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2008년 8월
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Influence of Dihydrexidine on Nicotinic Stimulation-Induced Catecholamine Release in the Rat Adrenal Medulla

조선대학교 대학원

의학과

이재황

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Dihydrexidine 이 흰쥐 부신수질에서 니코틴성 흥분작용에
의한 카테콜아민 유리에 미치는 영향

2008년 8월 25일

조선대학교 대학원

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지도교수 임 경 준

이 논문을 의학박사 학위 신청논문으로 제출함.

2008 년 4 월

조선대학교 대학원

의학과

이재황

이 재 황의 박사학위논문을 인준함

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이 재 황

(지도교수: 임 경 준)

조선대학교 대학원 의학과

도파민 D_1 수용체의 완전 작동제인 dihydrexidine (Lovenberg 등, 1989; Brewster 등, 1990)은 파킨슨질환 및 정신분열증의 치료를 위해 임상적으로 개발 중에 있는 약물이다. Dihydrexidine 의 임상적 개발에도 불구하고 흰쥐의 부신수질에서 카테콜아민 분비에 대한 영향에 대해서 알려진 바가 거의 없다. 따라서 본 연구의 목적은 카테콜아민 (CA)유리에 대한 dihydrexidine 의 도파민 D_1 수용체 작동제효과를 특성화하기 위해서 흰쥐 부신의 적출관류모델을 이용하여 카테콜아민 유리에 대한 dihydrexidine의 작용을 검색하고 그 작용의 본태를 규명하는데 있으며, 본 연구를 시행하여 다음과 같은 결과를 얻었다. Dihydrexidine 은 10-100 μM 을 부신정맥내로 60 분간 관류 시 비교적 용량 및 시간 의존적으로 ACh (5.32×10^{-3} M), 고칼륨 (5.6×10^{-2} M), DMPP (10^{-4} M) 및 McN-A-343 (10^{-4} M)에 의한 CA 분비반응을 유의하게 억제하였다. Dihydrexidine 자체는 기초 CA 분비량에 영향을 미치지 않았다. 또한, 30 μM dihydrexidine 존재

하에서, L 형 칼슘통로 활성화제인 Bay-K-8644 (10^{-5} M), 세포질의 내형질세망막에서 Ca^{2+}-ATPase 억제제인 cyclopiazonic acid (10^{-5} M) 및 나트륨통로 활성화제인 veratridine (10^{-4} M)에 의한 CA 분비반응이 뚜렷이 억제되었다. 그러나 선택성 도파민 D_1 수용체 차단제인 R(+)-SCH23390 (3 μM) 와 dihydrexidine (30 μM) 동시 존재 하에서 ACh, 고칼륨, DMPP 및 McN-A-343, 그리고 Bay-K-8644, cyclopiazonic acid 및 veratridine 의 CA 분비는 dihydrexidine 단독처치 시와 비교하여 대조치의 상당한 수준으로 회복되었다. 이와 같은 연구결과를 종합하여 보면, 흰쥐 적출 관류 부신수질에서 dihydrexidine 은 콜린성(니코틴 및 무스카린 수용체) 흥분작용 및 막탈분극에 의한 CA 분비작용을 유의하게 억제하였다. 이러한 dihydrexidine 의 억제작용은 흰쥐 적출 부신수질의 크롬친화세포에 있는 도파민 D_1 수용체의 흥분을 통해 매개되는 것으로 사료되며, 이는 전압의존성 Na^+ 및 Ca^{2+} 이온통로를 통한 세포내로 나트륨 및 칼슘이온의 유입을 차단하고 세포질내 칼슘저장고로부터 칼슘유리를 억제함으로서 나타나는 것으로 사료된다.

I. INTRODUCTION

The full D_1 receptor agonist dihydrexidine [(+/–)-trans-10,11-dihydroxy-5,6,6a, 7,8,12b-hexahydrobenzo[a]phenanthridine hydrochloride] (Fig. 1) has been available for over 15 years (Lovenberg et al. 1989; Brewster et al. 1990); however, little information is known about the discriminative stimulus effects of this compound despite its clinical development for the treatment of working memory and cognitive deficits in schizophrenia. A previous report demonstrated the ability of dihydrexidine to function as a discriminative stimulus in rats (Schechter 1995). Gleason and Witkin (2004) found that the D_1 receptor partial agonist, SKF 38393, fully substituted and the D_1 receptor antagonist SCH 23390 attenuated the discriminative stimulus effects of dihydrexidine. Furthermore, in rats trained to discriminate SKF 38393 from saline, dihydrexidine produced full SKF 38393-like responding. Recently, it has been known that the full dopaminergic D_1 receptor agonist dihydrexidine produces prominent dopamine D_1 receptor agonist effects in vivo and is likely to produce subjective effects in humans similar to other D_1 receptor agonists (Gleason & Witkin, 2006). A D1-like receptor (the D_1 receptor class is composed of the D_1 and D_5 dopaminergic receptors) has also been identified on bovine chromaffin cells by fluorescence microscopy (Artalejo et al., 1990). They have also found that stimulation of the D_1 receptors activates the facilitation of Ca^{2+} current in the absence of pre-depolarization or repetitive activity, and that activation by D_1 agonists is mediated by cyclic AMP and protein kinase A. The recruitment of facilitation of Ca^{2+} channels by dopamine in bovine chromaffin cells may form the basis of a positive feedback loop mechanism for

secretion of catecholamines [CA] (Artalejo et al., 1990). More recently, the D_1 agonist, SKF-38393, enhanced the number of exocytotic events as did prior exposure of the cell to epinephrine from bovine adrenal chromaffin cells (Villanueva and Wightman, 2007). In contrast to these findings, Dahmer and Senogles (1996) have observed that the D_1-selective agonists CI-APB and SKF-38393 inhibit DMPP-stimulated CA secretion in a concentration-dependent manner. Moreover, in bovine adrenal chromaffin cells, D_1-selective agonists are found to inhibit secretagogue-stimulated Na^+ uptake in a cyclic AMP-independent manner (Dahmer and Senogles, 1996).

It has also been reported that dopamine D_2 receptors are located on bovine chromaffin cells and their activation inhibited release (Bigornia et al., 1990). In chromaffin cells, inhibition of the CA secretion by dopamine agonists has been attributed to D_2 dopamine receptors based on the reversal of inhibition by D_2-selective antagonists such as haloperidol and butaclamol (Artalejo et al., 1985; Bigornia et al., 1988). In addition, binding sites for tritiated antagonists selective for the D_2 family of dopamine receptors were identified on these cells (Gonzalez et al., 1986; Lyon et al., 1987; Quik et al., 1987). At the time, these data were interpreted as evidence that dopamine D_2 receptors on the cells inhibited the CA release. Furthermore, results obtained *in vivo* mostly evidence an inhibitory influence of peripheral dopamine receptors on the CA release from adrenals. Hong et al. (2006) found that R-(-)-TNPA, a dopaminergic D_2 receptor agonist, inhibits catecholamine release from the rat adrenal medulla. It has also been demonstrated that apomorphine dose-dependently inhibits CA secretion by cholinergic receptor stimulation and also by membrane depolarization from the isolated perfused rat adrenal gland (Lim et al., 1994).

However, there is also the view that stimulation of peripherally located dopamine D_2-like receptors can enhance the rate of adrenal catecholamine synthesis in rat adrenal gland by stimulating the activity of tyrosine hydroxylase (Kujacic and Carlsson, 1995).

On the other hand, Huettl and his colleagues (1991) have demonstrated that functional dopamine D_2 receptors of the classical type are not existed on isolated bovine chromaffin cells. It has also been reported that peripheral D_2 receptors are not involved in the control of CA release from the adrenal medulla under in vitro conditions in dogs (Damase-Michel, et al., 1990). Moreover, it has been suggested that bipotential cells obtained from newborn rat adrenal medulla express both isoforms of the D_2 receptor, while D_3 receptor and D_4 receptor messenger RNAs (mRNAs) are not present (Sigala et al., 2000).

Thus, it is clear that there are still many controversial reports about the role of dopaminergic D_1-receptors in the CA release from the adrenal medulla. The purpose of the present study is to examine whether dihydrexidine, a full dopaminergic D_1 receptor agonist, can modify the CA release from the isolated perfused model of the rat adrenal gland, and to clarify its mechanism of action. This is the first report about the influence of dihydrexidine on the CA secretion form the rat adrenal medulla.

II. MATERIALS AND METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 200 to 300 grams, were anesthetized with thiopental sodium (50 mg/kg) intraperitoneally. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by the placement of three-hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37 $\pm 1^{\circ}$C (Fig. 2).

Perfusion of adrenal gland

The adrenal glands were perfused by means of peristaltic pump (ISCO$^{\circledR}$ pump,

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 catecholamines.

Drug administration

The perfusions of DMPP (10^{-4} M) for 2 minutes and/or a single injection of ACh (5.32×10^{-3} M) and KCl (5.6×10^{-2} M) in a volume of 0.05 ml were made into perfusion stream via a three-way stopcock, respectively. McN-A-343 (10^{-4} M), veratridine (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, the secretory responses to ACh, KCl, McN-A-343, veratridine, Bay-K-8644 and cyclopiazonic acid returned to preinjection level in about 4 min, but the responses to DMPP in 8 min.

Collection of perfusate

As a rule, prior to stimulation with various secretagogues, the perfusate was collected for 4 min to determine the spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, collection of the perfusates was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated sample's

perfusate 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 dihydrexidine or R(+)-SCH23390 on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing dihydrexidine or R(+)-SCH23390 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 dihydrexidine or R(+)-SCH23390, 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 (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 (SEM). 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: 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (dihydrexidine), (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol [R(+)-SCH23390], cyclopiazonic acid, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, methyl-1,4-dihydro-2, 6-dimethyl-3-nitro-4-(2 -trifluoro-methyl-phenyl)-pyridine-5 -carboxylate (BAY-K8644), veratridine hydrochloride, (Sigma Chemical Co., U.S.A.), and (3-(m-chloro-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, which was dissolved in 99.5 % ethanol and diluted appropriately with Krebs-bicarbonate solution (final concentration of alcohol was less than 0.1 %). Concentrations of all drugs are expressed in terms of molar base.

