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Naltrexone Inhibits Catecholamine Secretion Evoked by Nicotinic Receptor Stimulation in the Perfused Rat Adrenal Medulla

Byung-Sik Yu[1], Seon-Young Min[2], Yoo-Seok Seo[2], Cheol-Hee Choi[2], Eun-Hwa Lee[3], and Dong-Yoon Lim[2]

Departments of [1]Anesthesiology, [2]Pharmacology, College of Medicine, Chosun University, Gwangju 501-759, [3]Department of Medical and Pharmaceuticals, Chodang University, Jeonnam 534-701, Korea

The purpose of the present study was to examine the effect of naltrexone, an opioid antagonist, on secretion of catecholamines (CA) evoked by cholinergic nicotinic stimulation and membrane-depolarization from the isolated perfused rat adrenal gland and to establish the mechanism of its action. Naltrexone (3×10^{-6} M) perfused into an adrenal vein for 60 min produced time-dependent inhibition in CA secretory responses evoked by ACh (5.32×10^{-3} M), high K^+ (5.6×10^{-2} M), DMPP (10^{-4} M) and McN-A-343 (10^{-4} M). Naltrexone itself did also fail to affect basal CA output. In adrenal glands loaded with naltrexone (3×10^{-6} M), the CA secretory responses evoked by Bay-K-8644, an activator of L-type Ca^{2+} channels and cyclopiazonic acid, an inhibitor of cytoplasmic Ca^{2+}-ATPase, were also inhibited. However, in the presence of met-enkephalin (5×10^{-6} M), a well-known opioid agonist, the CA secretory responses evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid were also significantly inhibited. Collectively, these experimental results demonstrate that naltrexone inhibits greatly CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as that by membrane depolarization. It seems that this inhibitory effect of naltrexone does not involve opioid receptors, but might be mediated by blocking both the calcium influx into the rat adrenal medullary chromaffin cells and the uptake of Ca^{2+} into the cytoplasmic calcium store, which are at least partly relevant to the direct interaction with the nicotinic receptor itself.

Key Words: Naltrexone, Opioid receptors, Secretion of catecholamines, Adrenal gland, Nicotinic receptors

INTRODUCTION

Naltrexone and naloxone are selective antagonists for μ-receptors only at low doses; as the doses are increased, κ- and then δ-receptors are occupied. For example, in humans, low doses of naloxone (0.006~0.01 mg/kg), given intravenously, primarily block respiratory depression caused by μ-agonists (Reisine & Pasternak, 1996).

Bovine chromaffin cells co-release opioid peptides together with catecholamines (CA) following nicotinic receptor stimulation (Livett et al, 1981) and their membranes possess μ-, δ- and κ-opioid receptors (Lemaire et al, 1984). The secreted opioid peptides might inhibit nicotine-induced CA secretion from chromaffin cells (Kumakura et al, 1980), but this effect does not seem to be mediated by opioid receptors since opioid antagonists, like naloxone, have the same effect (Dean et al, 1982). Moreover, it has been shown that activation of opioid κ-receptors inhibits nicotine-induced Ca^{2+} entry into chromaffin cells but this effect is not prevented by opioid antagonists like Mr2266 (Bunn & Dunkley, 1991).

Marley & Livett (1987) have found that adrenal opioid peptides from cultured bovine adrenal chromaffin cells probably do not act on adrenal opioid binding sites characterized from ligand binding studies to prevent the nicotinic response from desensitizing. They are unlikely, therefore, to be involved in such a mechanism to maintain CA secretion during stress.

Dermitzaki & his colleagues (2001) have suggested that the suppressive effect of opioids on basal and nicotine-induced CA secretion in the PC12 (rat pheochromocytoma cell line) may result from an opioid-provoked stabilization of cortical actin. The cultured porcine adrenal chromaffin cells are found to possess μ-, δ- and κ-opioid receptors, and activation of opioid receptors mainly inhibits N-type voltage-dependent calcium channels (VDCCs) via pertussis toxin-sensitive G-proteins (Kitamura et al, 2002).

