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D-Amphetamine Causes Dual Actions on Catecholamine Release from the Rat Adrenal Medulla

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The present study was designed to examine the effect of d-amphetamine on CA release from the isolated perfused model of the rat adrenal gland, and to establish its mechanism of action. D-amphetamine (10~100 μM), when perfused into an adrenal vein of the rat adrenal gland for 60 min, enhanced the CA secretory responses evoked by ACh (5.32×10^{-3} M), excess K^+ (5.6×10^{-2} M, a membrane depolarizer), DMPP (10^{-4} M, a selective neuronal nicotinic N_n-receptor agonist) and McN-A-343 (10^{-4} M, a selective M_1-muscarinic agonist) only for the first period (4 min), although it alone has weak effect on CA secretion. Moreover, d-amphetamine (30 μM) in to an adrenal vein for 60 min also augmented the CA release evoked by BAY-K-8644, an activator of the dihydropyridine L-type Ca^{2+} channels, and cyclopiazonic acid, an inhibitor of cytoplasmic Ca^{2+} ATPase only for the first period (4 min). However, in the presence of high concentration (500 μM), d-amphetamine rather inhibited the CA secretory responses evoked by the above all of secretagogues. Collectively, these experimental results suggest that d-amphetamine at low concentrations enhances the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) as well as by membrane depolarization, but at high concentration it rather inhibits them. It seems that d-amphetamine has dual effects as both agonist and antagonist at nicotinic receptors of the isolated perfused rat adrenal medulla, which might be dependent on the concentration. It is also thought that these actions of d-amphetamine are probably relevant to the Ca^{2+} mobilization through the dihydropyridine L-type Ca^{2+} channels located on the rat adrenomedullary chromaffin cell membrane and the release of Ca^{2+} from the cytoplasmic store.

Key Words: d-Amphetamine, Adrenal medulla, Catecholamine release, Dual actions, Agonist and antagonist at nicotinic receptors

INTRODUCTION

It has been shown that d-amphetamine is a versatile drug that was used in the clinical treatment of such maladies as obesity, narcolepsy and attention deficit/hyperactivity disorders until its addictive potential was fully acknowledged (Seiden et al, 1993). Mundorf et al. (1999) reported that d-amphetamine induces Ca^{2+}-dependent CA release from the cultured bovine adrenal chromaffin cells. Thus, it seems that calcium signaling-related mechanisms might be involved in the actions of d-amphetamine. Dopamine (DA) is released via one of two mechanisms (Raiteri et al, 1979; Richter et al, 1995): (1) vesicular release, which is Ca^{2+}- and impulse-dependent, and (2) transporter-mediated release, which is much less dependent on Ca^{2+} and is impulse-independent (Hurd and Ungerstedt, 1989; Pierce and Kalivas, 1997). Studies have shown that d-amphetamine releases dopamine by means of a carrier-mediated process that depends on a plasmalemmal (Burnette et al, 1996; Sitte et al, 1998; Pifl et al, 1999) or vesicular monoamine transporter (VMAT) (Pifl et al, 1995). However, d-amphetamine-induced dopamine release far exceeds the relatively weak binding capacity of the dopamine transporter (DAT) (Andersen, 1987; Ritz et al, 1987; Wayment et al, 1998; Wu and Gu, 1999). Furthermore, d-amphetamine-induced dopamine release has been shown to have a weak correlation with the DAT density (Laruelle et al, 2000). It has been shown that d-amphetamine reduces the accumulation of monoamines in synaptic vesicle preparations that lack a plasma membrane transporter (Knepper et al, 1988). In adrenal chromaffin cells, which are derived from the same embryonic origin as sympathetic neurons,

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ABBREVIATIONS: CA, catecholamine; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide; DAT, dopamine transporter; VDCCs, voltage-dependent calcium channels; Dopamine (DA); VMAT, vesicular monoamine transporter; DHP, dihydropyridine; BAY-K-8644, ethyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate; McN-A-343, 4-(N-[3-Chlorophenyl] carbamoyloxy)-2-butynyltrimethyl ammonium chloride.