III. RESULTS

Effects of dihydrexidine on CA secretion evoked by ACh, high K^+, DMPP and McN-A-343 from the perfused rat adrenal glands

After the perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 21 ± 2 ng for 2 min (n=9). Since in bovine adrenal chromaffin cells, D_1-selective agonists are found to inhibit secretagogue-stimulated Na^+ uptake in a cyclic AMP-independent manner (Dahmer and Senogles, 1996), it was attempted initially to examine the effects of dihydrexidine itself on CA secretion from the perfused model of the rat adrenal glands. However, in the present study, dihydrexidine ($10^{-5} \sim 10^{-4}$ M) itself did not produce any effect on basal CA output from perfused rat adrenal glands (data not shown). Therefore, it was decided to investigate the effects of dihydrexidine on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15 min-intervals. Dihydrexidine was present for 60 minutes after the establishment of the control release.

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 1376 ± 52 ng for 4 min. However, in the presence of dihydrexidine in the range of $10^{-5} \sim 10^{-4}$ M for 60 min, ACh-stimulated CA secretion was inhibited in concentration- and time-dependent fashion. As shown in Fig. 3, in the presence of dihydrexidine, CA releasing responses were inhibited by 42% of the corresponding control release. Also, the depolarizing agent, high potassium markedly stimulated the CA secretion

(649±32 ng for 0-4 min). However, following the pretreatment with dihydrexidine (10^{-5} M ~ 10^{-4} M), high K^+ (5.6 x 10^{-2} M)-stimulated CA secretion was significantly inhibited by 36% of the control at last period (60-64 min) as shown in Fig. 4. DMPP (10^{-4} M), which is a selective nicotinic (N_N) receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (1278±41 ng for 0-8 min). However, as shown in Fig. 5, DMPP-evoked CA secretion after pretreatment with dihydrexidine was greatly reduced to 42% of the control release (100%). McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-receptor agonist (Hammer and Giachetti, 1982), perfused into an adrenal gland for 4 min also caused an increased CA secretion (514±27 ng for 0-4 min). However, in the presence of dihydrexidine, McN-A-343-evoked CA secretion was markedly depressed to 43% of the corresponding control secretion (100%) as depicted in Fig. 6.

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

Since Bay-K-8644 is known to be a calcium channel activator, which enhances basal Ca^{2+} uptake (Garcia et al., 1984) and CA release (Lim et al., 1992), it was of interest to determine the effect of dihydrexidine on Bay-K-8644-evoked CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10^{-5} M)-evoked CA secretion in the presence of dihydrexidine (30 μM) was greatly blocked to 53% of the control at 45-64 min period as compared to the corresponding control release (480±21 ng for 0-4 min) from 8 adrenal glands as

shown in Fig. 7.

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 dihydrexidine on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 8. In the presence of dihydrexidine from 8 adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was also inhibited to 57% of the control response (448±24 ng for 0-4 min).

The voltage-dependent Na^+ channels consist of the principal α-subunit, which is associated with a noncovalently attached $β_1$-subunits, and a disulfide-linked $β_2$-subunit (Catterall, 2000). It has also been known that veratridine-induced Na^+ influx mediated through Na^+ channels increased Ca^{2+} influx via activation of voltage-dependent Ca^{2+} channels and produced the exocytotic secretion of CA in cultured bovine adrenal medullary cells (Wada et al., 1985). To characterize the pharmacological action of dihydrexidine on voltage-dependent Na^+ channels, the effect of dihydrexidine on the CA secretion induced by veratridine was examined here. As shown in Fig. 9, veratridine greatly produced CA secretion (1072±48 ng for 0-4 min). However, in the presence of dihydrexidine (30 μM), veratridine (100 μM)-evoked CA secretion was greatly inhibited to 43% of the corresponding control release.

Effects of dihydrexidine plus R(+)-SCH23390 on CA release evoked by ACh, high K^+, DMPP and McN-A-343 from the perfused rat adrenal glands

As shown in Fig. 3~9, it has also been shown that dihydrexidine inhibits the CA secretory response evoked by cholinergic stimulation in the perfused rat adrenal gland. Therefore, to study the relationship between dopaminergic D_1 receptors and CA release, the effects of R(+)-SCH23390 on dihydrexidine-induced inhibitory responses of CA secretion evoked by cholinergic receptor-stimulation as well as membrane depolarization were examined. In the simultaneous presence of dihydrexidine (30 μM) and R(+)-SCH23390 (3 μM) for 60 min, ACh-evoked CA release was recovered by 79~90% of the corresponding control release compared to results after loading of dihydrexidine alone as illustrated in Fig. 10. High K^+ (56 mM)-evoked CA release in the simultaneous presence of dihydrexidine (30 μM) and R(+)-SCH23390 (3 μM) for 60 min was also recovered by 76~100% of the corresponding control release during all periods in comparison to data of treatment with dihydrexidine alone (Fig. 11).

As shown in Fig. 12, the simultaneous perfusion of dihydrexidine and R(+)-SCH23390 for 60 min got over the DMPP-evoked CA release to 78~90% of the correseponding control response in comparison to that of the dihydrexidine-treatment alone. Moreover, in the presence of dihydrexidine (30 μM) and R(+)-SCH23390 (3 μM), the CA secretory response evoked by McN-A-343 (10^{-4} M for 4 min) was recovered to 73~100% of the corresponding control release compared to results of the dihydrexidine-treatment alone, as shown in Fig. 13.

Effects of dihydrexidine plus R(+)-SCH23390 on CA release evoked by BAY-K-8644, cyclopiazonic acid and veratridine from the perfused

rat adrenal glands

As shown in Fig. 14, the simultaneous perfusion of dihydrexidine (30 μM) and R(+)-SCH23390 (3 μM) for 60 min made the CA release evoked by Bay-K-644 to 73~100% of the corresponding control response compared to the results of dihydrexidine-treatment alone. After the simultaneous perfusion with dihydrexidine and R(+)-SCH23390, cyclopiazonic acid-evoked CA reslease was also recovered by 74~100% of the control release in comparison to the results following the treatment with dihydrexidine alone (Fig. 15). As depicted in Fig. 16, in the simultaneous presence of dihydrexidine and R(+)-SCH23390, veratridine-evoked CA reslease was also recovered by 76~89% of the corresponding control release compared to the results of dihydrexidine-treatment alone.

IV. DISCUSSION

These results obtained from the present study suggest that dihydrexidine inhibits the CA secretion evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization from the rat adrenal medulla. This inhibitory effect of dihydrexidine seems to be mediated by blocking the influx of Na^+ and Ca^{2+} ions through their channels as well as by inhibiting the release of Ca^{2+} from cytoplasmic store through activation of dopaminergic D_1 receptors located on the rat adrenomedullary chromaffin cells, which are relevant to adrenal nicotinic receptor blockade.

In support of the present results, previously Artalejo and his co-workers (1990) found specific binding of the rhodamine conjugate of the D_1 antagonist SCH-23390 to almost all of the cells in chromaffin cell cultured. Because SCH-23390 will bind D_5 receptors as well as D_1 receptors, it is possible, given the results of RNA analysis by Dahmer and Senogles (1996), that D_5-receptors were labeled on the cells. These observations suggested that D_5 receptors on the cells are responsible for inhibition of secretion by D_1-selective agonists. Dahmer and Senogles (1996) have also shown that dopaminergic D_1-selective agonists inhibit secretagogue-stimulated Na^+ uptake into bovine adrenal chromaffin cells in a cyclic AMP-independent manner. Albillos and his colleagues (1992) have reached two conclusions: First, the cat adrenal medulla chromaffin cell possesses a dopamine D_1-receptor that seems to be coupled to an adenylyl cyclase. Second, this receptor regulates the muscarinic-mediated catecholamine release response through a negative feed-back loop which uses cyclic AMP as a second

messenger. In addition, D_1-like receptors have been reported to inhibit secretion (Schoors et al., 1991); however, such a function for members of the D_1 family of dopamine receptors is controversial.

These previous results are consistent with those obtained from the present study. In the simultaneous presence of R(+)-SCH23390 and dihydrexidine, the CA secretory responses evoked by ACh, high K^+, and DMPP were recovered to the similar level of their corresponding control responses compared to results of dihydrexidine-treatment alone. This finding confirms that dihydrexidine inhibits the CA secretory responses evoked by cholinergic stimulation as well as membrane depolarization through activation of inhibitory dopaminergic D_1-receptors on rat adrenal medullary chromaffin cells. Furthermore, electrophysiological studies in awake, behaving monkeys have also shown that iontophoresis of low concentrations of D_1 antagonists enhances memory-related neuronal firing (Williams and Goldman-Rakic, 1995). Infusions of the selective D_1 receptor antagonists R(+)-SCH23390 or SCH39166 into the the prefrontal cortex (PFC) of monkeys (Sawaguchi and Goldman-Rakic, 1991) or rats (Seamans et al., 1995) impaired spatial working memory performance, without altering performance of a control task with identical motor and motivational demands but little mnemonic component (Sawaguchi and Goldman-Rakic, 1991). In terms of these findings, it is plausible that dopaminergic D_1 receptors exist on the rat adrenomedullary chromaffin cells. It has also been reported that, in sinoaortic denervated dogs (i.e. animals deprived from baroreflex pathways), the fenoldopam-induced decrease in arterial blood pressure was more important than in normal dogs (Damase-Michel et al., 1995). Heart rate was unchanged. In these animals, D_1 stimulation induced a decrease in sympathetic tone, as shown by the significant

fall in plasma noradrenaline levels. These "in vivo" data clearly demonstrate the inhibitory role of ganglionic D_1 receptors. Furtheremore, recently, it has also been shown that the full dopaminergic D_1 receptor agonist dihydrexidine produces prominent dopamine D_1 receptor agonist effects in vivo and is likely to produce subjective effects in humans similar to other D_1 receptor agonists (Gleason & Witkin, 2006).