It has been shown that codeine is an autocrine regulator, which suppresses CA release via naloxone-insensitive re-

Corresponding to: Dong-Yoon Lim, Department of Pharmacology, College of Medicine, Chosun University, 375 Seoseok-dong, Dong-gu, Gwangju 501-759, Korea. (Tel) 82-62-230-6335, (Fax) 82-62-227-4693, (E-mail) dylim@chosun.ac.kr
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ABBREVIATIONS: CA, catecholamine; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide; BAY-K-8644, ethyl-1,4-dihydro-2, 6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5- carboxylate; VDCCs, voltage-dependent calcium channels; DHP, dihydropyridine; McN-A-343, 4-(N-[3-Chlorophenyl]carbamoyloxy)-2-butynyltrimethyl ammonium chloride.

ceptors from perifused chromaffin cells of the eel, and stimulates CA release via opiate receptor(s). Co-released morphine may modulate the action of codeine (Epple et al, 1994). In contrast, morphine, [D-Ala^2-D-Leu^5] enkephalin and U50, 488H, relatively selective ligands for the μ-, δ- and κ- receptors, injected intracerebroventricularly into conscious rats, produced dose-dependent elevations in levels of noradrenaline and adrenaline, the order of potency being enkephalin ~- morphine >U50,488H (Conway et al, 1984). These responses to morphine were blocked in the presence of naloxone but were not altered by RX 781094, a selective α_2-adrenoceptor antagonist. Therefore, to elucidate the mechanism of action on the inhibition of nicotinic stimulation-induced CA secretion by opioid antagonists, the present study was carried out to investigate the effect of naltrexone, a well-known opioid antagonist, on nicotinic stimulation-induced CA secretion from the isolated perfused model of the rat adrenal gland, in comparison with the responses to met-enkephalin, an opioid peptide.

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 the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by the placement of three-hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

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

Perfusion of adrenal gland

The adrenal glands were perfused by means of ISCO pump (WIZ Co.) 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), 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 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 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 naltrexone on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing naltrexone 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 naltrexone, and the perfusates were collected for the same period as that for the background sample. The adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayre (Anton & Sayre, 1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981) using fluorospectrophotometer (Kontron Co., Milano, Italy).

A volume of 0.2 ml of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by secretagogues used in the present work was high enough to obtain readings several folds greater than the reading of control samples (unstimulated). The sample blanks were also lowest for perfusates of stimulated and non-stimulated samples. The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statistical analysis

The statistical difference between the control and pretreated groups was determined by the Student's t and ANOVA tests. A P-value of less than 0.05 was considered to represent statistically significant changes unless specifically noted in the text. Values given in the text refer to means and the standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computer program described by Tallarida & Murray (1987).

Drugs and their sources

The following drugs were used: naltrexone hydrochloride (Jeil Pharmaceutical Co., Korea), ACh chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), methionine-enkephalin, norepinephrine bitartrate, methyl-1, 4-dihydro-2, 6-dimethyl-3-nitro-4- (2-trifluoromethyl-phenyl) -pyridine-5-carboxylate (BAY-K8644) (Sigma Chemical Co., USA), and cyclopiazonic acid, (3-(m-chlorophenyl-carbamoyl-oxy) -2-butynyltrimethyl ammonium chloride [McN-A-343] (RBI, USA). 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.