d-amphetamine can induce an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) and a Ca^{2+}-dependent secretion (Mundorf et al, 1999). However, it has been shown that d-amphetamine acts as an ion channel blocker of muscle-type nicotinic receptors (Spitzmaul et al, 1999). Recently, Liu and his colleagues (2003) have reported that d-amphetamine sulfate alone induced an increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) and [^{3}H]norepinephrine release in a dose-dependent and extracellular Ca^{2+}-dependent manner from cultured bovine adrenal chromaffin cells while d-amphetamine sulfate inhibited the DMPP-induced $[Ca^{2+}]_c$ rise and [^{3}H]norepinephrine release, but not the high K^+- or veratridine-induced $[Ca^{2+}]_c$ increase and [^{3}H] norepinephrine release. D-Amphetamine, in both doses used (2.5 and 5 mg/kg) induced a statistically significant decrease in forebrain DOPAC (3,,4-dihydroxyphenylacetic acid) between 30 min and 2 hr, and an increase in adrenal DA in rat adrenal glands (Kujacic and Carlsson, 1994). D-amphetamine has powerful central nervous system stimulant actions in addition to the peripheral α- and β-actions common to indirect-acting sympathomimetic drugs. Using a screening method based on comparison of the molecular structures of organic compounds, d-amphetamine suggested as a candidate to interact with the acetylcholine receptor (AChR) (Barrantes et al, 1999). Thus, there seems to be some controversy in the effect of d-amphetamine on the CA secretion. The present study was designed to investigate the effects of d-amphetamine on CA release evoked by stimulation of nicotinic receptors and by membrane depolarization in the perfused model of the isolated rat adrenal medulla, and also to establish its mechanism of the action.

METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180~300 g, were anesthetized intraperitoneally with thiopental sodium (50 mg/kg). 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. Before ligating vessels and cannulations, heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation. 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. The adrenal gland, along with ligated blood vessels and the cannula, was then carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at $37\pm1^{\circ}C$ (Fig. 1).

Perfusion of adrenal gland

The adrenal glands were perfused by means of a peristaltic pump (WIZ Co.) at a rate of 0.3 ml/min in rats and 0.8 ml/min in rabbits. 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 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 (100 μM) and McN-A-343 (100 μM) for 2 minutes, and Bay-K-8644 (10 μM) for 4 minutes were made into perfusion stream, respectively. A single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml was injected into perfusion stream via a three-way stopcock, 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

Prior to stimulation with various secretagogues, perfusate was routinely collected for 4 to 10 min to determine spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, the perfusates were continuously collected in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated samples were collected for 4 to 10 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 effects of d-amphetamine on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing d-amphetamine for 60 min immediately after the perfusate was collected for a certain minute (background sample). And the medium was then changed to the one containing the stimulating agent, and the perfusates were collected for the same period as that for the background sample. Generally, the adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

CA content of perfusate was fluorospectrophotometrically (Kontron Co. Italy) measured directly by the fluorometric method of Anton and Sayre (1962) without intermediate purification on alumina for the reasons described earlier (Wakade, 1981). A volume of 0.2 ml perfusate was used for the reaction. The CA content in the glands perfusate stimulated by secretogagues in the present work was high enough to obtain several folds greater readings than that of control samples (unstimulated). The sample blanks were also the lowest for perfusates of stimulated and non-stimulated samples. The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statistical analysis

The statistical significance between groups was determined by utilizing the Student's t-test. A P-value of less than 0.05 was considered to represent statistically significant changes, unless specifically noted in the text. Values given in the text refer to means and 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

D-amphetamine sulfate, acetylcholine chloride (ACh), 1.1- dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, ethyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro-methylphenyl)-pyridine-5-carboxylate (BAY-K-8644) and cyclopiazonic acid were purchased from Sigma Chemical Co., U.S.A. (4-[N-(3-Chlorophenyl) carbamoyloxy]-2-butynyltrimethylammonium chloride (McN-A-343) was purchased from RBI Co., U.S.A. Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644, which was dissolved in 99.5 % ethanol and diluted appropriately (final concentration of alcohol was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

Effect of 10~100 μM d-amphetamine on CA secretion evoked by ACh, high K^+, DMPP, and McN-A-343 from the perfused rat adrenal glands

When the adrenal gland was perfused with oxygenated Krebs-bicarbonate solution for 60 min before experimental protocol is initiated, the spontaneous CA secretion reached steady state. The basal CA release from the perfused rat adrenal medulla amounted to 21±2 ng for 2 min from 13 experiments. The CA releasing effects to the initial perfusion of d-amphetamine alone (10^{-6}~5×10^{-4} M) for 60 min into the adrenal vein were negligibly very weak in comparison with the corresponding background release (Data not shown). Recently, it has reported that d-amphetamine sulfate alone induced an increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) and [^{3}H]norepinephrine release in a dose-dependent and extracellular Ca^{2+}-dependent manner from cultured bovine adrenal chromaffin cells (Liu et al, 2003). Therefore, in order to examine the effects of d-amphetamine on CA release, the dose-dependent effects of d-amphetamine on the CA seretory responses evoked by ACh, high K^+, DMPP and McN-A-343 were examined. As illustrated in Fig. 2~5 d-amphetamine at various concentrations (10~100 μM) produced the effective enhancement of CA secretory responses evoked by ACh, DMPP and McN-A-343, although it did not at lower concentrations (<3 μM). In the present experiment, ACh (5.32×10^{-3} M)-evoked CA release prior to the perfusion with d-amphetamine was 463±49 ng (0~4 min). In the presence of d-amphetamine (10 ~100 μM) for 60 min, it was significantly increased by 111~157% only at first 0~4 min, but never affected at 15~64 min in comparison with the corresponding control release (Fig. 1- upper). Also, KCl, a direct membrane-depolarizing agent, sharply stimulates