The nicotinic receptor is a neurotransmitter-gated cation-conducting ion channel that is opened by binding of agonists such as ACh and DMPP (McGehee and Role, 1995). The opening of this channel triggers Ca^{2+} uptake and secretion of CA from chromaffin cells (Wada et al., 1985). To determine if the inhibition of DMPP-stimulated secretion by dopaminergic D_1 agonist was due to an effect on the activity of the nicotinic receptor, the effect of dihydrexidine, a full D_1-selective agonist, on DMPP-stimulated CA secretion was examined. As shown in Fig. 5, treatment with dihydrexidine greatly inhibited DMPP-evoked CA secretion, reducing by 42% of the control release. The present data are very similar to the result that C1-APB, a D_1-selective agonist, inhibited DMPP-stimulated Na^+ uptake in bovine chromaffin cells (Dahmer and Senogles, 1996). Previous studies have demonstrated that in bovine adrenal chromaffin cells both D_1- and D_2-selective dopamine receptor agonists inhibit CA secretion and Ca^{2+} uptake stimulated by the nicotinic ACh receptor agonist DMPP (Dahmer and Senogles, I996). Both D_1-and D_2-selective agonists are found to inhibit CA release stimulated by veratridine, an agent that opens voltage-sensitive Na^+ channels (Dahmer and Senogles, 1996). It is likely plausible that dihydrexidine can activate a signal transduction pathway that is altering the activity of both nicotinic receptors and voltage-sensitive Na^+ channels. There have been reports that D_1-like dopamine

receptors are linked to phosphoinositide metabolism (Felder et al., 1989; Andersen et al., 1990; Undie and Friedman, 1990). Activation of such a pathway could result in elevated levels of Ca^{2+}, diacylglycerol, and inositol bisphosphate in the cells. Consequently Ca^{2+}-dependent and protein kinase C-dependent pathways may be activated. Protein kinase C has been reported to attenuate the activity of both nicotinic receptors (Swope et al., 1992) and voltage-sensitive Na^+ channels (Catterall, 1992). It has been proposed that activation of D_1 receptors on chromaffin cells causes activation of facilitation Ca^{2+} channels on the cells in a cAMP-dependent manner (Artalejo et al., 1990). However, Dahmer and Senogles (1996) could find no evidence of message for D_1 dopamine receptors in chromaffin cells by either PCR analysis or northern blot analysis of RNA. In the present study, dihydrexidine, a D_1-selective agonist inhibited the CA secretory responses by high potassium as well as by Bay-K-8644, an activator of L-type Ca^{2+} channels, which facilitates the influx of Ca^{2+} into the cells. The observation that D_1-selective agonists inhibited the CA secretion evoked by Bay-K-8644 was surprising, as Artalejo et al. (1990) have reported that D_1-selective agonists recruit a facilitation Ca^{2+} current in bovine chromaffin cells. Although Ca^{2+} uptake measurements are clearly not the same as measuring Ca^{2+} channel activity, it is difficult to reconcile data indicating that D_1-selective agonists can inhibit Ca^{2+} uptake and facilitate Ca^{2+} channel activity. Again, one possible explanation is that only a subpopulation of chromaffin cells responds to dopamine agonists by recruiting the facilitation channels.

It is unclear how activation of dopamine receptors results in the inhibition of secretion seen in these cells. The simplest interpretation is that the decrease in Ca^{2+} uptake by D_1-selective agonist is responsible for the observed inhibition of

the CA secretion. However, such an interpretation is complicated by the complexity of the relationship between the CA secretion and intracellular free Ca^{2+} levels. Both the intracellular location of the Ca^{2+} level increase (Cheek, 1989; Ghosh and Greenberg, 1995) and the magnitude of the Ca^{2+} level increase (Holz et al., 1982) can affect the relationship between intracellular free Ca^{2+} levels and secretion. Holz et al. (1982) have reported that when Ca^{2+} uptake is large, changes in Ca^{2+} uptake resulted in less than proportional changes in CA secretion. Consequently, although the decrease in Ca^{2+} uptake (influx) into the adrenal chromaffin cells may explain the decrease by dihydrexidine in CA secretion, it is still unclear whether this is only or even most important factor contributing to the inhibition of CA secretion by dopaminergic D_1 agonists. However, in view of the results so far obtained from the present study, it is felt that the voltage-sensitive Ca^{2+} channels located on chromaffin cell membrane of the rat adrenal medulla could be the target site for dihydrexidine-mediated inhibition of CA secretion.

In the present study, dihydrexidine also inhibited the CA secretory responses evoked by cyclopiazonic acid, which is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Goeger & Riley, 1989; Seidler et al., 1989). Therefore, it is felt that the inhibitory effect of dihydrexidine on CA secretion evoked by cholinergic stimulation as well as by membrane-depolarization may be associated with the mobilization of intracellular Ca^{2+} in the chromaffin cells. This indicates that the activation of dopaminergic D_1-receptors has an inhibitory effect on the release of Ca^{2+} from the intracellular pools induced by stimulation of muscarinic ACh receptors, which is weakly responsible for the secretion of CA. In the present work, dihydrexidine time- and

concentration-dependently produced the inhibition of CA secretion evoked by McN-A-343, a selective muscarinic M_1-agonist. This fact suggests new other concept that dihydrexidine can modulate the CA secretory process induced by activation of muscarinic M_1-receptors as well as neuronal nicotinic receptors in the rat adrenal medulla. In supporting this finding, it has been 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 (Suzuki et al., 1992). Moreover, in bovine adrenal chromaffin cells, stimulation of muscarinic ACh receptors is also proposed to cause activation of phosphoinositide metabolism, resulting in the formation of inositol 1,4,5-trisphosphate, which induces the mobilization of Ca^{2+} from the intracellular pools (Cheek et al., 1989; Challiss et al., 1991). However, in the present study, it is uncertain whether the inhibitory effect of the dihydrexidine 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 D_1-receptor activation by dihydrexidine. Based on these previous results, this finding of the present work suggests that the inhibitory dopaminergic D_1-receptors may be involved in regulating CA secretion evoked by muscarinic M_1-receptor stimulation in the rat adrenal medullary chromaffin cells.

On the other hand, Collet and Story (1982) have found that dopamine inhibited the electrically evoked release of [^{3}H] NE from isolated perfused rabbit adrenal glands. This inhibition could be reversed completely by the dopamine D_2 selective antagonist metoclopramide. In the previous experiments, it has been known that metoclopramide enhances the CA secretion in the perfused rat adrenal gland (Lim et al., 1989). Moreover, it has also been demonstrated that

apomorphine causes a dose-dependent inhibition of CA secretion by cholinergic receptor stimulation and also by membaren depolarization from the isolated perfused rat adrenal gland, and this inhibition is depressed by the pretreatment with metoclopramide (Lim et al., 1994). Thus, the inhibition of CA secretory responses through D_2 dopaminergic activation is supported by several previous studies (Gonzalez et al., 1986; Lyon et al., 1987; Quick et al., 1987; Damase-Michel et al., 1999). This inhibitory D_2 dopaminergic effect has been also shown not to interact with D_1 receptors as described previously (Bigornia et al., 1988; 1990). Moreover, Bigornia and his colleague (1990) have demonstrated that, in the same preparation of adrenomedullary samples where significant numbers of D_2 receptors are found there is no statistical significant specific binding of the D_1 receptor ligand, [^{3}H] SCH 23390. Dopaminergic inhibition of CA secretion from adrenal medulla of conscious male beagle dogs is mediated by D_2-like but not by D_1-like dopaminergic receptors (Damase-Michel et al., 1999). Based on these findings as well as the previous results obtained from the rat adrenal medulla (Lim et al., 1994), it is likely clear that dopaminergic D_2 receptors are involved in regulation of the CA secretion from the rat adrenomeduullary chromaffin cells.

In contrast with the present experimental results, Huettl and his colleagues (1991) concluded that pergolide and apomorphine act in a nonreceptor-mediated manner to inhibit CA release from bovine chromaffin cells, and that functional dopaminergic D_2 receptors of the classical type do not exist on isolated bovine chromaffin cells. Because the inhibitory effect of the selective dopaminergic D_2 agonists pergolide as well as apomorphine on CA release from the chromaffin cells was neither reversed nor antagonized by the selective dopaminergic D_2

receptor antagonists such as haloperidol, domperidone, metoclopramide, fluphenazine, flugintixol and sulpiride (Huettl et al., 1991). It has also been shown that stimulation of dopaminergic D_1-receptors activates the facilitation of Ca^{2+} currents in the absence of pre-depolarizations or repetitive activity from bovine chromaffin cells, and that activation by D_1 agonists is mediated by cAMP and protein kinase A (Artalejo et al., 1990). This recruitment of facilitation of Ca^{2+} channels by dopamine may form the basis of a positive feedback loop mechanism that augments CA secretion. In anesthetized dogs, both quinpirole and apomorphine, selective D_2 dopaminergic agonists, did fail to modify release of epinephrine and norepinephrine from the adrenal medulla whatever the stimulation frequencies of the sectioned splanchnic nerve. This fact indicates that peripheral dopaminergic D_2 receptors are not involved in the control of CA release from the adrenal medulla under *in vivo* conditions (Damase-Michel et al., 1990).

Taken together, these experimental results suggest that dihydrexidine inhibits the CA secretion evoked by cholinergic (both nicotininc and muscarinic) receptor stimulation and membrane depolarization from the rat adrenal medulla. It seems that this inhibitory of dihydrexidine is due to activation of dopaminergic D_1 receptors located on the rat adrenomedullary chromaffin cells, which are relevant to the blockade of influx of both Na^+ and Ca^{2+} ions as well as to the inhibition of Ca^{2+} release from the cytoplasmic store.