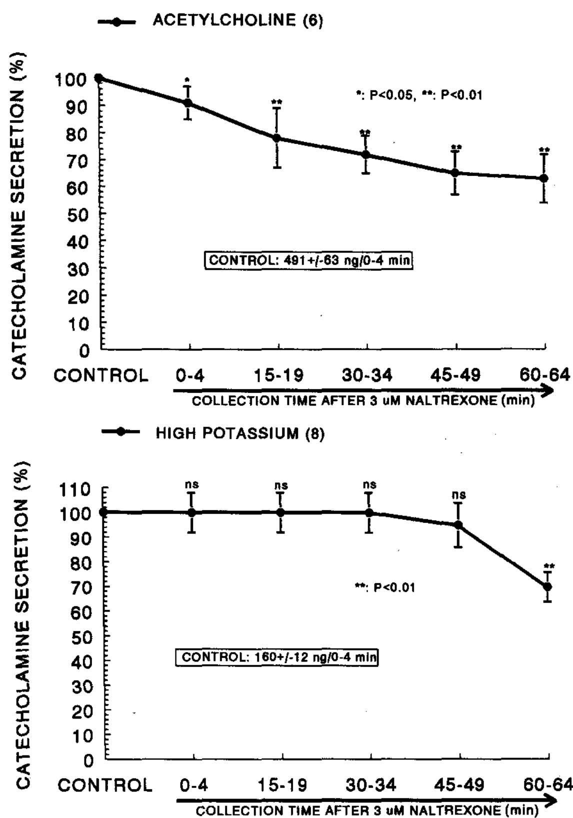

Fig. 1. Time course effect of naltrexone on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh, Upper) and by high K^+ (Lower) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) or K^+ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 3 μM naltrexone 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 naltrexone. Pefusates induced by ACh and high K^+ were collected for 4 minutes, respectively. ns: statistically not significant.

RESULTS

Effect of naltrexone 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 20 ± 2 ng for 2 min (n=8). Since nicotine-induced CA secretion and inward ionic currents were inhibited in a dose-dependent manner by the opioid antagonist naltrexone in cultured bovine chromaffin cells (Tome et al, 2001), it was attempted initially to examine the effects of naltrexone itself on CA secretion from the perfused model of the rat adrenal glands. However, in the present study, naltrexone (10^{-6} M) itself did not produce any effect on basal CA output from perfused rat adrenal glands (data not shown). Therefore, it was of interest to investigate the effects of naltrexone on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15 min-intervals. Naltrexone was present 15 min before initiation of stimulation.

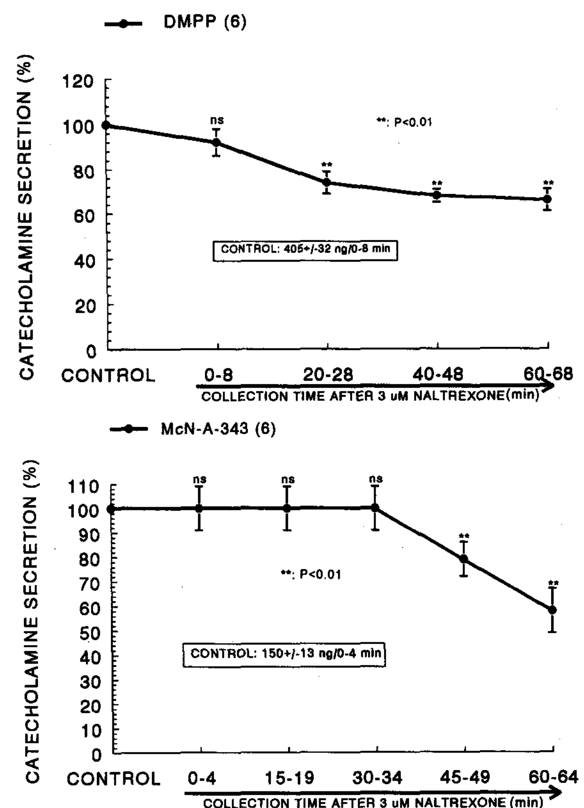

Fig. 2. Time course effect of naltrexone on the secretory responses of catecholamines (CA) evoked by DMPP (Upper) and McN-A-343 (Lower) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DPPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min at 20 and 15 min intervals were induced after preloading with 3 μM naltrexone 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. ns: statistically not significant.