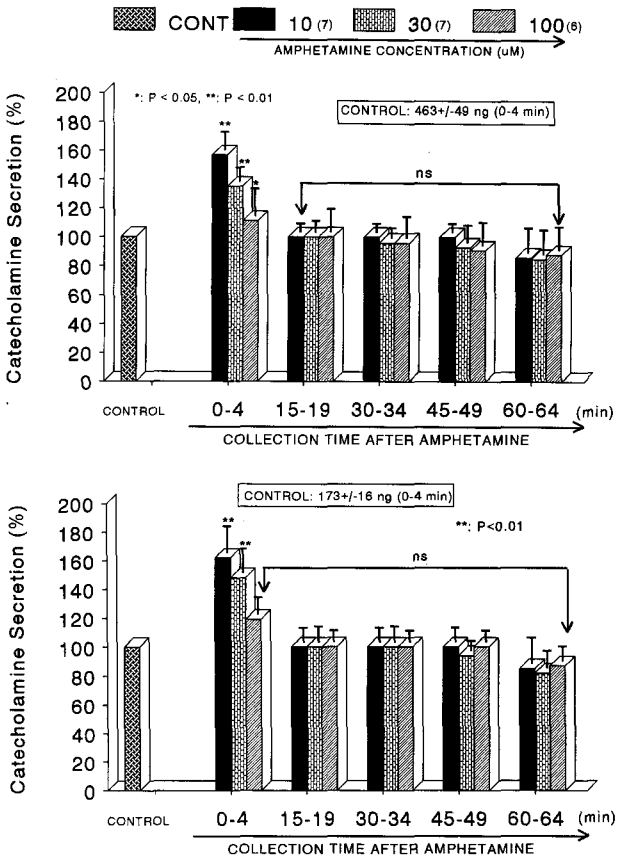

Fig. 1. Dose-dependent effects of d-amphetamine on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh, Upper) and by high K^+ (Lower) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) or K^+ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 10, 30, 100 μM of d-amphetamine for 60 min as indicated with an arrow mark. Numbers in the parenthesis indicate number of rat adrenal glands. Vertical bars on the columns represent the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from the adrenal gland (% of control). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control (CONT) with each concentration-pretreated group of d-amphetamine. Pefusates induced by ACh and high K^+ were collected for 4 minutes, respectively. *: P<0.05, **: P<0.01. ns: Statistically not significant.

CA secretion (173±16 ng, 0~4 min). In the present work, high K^+ (5.6×10^{-2} M)-evoked CA release in the presence of d-amphetamine (10~100 μM) for 60 min was also enhanced by 119~162% only at first 0~4 min in comparison to the corresponding control secretion, as shown in Fig. 1 (lower). DMPP (10^{-4} M), a selective nicotinic receptor agonist in autonomic sympathetic ganglia, when perfused through the rat adrenal gland, evoked a sharp and rapid increase in CA secretion. As shown in Fig. 2 (upper), DMPP (10^{-4} M)-stimulated CA secretion following the loading with d-amphetamine (10~100 μM) was greatly potentiated by 115~146% compared to the corresponding control secretion (338±22 ng, 0~8 min), which was also the peak release only at first 0~8 min. As illustrated in Fig. 2 (lower), McN-A-343 (10^{-4} M), which is a selective