V. SUMMARY

The full D_1 receptor agonist, dihydrexidine (Lovenberg et al., 1989; Brewster et al., 1990) is under clinical development for Parkinson's disease and schizophrenia. Despite the clinical development of dihydrexidine, very little is known about its stimulus properties to release catecholamines (CA) from the rat adrenal medulla. To more fully characterize D_1 receptor agonist effect of dihydrexidine on the CA release, the present study was designed to investigate the effect of dihydrexidine on the CA release from the perfused model of the rat adrenal gland, and to establish the mechanism of action.

Dihydrexidine (10 ~ 100 µM), perfused into an adrenal vein for 60 min, relatively produced dose- and time-dependent inhibition in the CA secretory responses evoked by ACh (5.32 mM), high K^+ (56 mM), DMPP (100 µM) and McN-A-343 (100 µM). Dihydrexidine itself did fail to affect basal CA output. Also, in adrenal glands loaded with dihydrexidine (30 µM), the CA secretory responses evoked by Bay-K-8644 (10 µM), an activator of L-type Ca^{2+} channels, cyclopiazonic acid (10 µM), an inhibitor of cytoplasmic Ca^{2+}-ATPase, and veratridine, an activator of voltage-dependent Na^+ channels (10 µM), were also markedly inhibited, respectively. However, in the simultaneous presence of dihydrexidine (30 µM) and R(+)-SCH23390 (a selective antagonist of D_1 receptor, 3 µM), the CA secretory responses evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644, cyclpiazonic acid and veratridine were considerably recovered to the extent of the corresponding control secretion compared with the inhibitory responses by dihydrexidine-treatment alone.

In conclusion, these experimental results suggest that dihydrexidine significantly inhibits the CA secretion evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization from the rat adrenal medulla. It seems that this inhibitory effect of dihydrexidine may be mediated by inhibiting influx of both Ca^{2+} and Na^+ into the cytoplasm as well as by suppression of Ca^{2+} release from cytoplasmic calcium store through activation of dopaminergic D_1 receptors located on the rat adrenomedullary chromaffin cells.

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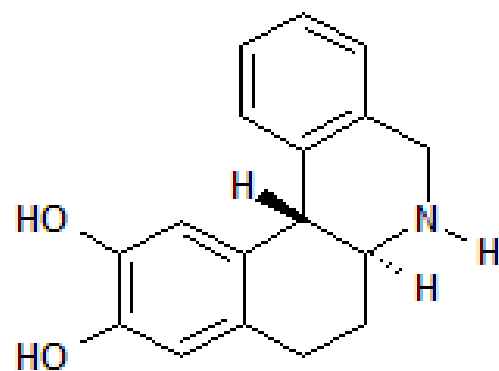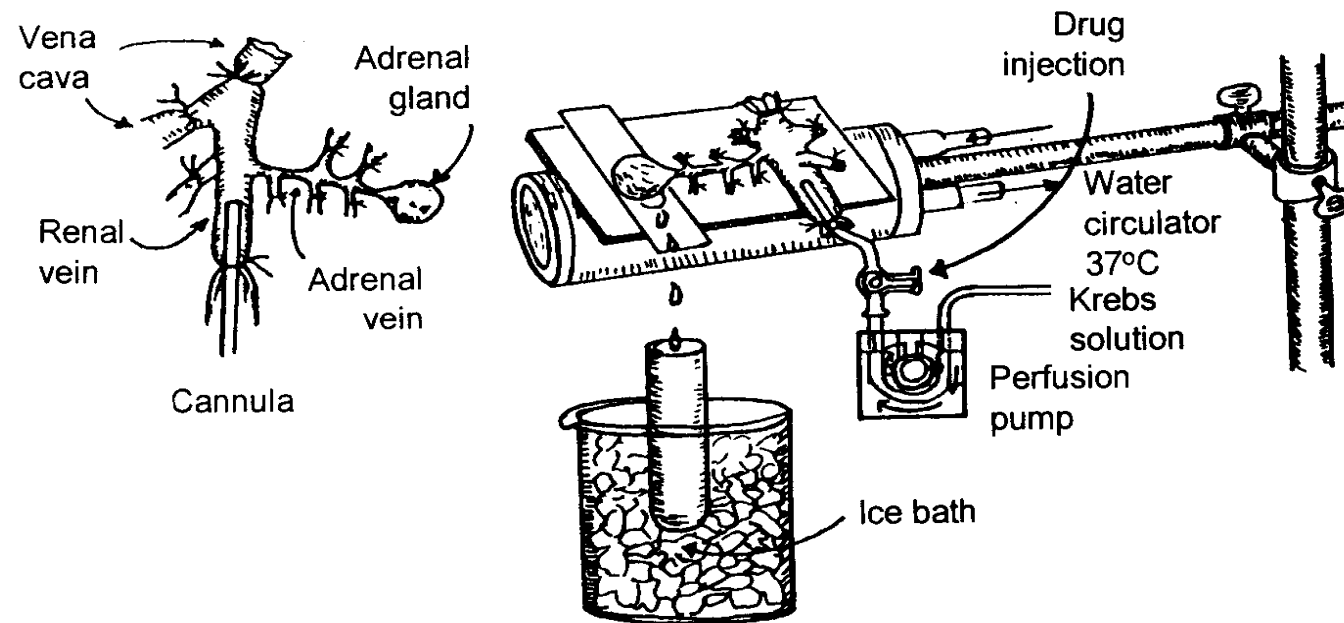
Fig. 1. Chemical structure of dihydrexidine

Fig. 2. Schematic drawing of the preparation used to study secretion of catecholamines in the isolated perfused adrenal gland of the SHRs.
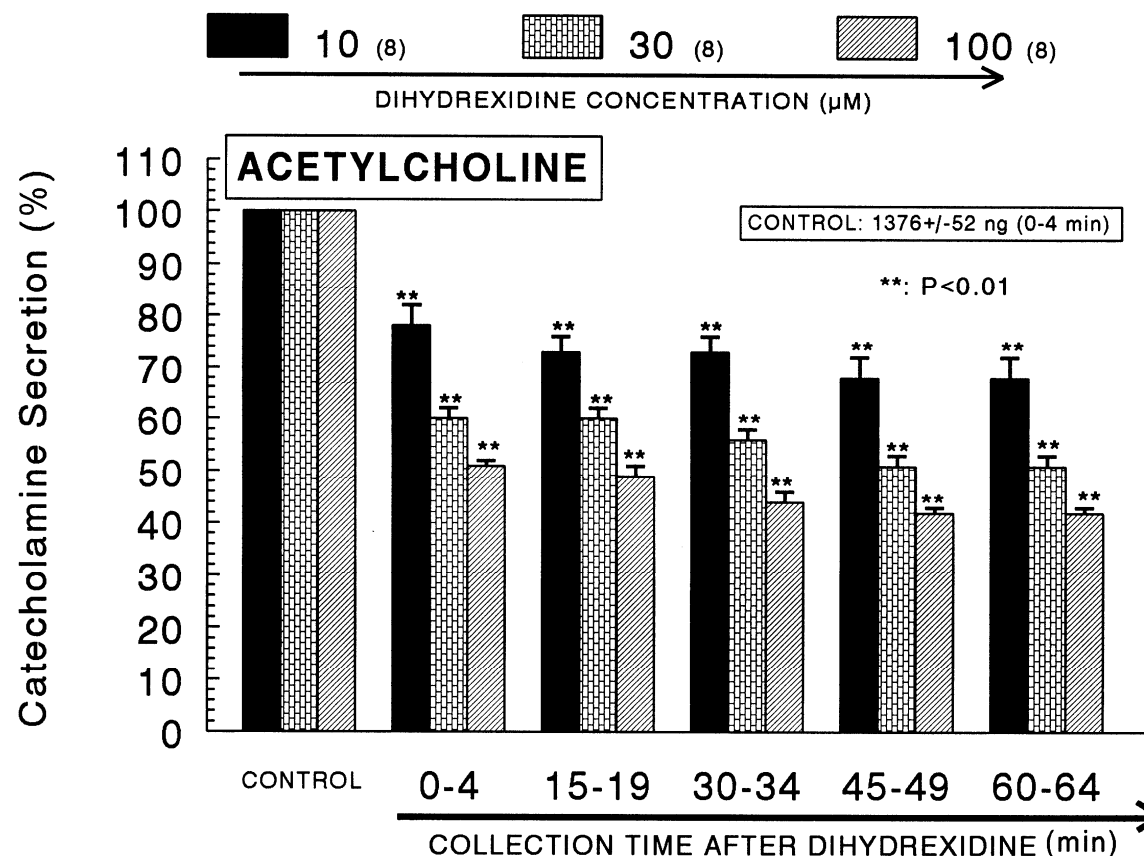
Fig. 3. Dose-dependent effect of dihydrexidine on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh $(5.32 \times 10^{-3}$ M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 10, 30, 100 μM of dihydrexidine for 60 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 for 4 min). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control (CONTROL) with each concentration-pretreated group of dihydrexidine. ACh-induced perfusate was collected for 4 minutes. **: P < 0.01.
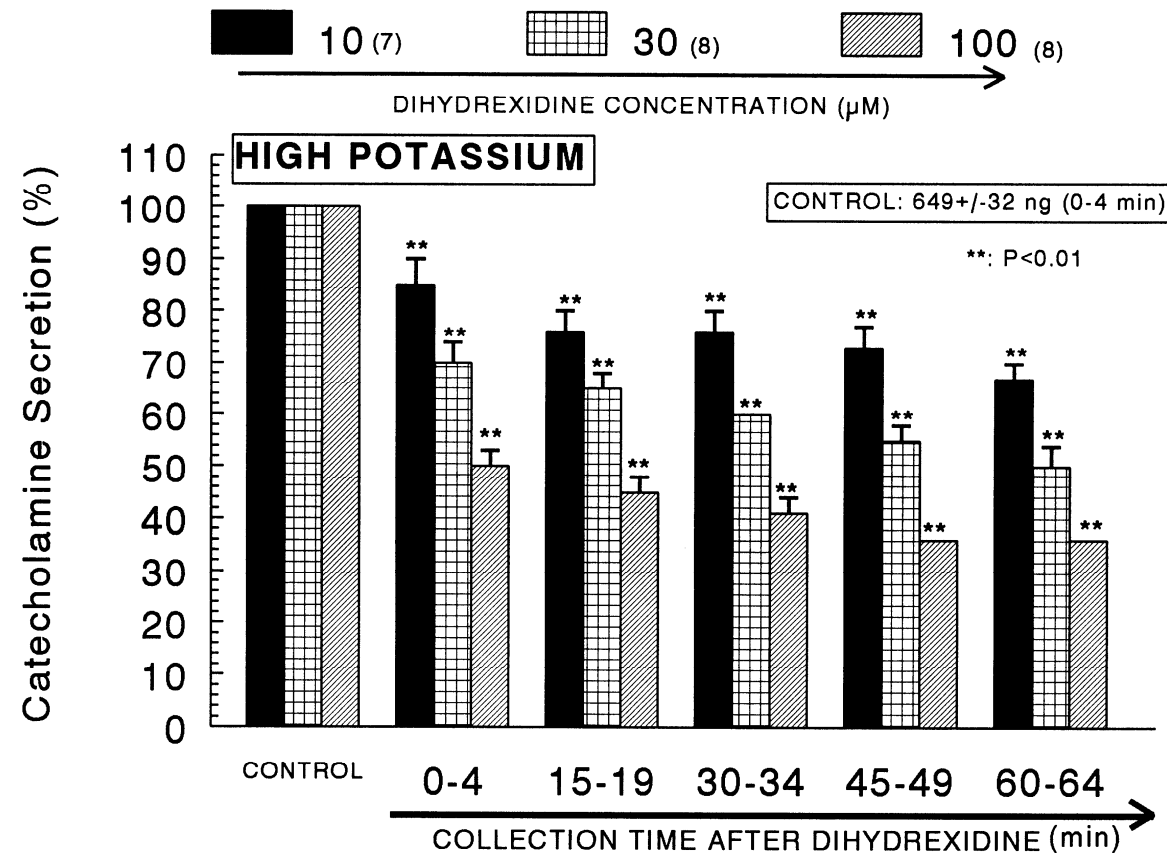
Fig. 4. Dose-dependent effect of dihydrexidine on the secretory responses of catecholamines (CA) evoked by high K⁺ from the isolated perfused rat adrenal glands. CA secretion by a single injection of K^+ (56 mM) was injected in a volume of 0.1 ml at 15 min intervals after preloading with 10, 30, 100μM of dihydrexidine for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control (CONT) with each concentration-pretreated group of dihydrexidine. K^+-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 3. **: P < 0.01.