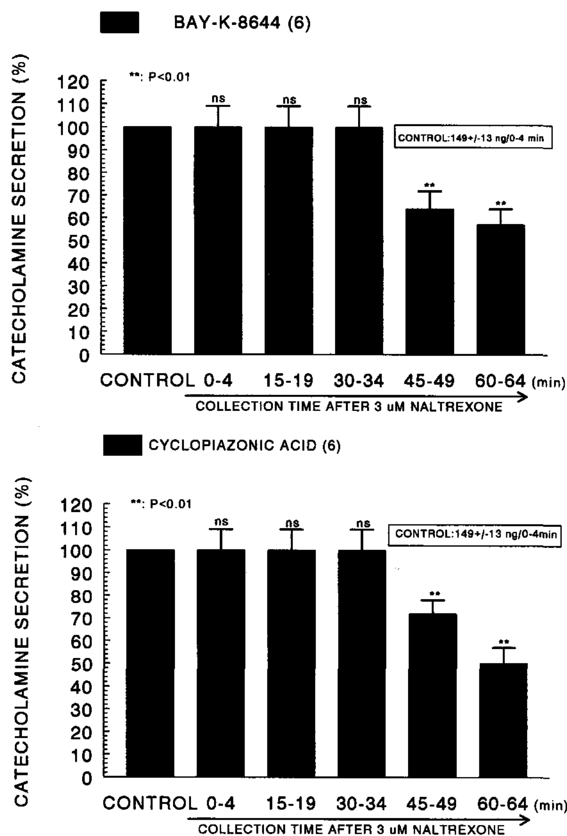

Fig. 3. Time course effect of naltrexone on CA release evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower) from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of naltrexone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

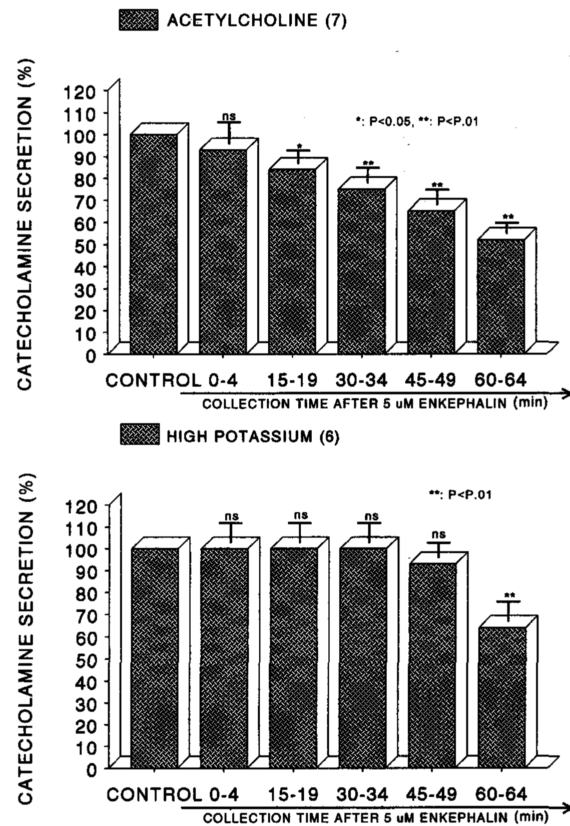

Fig. 4. The effects of met-enkephalin on the secretory responses of catecholamines (CA) evoked by acetylcholine (Upper) and by high K^+ (Lower) from the isolated perfused rat adrenal glands. The CA secretory responses by a single injection of ACh (5.32×10^{-3} M) or K^+ (56 mM) in a volume of 0.05 ml were induced before (CONTROL) and after preloading with 5 μM met-enkephalin for 60 min. Perfusate was collected for 4 minutes at 15 min-intervals. Other legends are the same as in Fig. 1. ns: statistically not significant.

When ACh (5.32×10^{-2} M) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 491±63 ng for 4 min. However, the pretreatment with naltrexone (3×10^{-6} M) for 60 min time-dependently inhibited ACh-stimulated CA secretion. As shown in Fig. 1 (upper), in the presence of naltrexone, CA releasing responses evoked by ACh were inhibited by 63% of the corresponding control release. Also, it has been found that depolarizing agent like KCl stimulates markedly CA secretion (160±12 ng for 0~4 min). Excess K^+ (5.6×10^{-2} M)-stimulated CA secretion, following the pretreatment with naltrexone (3×10^{-6} M), was significantly inhibited by 70% of the control after 45 min period, although it was not initially affected by naltrexone. DMPP (10^{-4} M), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (405±32 ng for 0-8 min). However, as shown in Fig. 2 (upper), DMPP-stimulated CA secretion after pretreatment with naltrexone was greatly reduced to 66% of the control release (100%). McN-A-343 (10^{-4} M), a selective muscarinic M_1-agonist (Hammer & Giachetti, 1982), perfused into an adrenal gland for 4 min caused an increased CA secretion (150±13 ng for 0~4 min). However, McN-A-343-stimulated CA secretion in the presence of naltrexone was markedly depressed to 58% of the corresponding control secretion (100%) as depicted in Fig. 2 (lower).