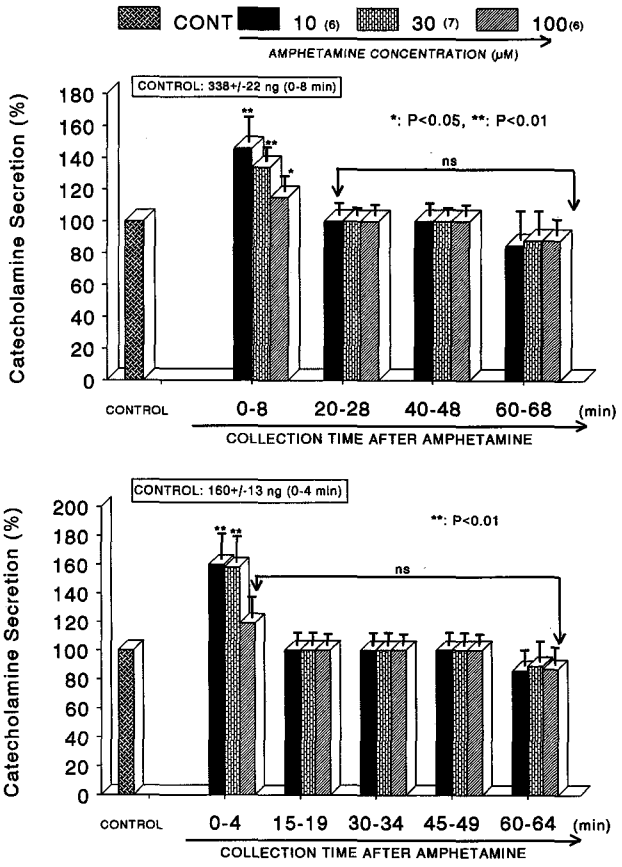

Fig. 2. Dose-dependent effects of d-amphetamine on the secretory responses of catecholamines (CA) evoked by DMPP (Upper) and McN-A-343 (Lower) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DPPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min at 20 and 15 min intervals were induced after preloading with 10, 30, 100 μM of d-amphetamine for 60 min, respectively. Pefusates induced by DMPP and McN-A-343 were collected for 8 and 4 minutes, respectively. Other legends are the same as in Fig. 1. *: P<0.05, **: P<0.01. ns: Statistically not significant.

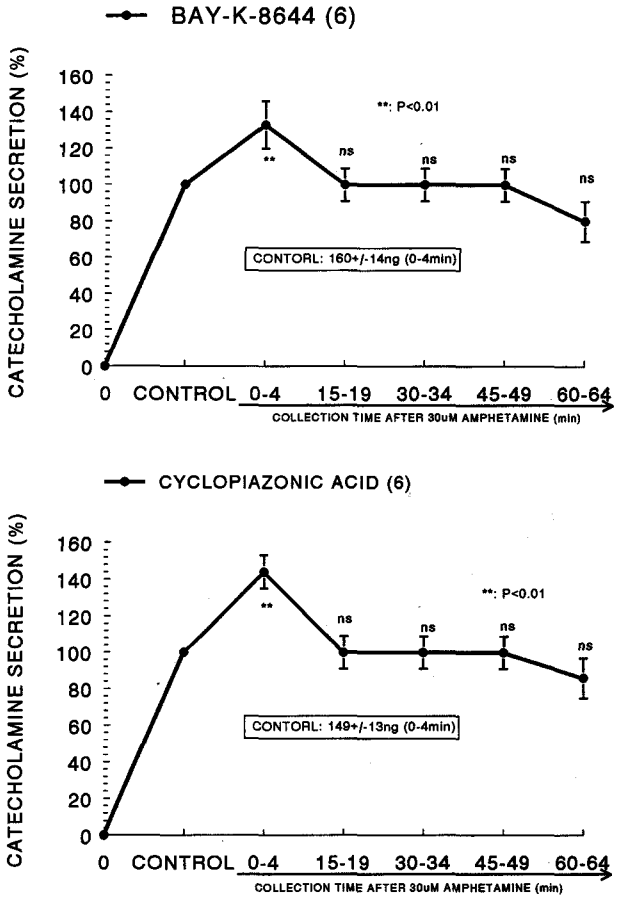

Fig. 3. Effects of d-amphetamine on CA release evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower) from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of d-amphetamine (30 μM) for 60 min, respectively. Other legends are the same as in Fig. 1. **: P<0.01. ns: Statistically not significant.

muscarinic M_1-receptor agonist (Hammer and Giachetti, 1982), perfused into an adrenal vein for 4 min caused an increased CA secretion to 160±13 ng (0~4 min). In the presence of d-amphetamine (10~100 μM), McN-A-343-evoked CA secretion was significantly increased by 119~160% only at first 0~4 min of the corresponding control release.

Effect of 30 μM d-amphetamine on CA secretion evoked by Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

It has been found that Bay-K-8644 is a selective L-type calcium channel activator, which causes positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm et al, 1982; Wada et al, 1985) and enhances basal Ca^{2+} uptake (Garcia et al, 1984) and CA release (Lim et al, 1992). Therefore, it was of interest to determine the effects of d-amphetamine on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. In the absence of d-amphetamine, Bay-K-8644 (10^{-5} M) given into the perfusion stream produced CA secretion of 160±14 ng (0~4 min). However, in the presence of d-amphetamine (10~100 μM), Bay-K-8644-stimulated CA secretion was significantly increased by 133% only at first 0~4 min of the corresponding control secretion, but during the period of 15~64 min it was not affected, as shown in Fig. 3 (upper).