Fig. 5. Dose-dependent effect of dihydrexidine on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. CA secretion by the perfusion of DMPP (10^{-4} M) was infused for 2 min at 20 min intervals after preloading with 10, 30, 100μM of dihydrexidine for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control with each concentration-pretreated group of dihydrexidine. DMPP-induced perfusate was collected for 8 minutes. Other legends are the same as in Fig. 3. **: P < 0.01.
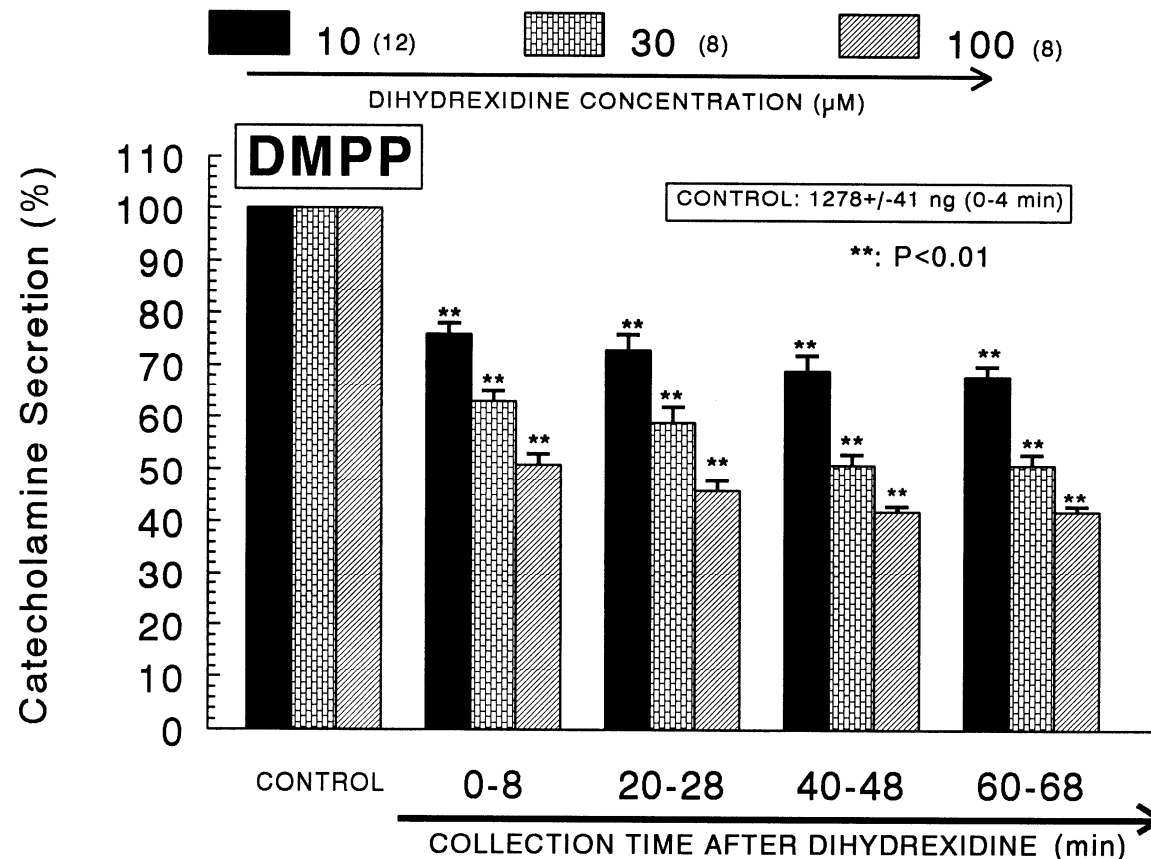
Fig. 6. Dose-dependent effect of dihydrexidine on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. CA secretion by the perfusion of McN-A-343 (10^{-4} M) was infused for 4 min at 15 min intervals after preloading with 10, 30, 100μM of dihydrexidine for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control with each concentration-pretreated group of dihydrexidine. McN-A-343-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 3. *: $P < 0.05$, **: $P < 0.01$.
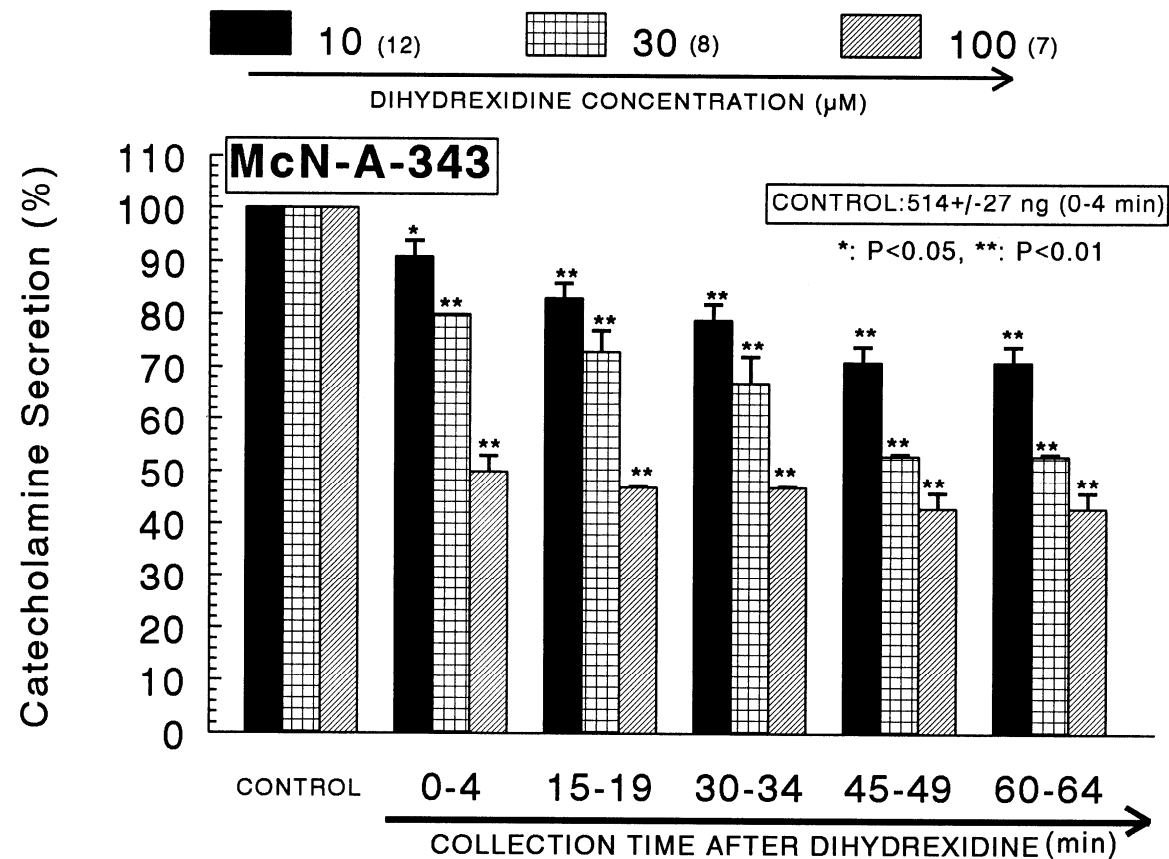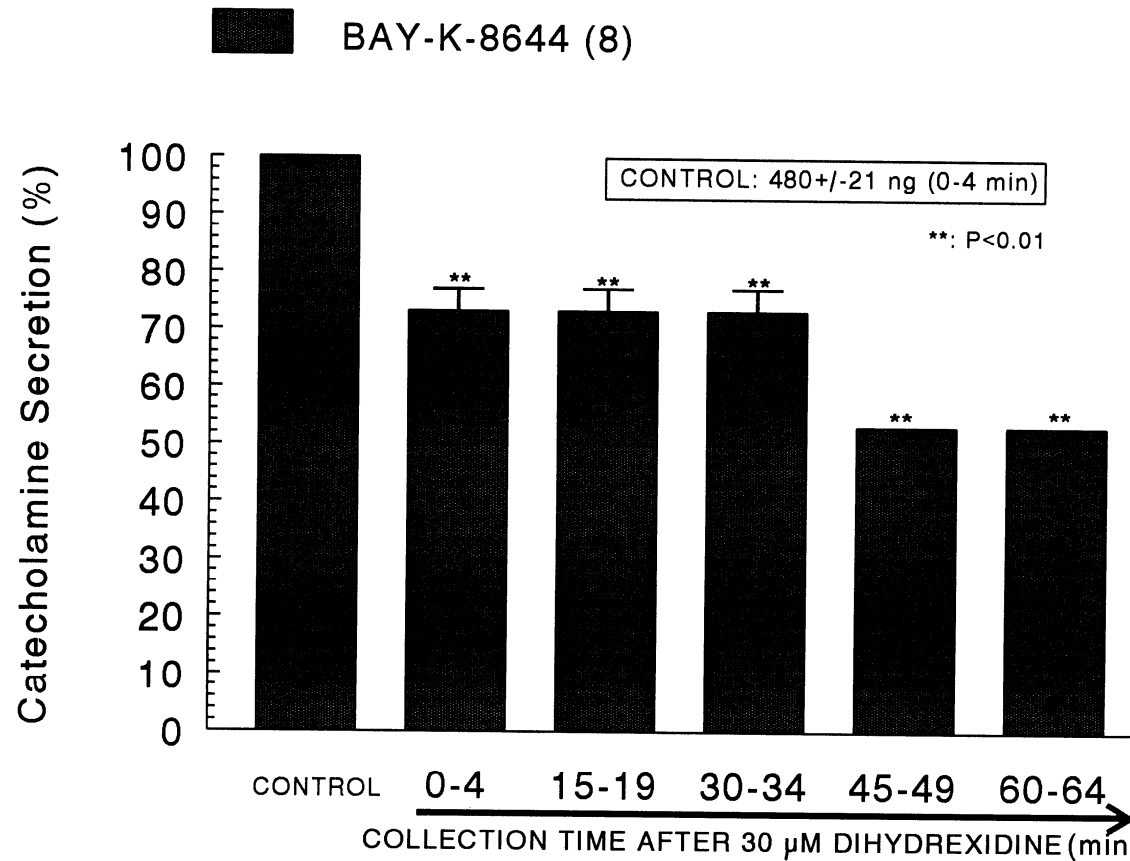
Fig. 7. Time-course effects of dihydrexidine on CA release evoked by Bay-K-8644 from the rat adrenal glands. Bay-K-8644 (10^{-5} M) was perfused into an adrenal vein for 4 min at 15 min intervals after preloading with dihydrexidine (30 μM) for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control with each period after pretreatment with dihydrexidine. Other legends are the same as in Fig. 3. **: P < 0.01.