Effect of naltrexone on CA secretion evoked by Bay-K-8644 and cyclopiazonic acid 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 effects of naltrexone 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 naltrexone was greatly blocked to 57% of the control except for the early 30 min as compared to the corresponding control release (149±13 ng for 0−4 min) from 6 rat adrenal glands as shown in Fig. 3 (upper).

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Goeger & Riley, 1989; Seidler et al, 1989). The inhibitory action of naltrexone on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 3

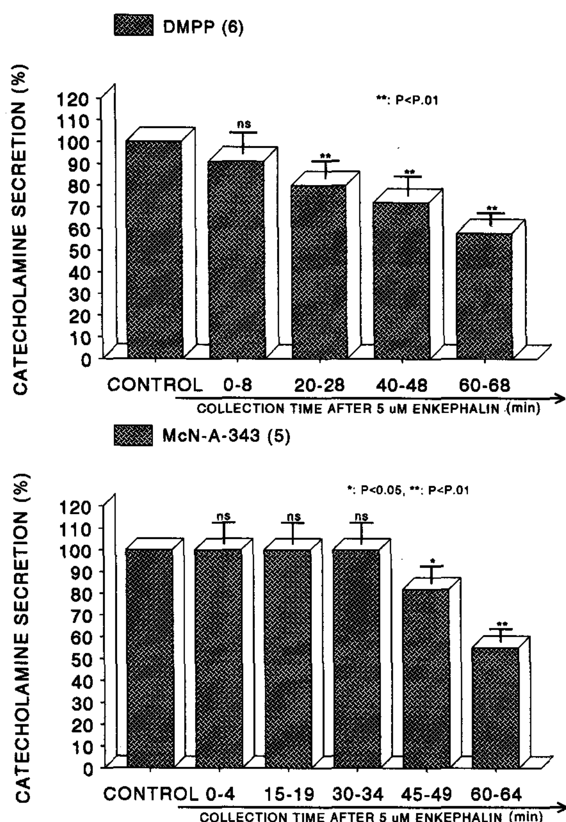

Fig. 5. The effects of met-enkephalin on the secretory responses of catecholamines (CA) evoked by DMPP (Upper) and McN-A-343 (Lower) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DPPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min at 20 and 15 min intervals were induced before (CONTROL) and after preloading with 5 μM met-enkephalin 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. ns: statistically not significant.

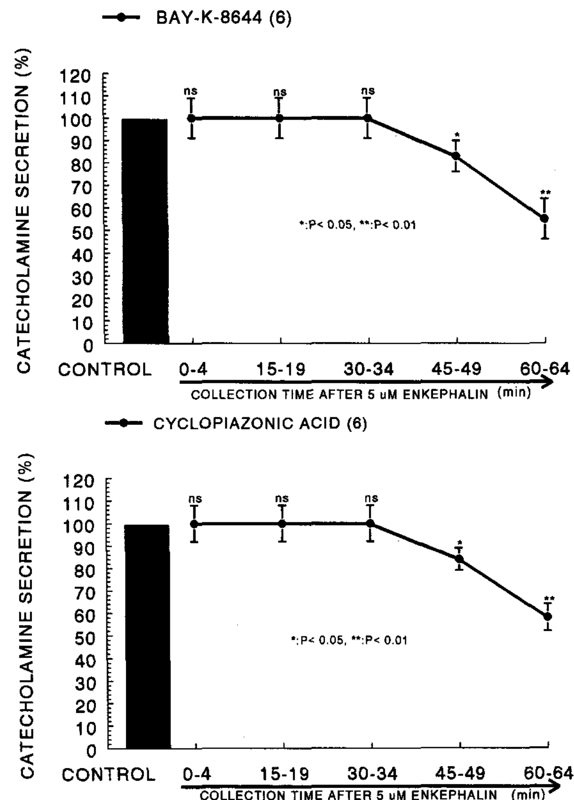

Fig. 6. The effect of met-enkephalin on CA release evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower) from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of met-enkephalin (5 μM) for 60 min, respectively. Other legends are the same as in Fig. 1. ns: statistically not significant.