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Goerger & Riley, 1989; Seidler et al, 1989). It may be extremely valuable pharmacological tool for investigating intracellular Ca^{2+} mobilization and ionic current regulated by intracellular calcium (Suzuki et al, 1992). When cyclopiazonic acid (10^{-5} M) was given into the perfusion stream, the CA secreted from the gland amounted to 149± 13 ng for 0~4 min. However, as shown in Fig. 3 (lower), the pretreatment with d-amphetamine (10~100 μM) enhanced cyclopiazonic acid (10^{-5} M)-evoked CA secretion by 144% only at first 0~4 min of the control response (100%).

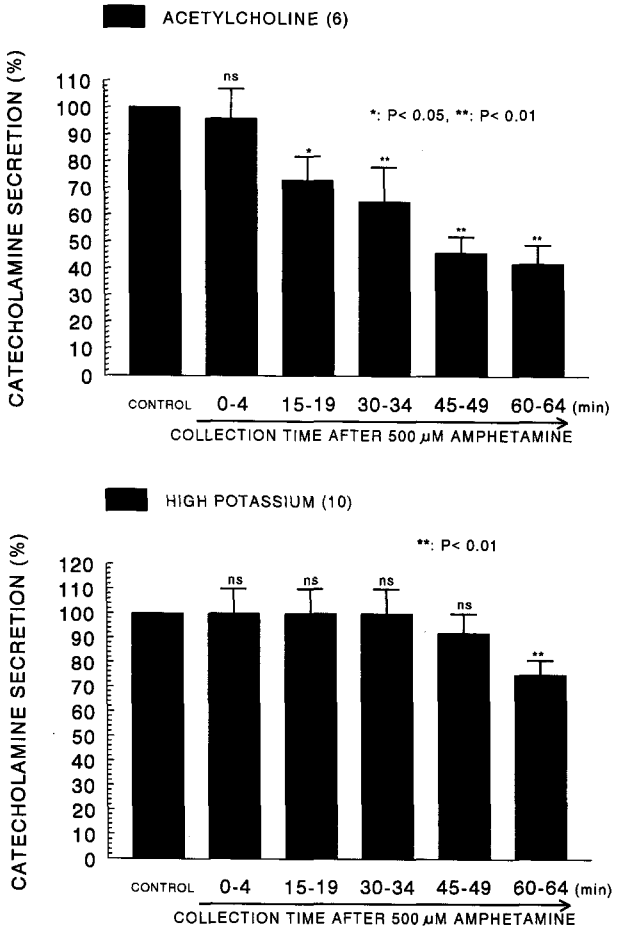

Fig. 4. Time course effect of high dose d-amphetamine on the secretory responses of catecholamines (CA) evoked by acetylcholine (Upper) and by high K^+ (Lower) from the isolated perfused rat adrenal glands. The CA secretory responses by a single injection of ACh (5.32×10^{-3} M) or K^+ (56 mM) in a volume of 0.05 ml were induced before (CONTROL) and after preloading with 500 μM d-amphetamine for 60 min. Perfusate was collected for 4 minutes at 15 min-intervals. Other legends are the same as in Fig. 1. *: $P<0.05$, **: $P<0.01$. ns: Statistically not significant.