CYCLOPIAZONIC ACID (8)
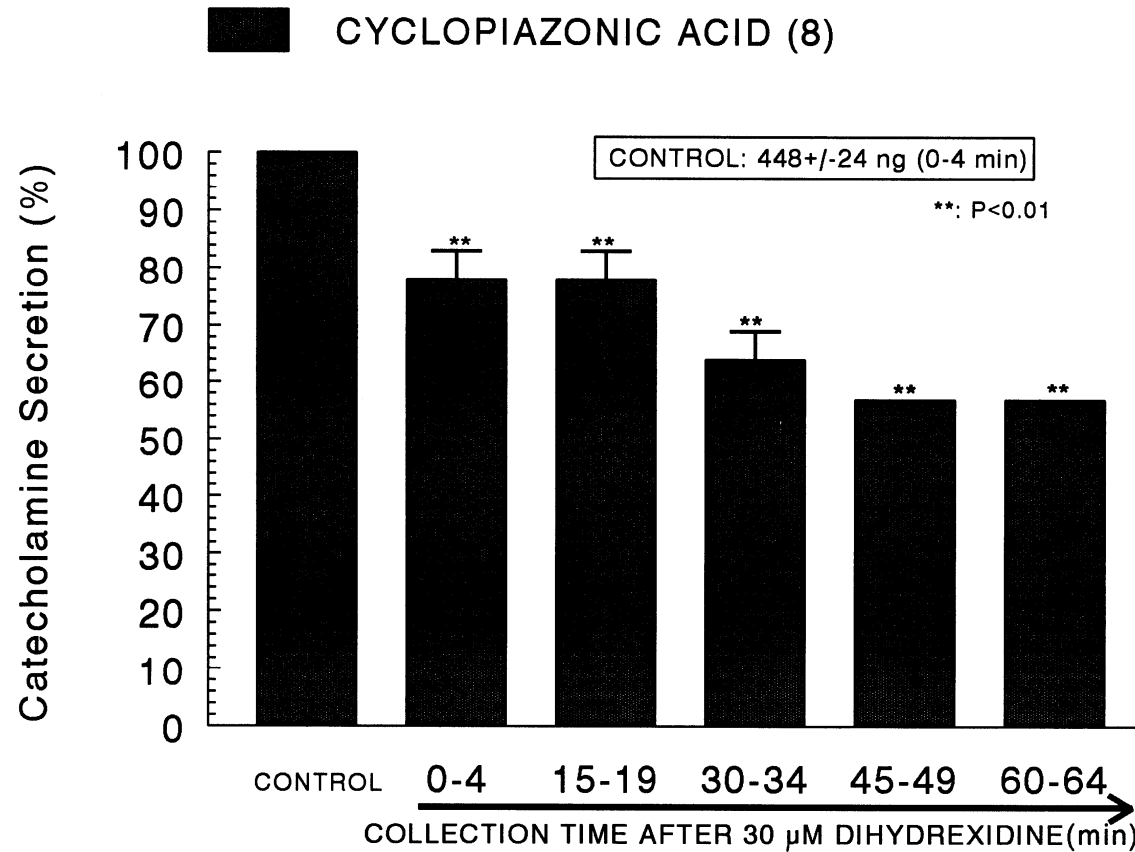
Fig. 8. Time-course effects of dihydrexidine on CA release evoked by cyclopiazonic acid from the rat adrenal glands. Cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with dihydrexidine (30 μM) for 60 min, respectively. Statistical difference was obtained by comparing the corresponding control with each period after pretreatment with dihydrexidine. Other legends are the same as in Fig. 3. **: $P < 0.01$.
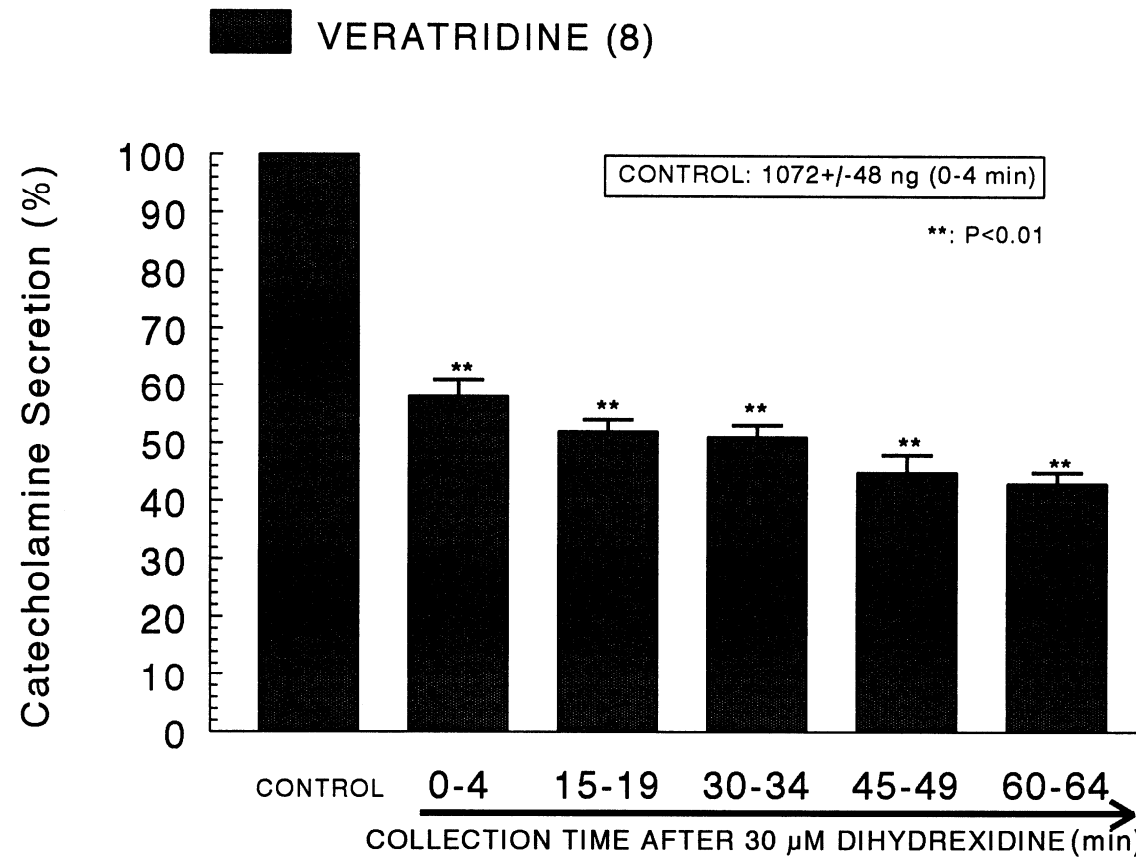
Fig. 9. Time-course effects of dihydrexidine on the CA release evoked by veratridine from the rat adrenal glands. Veratridine (10^{-4} M) was perfused into an adrenal vein for 4 min at 15 min intervals after preloading with dihydrexidine (30 µM) for 60 min, respectively. Other legends are the same as in Fig. 3. **: P < 0.01.
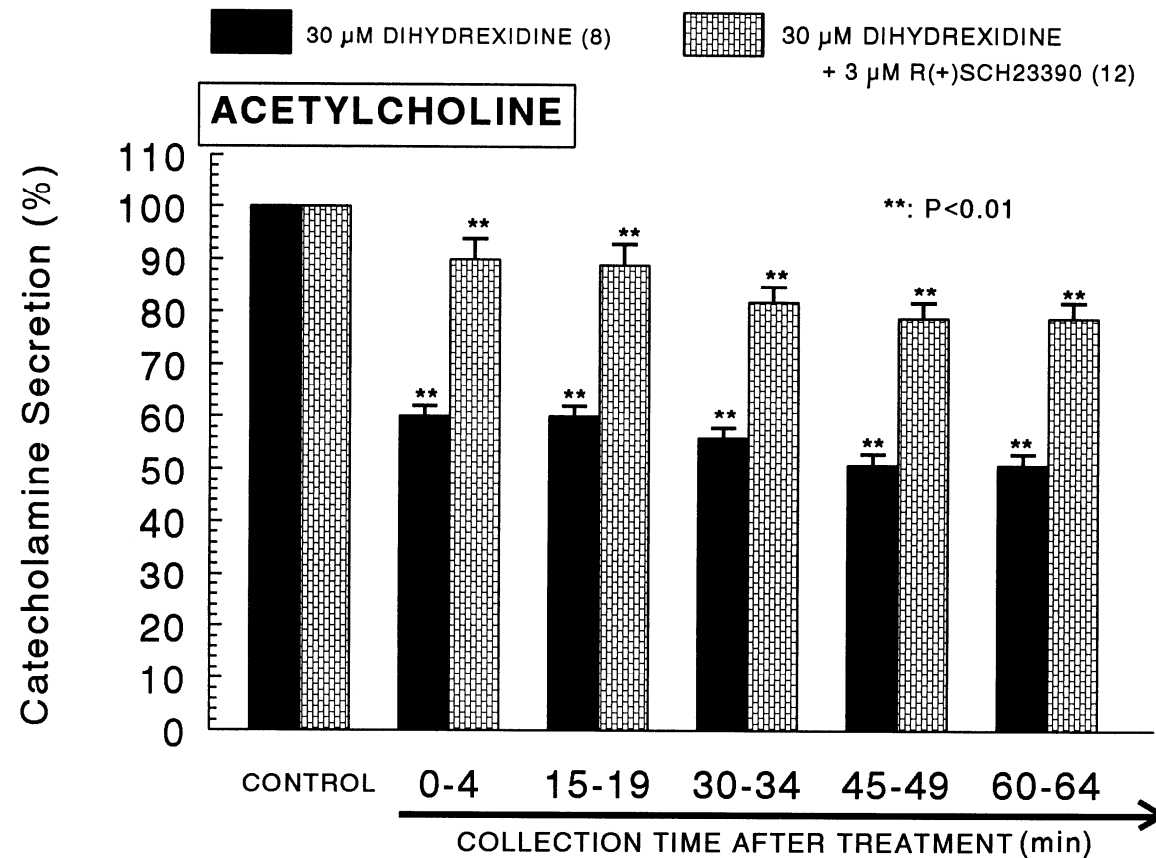
Fig. 10. Effects of dihydrexidine plus R(+)-SCH 23390 on the CA secretory responses evoked by acetylcholine from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml was evoked at 15 min intervals after simultaneous preloading with 30 μM dihydrexidine plus 3 μM R(+)-SCH 23390 for 60 min as indicated at an arrow mark. ACh-induced perfusates were collected for 4 minutes. **: P<0.01.
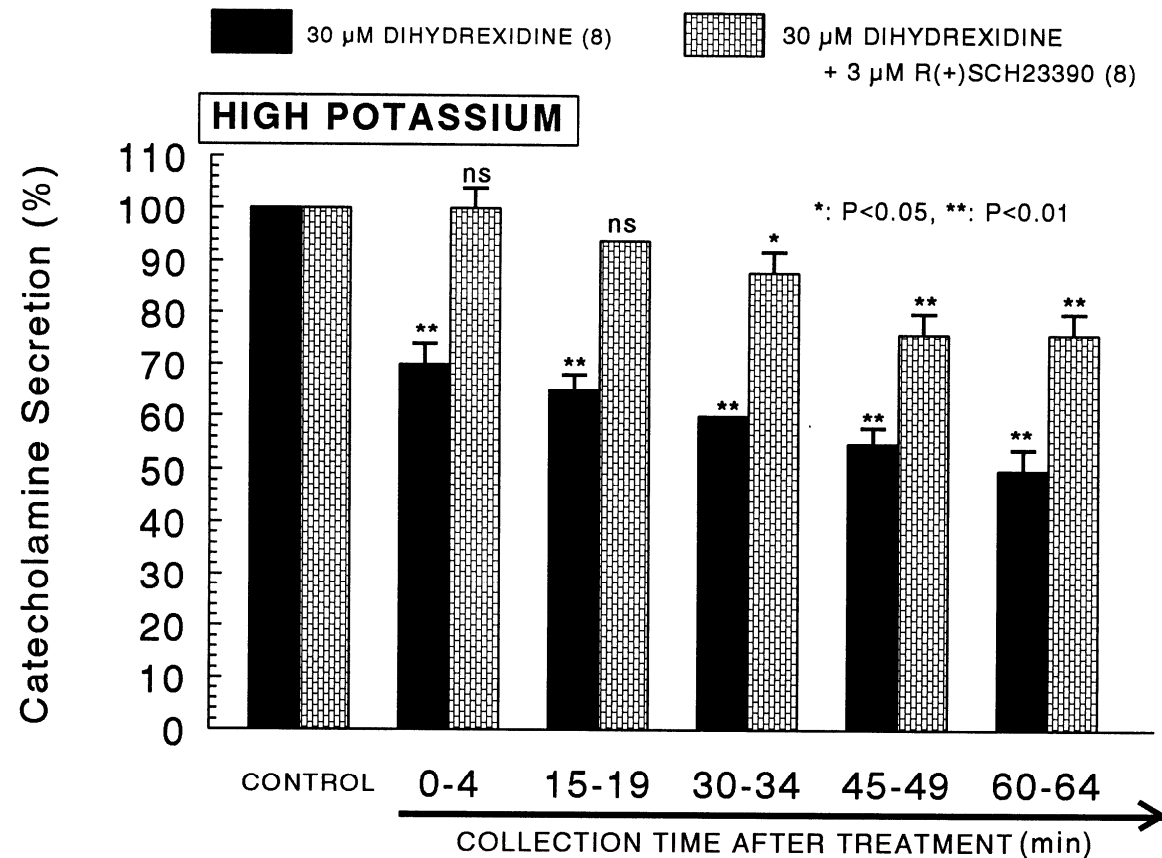
