic neurones." *J Physiol (Lond.)* ***356:***115-133, 1984.

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

Garcia-Barrado, M.J., Jonas, J.C., Gilon, P., Henquin, J.C.. "Sulphonylureas do not increase insulin secretion by a mechanism other that a rise in cytoplasmic Ca^{2+} in pancreatic β cells." *Eur J Pharmacol* ***298*****:**279-286, 1996.

Goeger, D.E., Riley, R.T.. "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, B.R., Frishman, W.H.. "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: M_1 and M_2 biochemical and functional characterization." *Life Sci* ***31*****:**2992-2998, 1982.

Harish, O.E., Kao, L.S., Raffaniello, R., Wakade, A.R., Schneider, A.S.. “Calcium dependence of muscarinic receptor-mediated catecholamine secretion from the perfused rat adrenal medulla.” *J Neurochem* ***48:***1730-1735, 1987.

Heldman, E., Levine, M., Rabeh, L., Pollard, H.B.. “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, W.R., 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.

Inoue, M., Kuriyama, H.. **“**Muscarinic receptor is coupled with a cation channel through a GTP-binding protein in guinea-pig chromaffin cells.” *J Physiol (Lond.)* ***436:***511-529, 1991.

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(6):*** 522-527, 2002.

Kemp, B.K., McPherson, G.A.. “Interspecies differences in thromboxane A2 receptors are distinguished by glibenclamide.” *Eur J Pharmacol* ***354*****:**173-178, 1998.

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

Knight, D.E., Baker, P.F.. **“**Observations on the muscarinic activation of catecholamine secretion in the chicken adrenal.” *Neuroscience* ***19:***357-366, 1986.

Kukovetz, W.R., 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, M.G., Aunis, D., Gandia, A.G., Garcia, A.G.. “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, J.J., Gandia, L., Garcia, A.G.. “Density of apamin-sensitive Ca^{2+}-dependent K^{+} channels in bovine chromaffin cells: Relevance to secretion.” *Biochem Pharmacol* ***49(10):***1459-1468, 1995.

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

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

Lim, D.Y., Park, G.H., Park, S.H.. “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.

Liu, Y., Oiki, S., Tsumura, T., Shimizu, T., Okada, Y.. "Glibenclamide blocks volume-sensitive Cl^- channels by dual mechanisms." *Am J Physiol* ***275*****:**C343-C351, 1998.

Liu, Y., Ren, G., O'Rourke, B., Marban, E., Seharaseyon, J.. "Pharmacological comparison of native mitochondrial K(ATP) channels with molecularly defined surface K(ATP) channels." *Mol Pharmacol* ***59(2):***225-230, 2001.

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(10):***1751-1758, 1994.

Meisheri, K.D., Cipkus-Dubray, L.A., Hosner J.M., 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.

Misbahuddin, M., Isosaki, M., Houchi, H., Oka, M.. **"**Muscarinic receptor-mediated increase in cytoplasmic free Ca^{2+} in isolated bovine adrenal medullary cells. Effects of TMB-8 and phorbol ester TPA." *FEBS Lett* ***190:*** 25-28, 1985.

Montiel, C., Lopez, M.G., Sanchez-Garcia, P., Maroto, R., Zapater, P., Garcia, A. G.. "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, K.P., Greenfield, S.A.. “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 SK_{Ca} 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.

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

Neely, A., Lingle, C.J.. “Effects of muscarine on single rat adrenal chromaffin cells.” *J Physiol (Lond)* ***453:***133-166, 1992.

Noma, A.. “ATP-regulated single K channels in cardiac muscle.” *Nature* ***305*****:** 147-148, 1983.

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

Perez-Vizcaino, F., Casis, O., Rodriguez, R., Comez, L.A., 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, O.H., Maruyama, Y.. “Calcium-activated potassium channels and their role in secretion.” Nature ***307(5953):***693-696, 1984.

Pierrefiche, O., Bischoff, A.M., Richter, D.W.. “ATP-sensitive K^+ channels are functional in expiratory neurones of normoxic cats.” *J Physiol (Lond)* ***494*****:**399-409, 1996.

Quast, U., Cook, N.S.. “Moving together: K+ channel openers and ATP-sensitive K+ channels.” *Trends Pharmacol Sci* ***10*****:**431-435, 1989.

Reeve, H.L., Vaughan, P.F.T., Peers, C.. “Glibenclamide inhibits a voltage-gated K^+ current in the human neuroblastoma cell line SH-SY5Y.” *Neurosci Lett* ***135*****:**137-140, 1992.

Rudy, B.. “Diversity and ubiquity of K channels.” *Neuroscience* ***25:***729-749, 1988.

Sadraei, H., Beech, D.J.. “Ionic currents and inhibitory effects of glibenclamide in seminal vesicle smooth muscle cells.” *Br J Pharmacol* ***115*****:**1447-1454, 1995.

Schmid-Antomarchi, H., Amoroso, S., Fosset, M., Lazdunski, M.. “K^+ channel openers activate brain sulphonylurea-sensitive K^+ channels and block neurosecretion.” *Proc Natl Acad Sci USA* ***87:***3489-3492, 1990.

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

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

1991.

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(4):***419-423, 1997.

Standen, N.B., Quatly, J.M., Davies, N.W., Brayden, J.E., Huang, Y., Nelson, M.T.. “Hyperpolarizing vasodilators activate ATP-sensitive K+ channels in arterial smooth muscle.” *Science* ***245(4914):***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, R.J., Murray, R.B.. "Manual of pharmacologic calculation with computer programs." *2nd Ed New York Speringer-Verlag* 132, 1987.

Terbush, D.R., Holz, R.W.. “Barium and calcium stimulate secretion from digitonin-permeabilized bovine adrenal chromaffin cells by similar pathways.” *J Neurochem* ***58*:**680-687, 1992.

Tian, Y.A., Johnson, G., Ashcroft, S.J.. “Sulphonylureas enhance exocytosis from pancreatic β-cells by a mechanism that does not involve direct activation of protein kinase C.” *Diabetes* ***47*:**1722-1726, 1998.

Uceda, G., Artalejo, A.R., de la Fuente, M.T., Lopez, M.G., Albillos, A., Michelena, P., Garcia, A.G., 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, A.R., Lopez, M.G., Abad, F., Neher, E., Garcia, A.G.. “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(3)*****:**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, A.R.. "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, A.H., Longmore, J., Newgreen, D.T., Edwards, G., Bray, K.M., Duty, S.. "The potassium channel openers: a new class of vasorelaxants." *Blood Vessels* ***27*****:**306-313, 1990.

Weston, A.H., Southerton, J.S., Bray, K.M., Newgreen, D.T., Taylor, S.G.. "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, C.W., Leung, C.K., Yung, W.H.. “Sulphonylureas reverse hypoxia induced K^+ conductance increase in substantia nigra pars reticulata neurones.” *Neuro Report* ***7:***2513-2517, 1996.