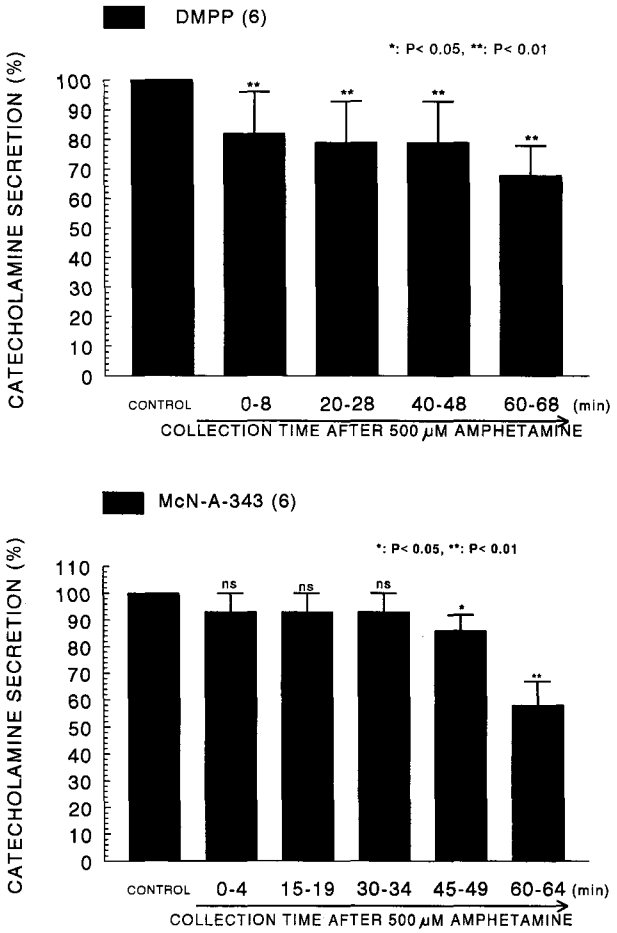

Fig. 5. Time course effect of high dose d-amphetamine on the secretory responses of catecholamines (CA) evoked by DMPP (Upper) and McN-A-343 (Lower) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DPPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min at 20 and 15 min intervals were induced before (CONTROL) and after preloading with 500 μM d-amphetamine for 60 min, respectively. Pefusates induced by DMPP and McN-A-343 were collected for 8 and 4 minutes, respectively. Other legends are the same as in Fig. 1. *: $P<0.05$, **: $P<0.01$. ns: Statistically not significant.

Effect of 500 μM d-amphetamine on CA secretion evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

It has been known that although main action of d-amphetamine is an open-channel blockade, it may also produce, at higher concentrations, a closed channel block (Spitzmaul et al, 1999). Induction of a closed channel block has been reported for other open channel blockers such as ephedrine (Bouzat, 1996; Milone and Engel, 1996), MK-801 (Amador and Dani, 1991) and barbiturates (Dilger et al, 1997). Therefore, it was of interest to study the mechanism of d-amphetamine-induced potentiation on CA secretion in the perfused model of the adrenal gland. As shown in Fig. 4 (upper), in the presence of a high concentration of d-amphetamine (500 μm), ACh (5.32×10^{-3} M)-evoked CA secretory responses were relative time-dependently reduced by maximal 42% of the corresponding control release (100%). However, high K^+ (5.6×10^{-2} M)-evoked CA secretion following the treatment with 500 μM d-amphetamine was inhibited by 75% at 60~64 min period in comparison to the corresponding control responses (100%), although it was not affected for 0~49 min period (Fig. 4-lower). DMPP (10^{-4} M)-evoked CA secretion, a selective neuronal nicotinic receptor agonist, in the presence of 500 μM d-amphetamine was reduced by 68% compared to the corresponding control secretion (100%), which was no enhancement (Fig. 5-upper). As illustrated in Fig. 5 (lower), the CA secretory response of McN-A-343 (10^{-4} M), a selective muscarinic M_1-receptor agonist, in the presence of 500 μM d-amphetamine, was significantly decreased by 58% of the corresponding control release (100%) without the enhancement of the CA release. As shown in Fig. 6, the simultaneous treatment with 500 μM d-amphetamine no longer enhanced the CA secretory responses evoked by Bay-K-8644 and cyclopiazonic acid in comparison to the corresponding control

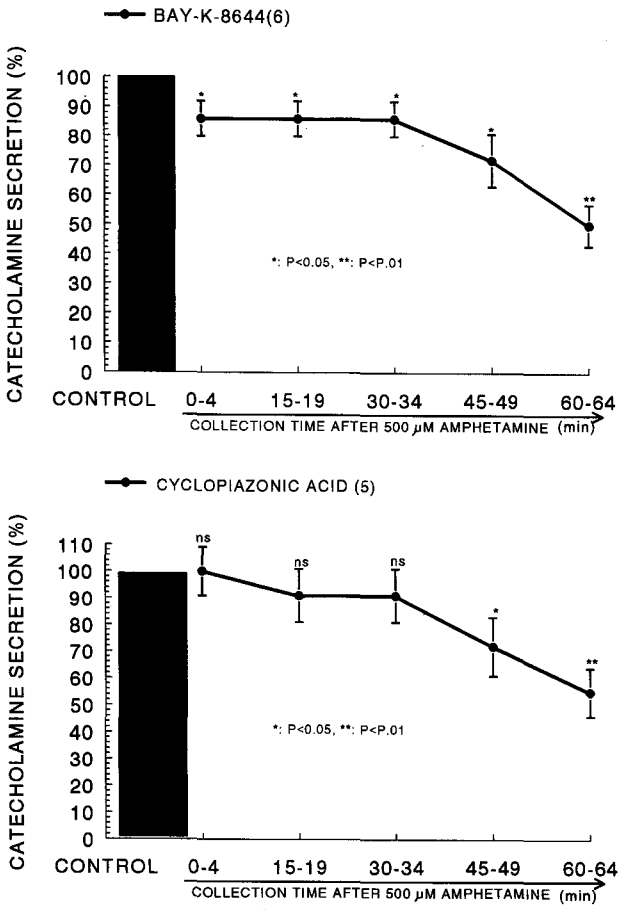

Fig. 6. Time course effect of high dose d-amphetamine on CA release evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower) from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of d-amphetamine (500 μM) for 60 min, respectively. Other legends are the same as in Fig. 1. *: $P<0.05$, **: $P<0.01$. ns: Statistically not significant.

responses (100%), which were rather inhibited by 50% and 55% of the control at later periods, respectively.