Fig. 11. Effect of dihydrexidine plus R(+)-SCH 23390 on the CA secretory responses evoked by high potassium from the isolated perfused rat adrenal glands. CA secretion by a single injection of high potassium (5.6×10^{-2} M) in a volume of 0.1 ml was evoked at 15 min intervals after simultaneous preloading with 30 μM dihydrexidine plus 3 μM R(+)-SCH 23390 for 60 min as indicated at an arrow mark. Other legends and methods are same as in Fig. 3. High potassium-induced perfusates were collected for 4 minutes. *: P< 0.05. **: P<0.01. ns: Statistically not significant.
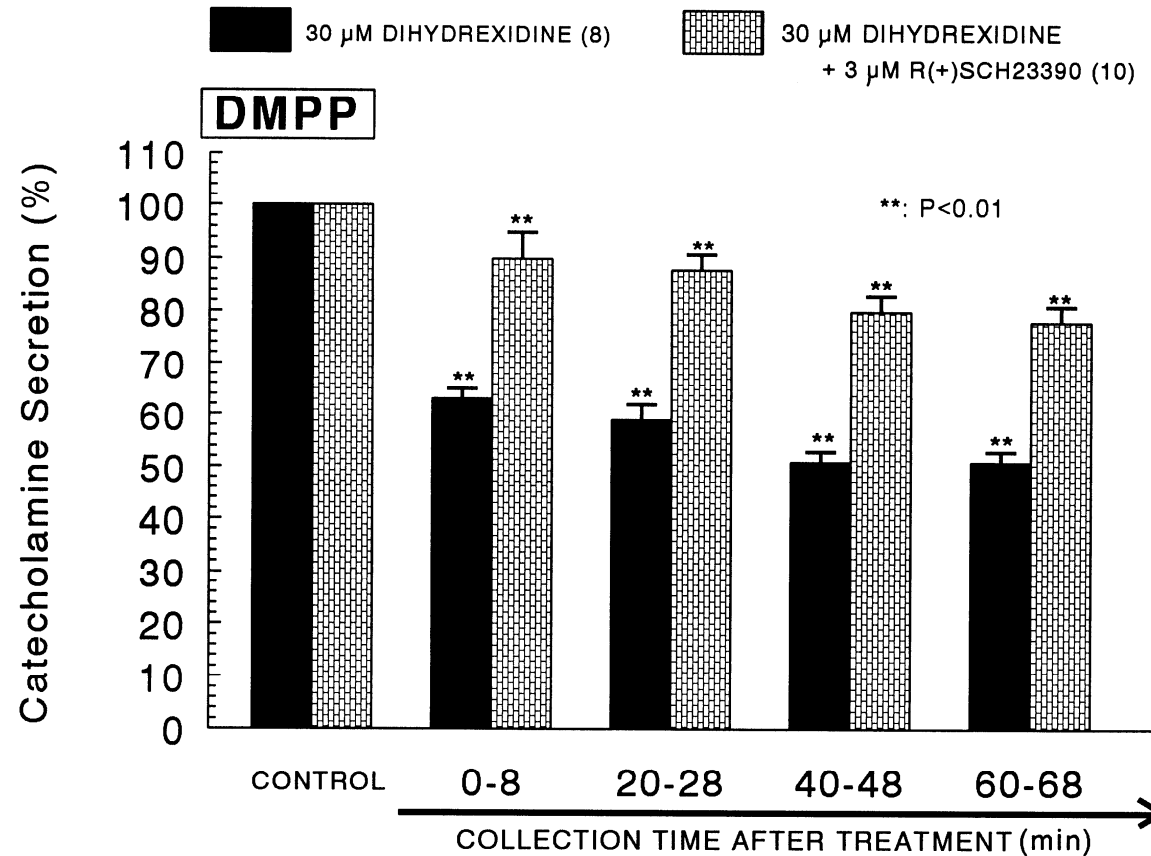
Fig. 12. Effect of dihydrexidine plus R(+)-SCH 23390 on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. CA secretion by the infusion of DMPP (10^{-4} M) for 2 min at 20 min intervals after simultaneous preloading with 30 μM dihydrexidine plus 3 μM R(+)-SCH 23390 for 60 min. DMPP-induced perfusates were collected for 8 minutes. Other legends are the same as in Fig. 3. **: P<0.01.
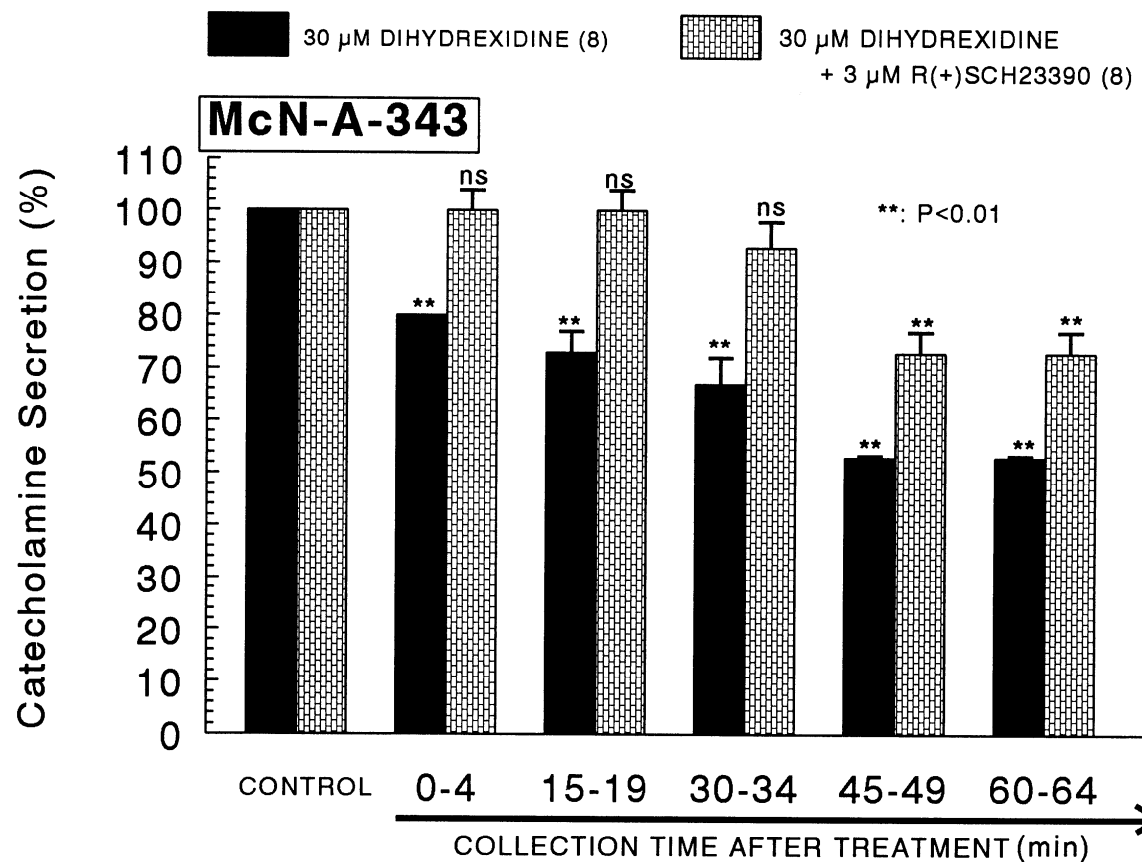
Fig. 13. Effect of dihydrexidine plus R(+)-SCH 23390 on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. CA secretion by the infusion of McN-A-343 $(10^{-4}$ M) for 2 min at 15 min intervals after simultaneous preloading with 30 μM dihydrexidine plus 3 μM R(+)-SCH 23390 for 60 min. McN-A-343-induced perfusates were collected for 4 minutes. Other legends are the same as in Fig. 3. **: P<0.01, ns: Statistically not significant.

Fig. 14. Effect of dihydrexidine plus R(+)-SCH 23390 on catecholamine (CA) release evoked by Bay-K-8644 from the rat adrenal glands. Bay-K-8644 (10^{-5} M) was perfused into an adrenal vein for 4 min at 15 min intervals after simultaneous preloading with 30 μM dihydrexidine plus 3 μM R(+)-SCH 23390 for 60 min. Other legends are the same as in Fig. 3. **: P<0.01, ns: Statistically not significant

Fig. 15. Effect of dihydrexidine plus R(+)-SCH 23390 on catecholamine (CA) release evoked by cyclopiazonic acid from the rat adrenal glands. Cyclopiazonic acid (10^{-5} M) was perfused into an adrenal vein for 4 min at 15 min intervals after simultaneous preloading with 30 µM dihydrexidine plus 3 µM R(+)-SCH 23390 for 60 min. Other legends are the same as in Fig. 3. **: P<0.01. ns: Statistically not significant.

Fig. 16. Effect of dihydrexidine plus R(+)-SCH 23390 on catecholamine (CA) release evoked by veratridine from the rat adrenal glands. Veratridine (10^{-4} M) was perfused into an adrenal vein for 4 min at 15 min intervals after simultaneous preloading with 30 µM dihydrexidine plus 3 μM R(+)-SCH 23390 for 60 min. Other legends are the same as in Fig. 3. **: P<0.01.
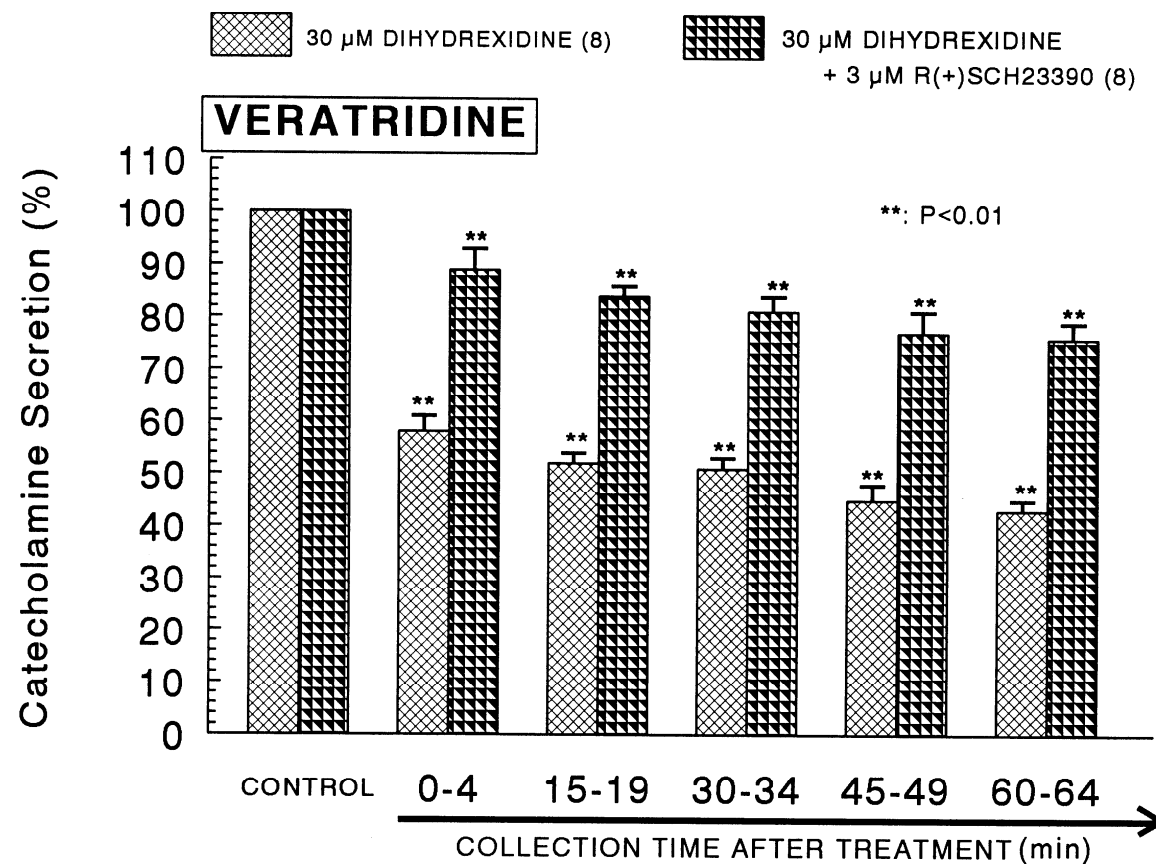
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논문제목	한글 : Dihydrexidine이 흰쥐 부신수질에서 니코틴성 흥분작용에 의한 카테콜아민 유리에 미치는 영향 영어 :Influence of Dihydrexidine on Nicotinic Stimulation -Induced Catecholamine Release in the Rat Adrenal Medulla

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다   음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(O)   반대( )

2008년 6월 일

저작자: 이 재 황   (서명 또는 인)

조 선 대 학 교 총 장 귀 하