DISCUSSION

The present experimental results suggest that d-amphetamine at low concentrations (10~100 μm) enhances the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) as well as by membrane depolarization, but at high concentration (500 μm) it rather greatly inhibits them. It seems that d-amphetamine acts as both agonist and antagonist at nicotinic receptors of the isolated perfused rat adrenal medulla, which are might be dependent on the concentration. It is also thought that these actions of d-amphetamine are probably relevant to the Ca^{2+} mobilization through the dihydropyridine L-type Ca^{2+} channels located on the rat adrenomedullary chromaffin cell membrane and the release of Ca^{2+} from the cytoplasmic store. The effectiveness of d-amphetamine in stimulating the release of endogenous CA, although it was very weak and transient in the rat adrenal gland, may be due to the simultaneous actions of several intracellular mechanisms provoking transmembraneous Ca^{2+} influx, which acts as the most important mediator exocytosis (Kim and Westhead 1989). On the one hand, it has been found that the adrenal medulla possesses characteristics of postganglionic sympathetic neurons, and both L- and N-type voltage-dependent Ca^{2+} channels (VDCCs) have been identified in medullary chromaffin cells (Gandia et al, 1995). Adrenal CA secretion is found to be mediated by muscarinic receptors as well as nicotinic receptors in various species (Harish et al, 1987; Nakazato et al, 1988), including the dog (Kimura et al, 1992).

It has been shown that the influx current of muscle nicotinic receptors is blocked by d-amphetamine sulfate and, therefore, suggested that d-amphetamine sulfate acts as a nicotinic receptor channel blocker (Spitzmaul et al, 1999). Based on this result, in the present study, the findings that d-amphetamine at low concentrations (10~100 μm) enhances the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors), but at high concentration (500 μM) it rather greatly inhibits them suggest that d-amphetamine not only blocked nicotinic receptor responses induced by cholinomimetic agonists, but also induced nicotinic receptor responses. These results, while demonstrating the suggestion of Spitzmaul that d-amphetamine acts as a nicotinic receptor blocker, also support the idea that d-amphetamine acts as a nicotinic receptor agonist. However, the differences between studies of Spitzmaul and the present work may be due to the nicotinic receptor subtypes, tissue cells (cultured chromaffin cells or perfused adrenal medulla) and d-amphetamine derivatives that were used: Spitzmaul et al. used the muscle nicotinic receptor expressed in human embryonic kidney cells, while we used the isolated perfused model of the adrenal glands that do not contain any subtype of muscle nicotinic receptors. In addition, different forms of d-amphetamine were used: d-amphetamine sulfate was used in the present work, while Spitzmaul et al. (1999) used an l-enantiomer of d-amphetamine.

It has been previously reported that d-amphetamine stimulates CA release from perfused isolated cow adrenal gland (Schneider, 1972), cultured bovine adrenal chromaffin cells (Mundorf et al, 1999; Liu et al, 2003) in a manner highly dependent on extracellular Ca^{2+}. Kim and Westhead (1989) have demonstrated that Ca^{2+} entering across the plasma membrane was much more effective at triggering exocytosis than the Ca^{2+} released from internal stores. This led them to claim that Ca^{2+} released from intracellular storage sites does not induce exocytosis. Thus, it is possible that mobilization of Ca^{2+} from extracellular as well as intracellular pools may contribute to CA secretion induced by d-amphetamine. In the present investigation, the results that d-amphetamine enhanced CA secretion evoked by stimulation of muscarinic receptors with McN-A-343, a selective muscarinic M_1-receptor agonist, suggest that Ca^{2+} mobilization from intracellular store by the activation of muscarinic M_1-receptors might be involved in the d-amphetamine-induced enhancement of the CA secretory response in the rat adrenal medulla. In support of this hypothesis, the muscarinic receptor-mediated secretion of adrenal CA has been thought to be caused by Ca^{2+} mobilized from intracellular storage sites (Cheek and Burgoyne, 1987; Nakazato et al, 1988; Misbahuddin and Oka, 1988; Yamada

et al, 1988). Furthermore, 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), guinea pigs (Inoue and Kuriyama, 1991) and the perfused rat adrenal gland (Lim and Hwang, 1991). These observations are in line with a previous report showing that Bay-K-8644 almost trippled the peak secretory response to muscarine in perfused cat adrenal glands (Ladona et al, 1987; Uceda et al, 1994). In the present experiment, d-amphetamine also potentiated the CA secretion induced by Bay-K-8644, which is found to evoke the release of CA by increasing Ca^{2+} influx through L-type VDCCs in chromaffin cells (Garcia et al, 1984). These findings that d-amphetamine potentiated CA secretion evoked by Bay-K-8644 as well as by high K^+, suggest that d-amphetamine activates directly the VDCCs. 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 VDCCs (Burgoyne, 1984; Oka et al, 1979). In the light of this notion, the present finding that d-amphetamine enhances DMPP-evoked CA secretion is thought to be due to the increased Ca^{2+} influx through VDCCs activated by nicotinic ACh receptors, although it at high concentration rather inhibits DMPP-evoked CA secretion. It is felt that the facilitatory effect of d-amphetamine on CA secretory responses evoked by cholinergic stimulation may be associated at least partly with the mobilization of intracellular Ca^{2+} from the cytoplasmic calcium store. This indicates that d-amphetamine increases the release of Ca^{2+} from the intracellular pools induced by stimulation of muscarinic ACh receptors, which is weakly responsible for the secretion of CA. It has been shown that Ca^{2+}-uptake into intracellular storage sites susceptible to caffeine (Ilno, 1989) is almost completely abolished by treatment with cyclopiazonic acid during the proceeding Ca^{2+} load (Suzuki et al, 1992). This is consistent with the findings obtained in skinned smooth muscle fibers of the longitudinal layer of the guinea-pig ileum, 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. 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). In the light of this finding, it is plausible that d-amphetamine enhances the CA secretory responses evoked by stimulation of muscarinic ACh receptors through the mobilization of the intracellular Ca^{2+}. However, in the present study, it is uncertain whether the stimulatory effect of d-amphetamine on Ca^{2+} movement from intracellular pools is due to its direct effect on the PI response or the indirect effects.

D-amphetamine has been shown to inhibit high K^+-stimulated dopamine release from rat synaptosomes (Bowyer et al, 1987) as well as action potential discharges in rat brains (Mercuri et al, 1989). Mahata et al. (1996) found that some vesicular monoamine transport inhibitors (e.g., reserpine) suppress nicotine- and membrane depolarization-induced secretion by inhibiting VDCCs. In the present study, it was shown that d-amphetamine at high concentration inhibited both nicotinic receptor stimulation- and membrane depolarization-evoked CA secretion as well as L-type VDCC-activated CA secretion. However, in cultured bovine chromaffin cells, it has been found that d-amphetamine suppressed nicotinic receptor-associated secretion, but did not significantly affect either high K^+- or veratridine-induced secretion (Liu et al, 2003). It, therefore, seems that the inhibitory effect of d-amphetamine on the nicotinic receptor response involves voltage-gated ion channels. The VDCCs found in various species have different amino acid sequences and different manners of modulation (Catterall, 2000). In chromaffin cells, studies have shown that voltage-gated Ca^{2+} channel activity differs according to species (Hernandez-Guijo et al, 1997; Gandia et al, 1995). Thus, these conflicting results may be the result of differences in tissue type, the d-amphetamine derivative used, and/or type of voltage-gated ion channel. In terms of these findings, the present experimental results suggest that d-amphetamine can act as an antagonist at neuronal nicotinic receptors. In support of this idea, Skau and Gerald (1977) found that d-amphetamine inhibits α-bungarotoxin binding at the neuromuscular junction in mice, and Sulzer et al. (1995) also reported that d-amphetamine suppresses nicotine-induced dopamine release in PC12 cells. In addition, Karler et al (1996) showed that nicotinic antagonists blocked the induction and expression of d-amphetamine sensitization. It has also been found that treatment with either nicotine or d-amphetamine suppresses asphyxia-induced changes, and that d-amphetamine counteracts the inhibitory effects of nicotine on these changes (Chen et al, 1995).

In conclusion, these experimental results suggest that d-amphetamine at low concentrations enhances the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) as well as by membrane depolarization, but at high concentration it rather inhibits them. It seems that d-amphetamine acts as both agonist and antagonist at nicotinic receptors of the isolated perfused rat adrenal medulla, which might be dependent on the concentration. It is also thought that these actions of d-amphetamine are probably relevant to the Ca^{2+} mobilization through the dihydropyridine L-type Ca^{2+} channels located on the rat adrenomedullary chromaffin cell membrane and the release of Ca^{2+} from the cytoplasmic store.

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