(lower). In the presence of naltrexone in 6 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was inhibited to 50% of the control response (149±13 ng for 0~4 min).

Effect of met-enkephalin on CA secretion evoked by ACh, excess K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

It has been found that opioids suppress CA secretion in bovine, human, and rodent adrenals (Livett & Boksa, 1984; Mannelli et al, 1986; Marley et al, 1986; Jarry et al, 1989; Twitchell & Rane, 1993). This inhibitory effect of opioids is retained in two pheochromocytoma cell lines, rat PC12 (Venihaki et al, 1996) and human KAT45 (Venihaki et al, 1998). Therefore, to study the relationship between naltrexone effect and opioid receptors, the effect of met-enkephalin, an opioid peptide, on CA secretory responses evoked by cholinergic receptor-stimulation as well as membrane depolarization was examined. In the present study, ACh (5.32 mM)-evoked CA release before pretreatment with met-enkephalin was 402±47 ng (0~4 min) from 7 rat adrenal glands. In the presence of met-enkephalin (5 μM) for 60 min, it was not affected at first 0~4 min, but later rather inhibited by 52% of the corresponding control release as shown in Fig. 4 (upper). High K^+ (56 mM)-evoked CA release in the presence of met-enkephalin (5 μM) for 60 min was also not changed at 0~45 min period in comparison to the control secretion (155±18 ng, 0~4 min) from 6 glands (Fig. 4-lower). However, it was greatly inhibited by 65% of the control release only at last 60~64 min period. As shown in Fig. 5 (upper), DMPP-evoked CA release prior to the perfusion with met-enkephalin was 396±55 ng (0~8 min). In the presence of met-enkephalin (5 μM) for 60 min, DMPP-evoked CA release was also depressed by 58% of the corresponding control from 6 experiments. Moreover McN-A-343 (10^{-4} M)-evoked CA release in the presence of met-enkephalin (5 μM) was also reduced by 55% of the control release (161±21 ng, 0~4 min) from 5 rat glands, as shown in Fig. 5 (lower).

As shown in Fig. 6, the simultaneous perfusion met-enkephalin (5 μM) for 60 min depressed the CA secretory responses evoked by Bay-K-644 and cyclopiazonic acid at 45~60 min period by 55% and 58% in comparison to their corresponding control responses (152±18 ng/0~4 min and 143±15 ng/0~4 min), respectively. However, they were not

affected initially at 0~30 min period.

DISCUSSION

Generally, chromaffin cells synthesize, store and secrete CAs (dopamine, noradrenaline and adrenaline) in nerve stimulations. The cells have an excitable action in response to ACh or to electrical stimulation of the splanchnic nerve. Following activation of ACh nicotinic receptors, ACh causes the opening of the receptor-mediated ion channel, allowing the influx of Na^+, and to a lesser extent Ca^{2+} (Douglas et al, 1967). This influx into the chromaffin cells of Na^+ results in a mild depolarization of the cell membrane sufficient to activate voltage-dependent Na^+ channels (Cena et al, 1983). The opening of Na^+ channels induces the activation of voltage-dependent Ca^{2+} channels (Garcia et al, 1984). The opening of Na^+ and Ca^{2+} channels causes the firing of action potentials and the entry of Ca^{2+} from extracelluar spaces (Artalejo et al, 1994). An increase in $[Ca^{2+}]_i$ is the trigger for exocytosis of chromaffin granules (secretion of CA) and stimulation of biosynthesis of CA. Cholinergic nicotinic and muscarinic receptors are present in chromaffin cells, and these receptors in most species stimulate secretion of CA. In bovine adrenal chromaffin cells, however, only nicotinic receptor stimulation evokes secretion of CA. The CA secretion can also be evoked by high K^+, which directly activate the voltage-dependent channels. The depolarization induced by high K^+ directly opens the Ca^{2+} channels without the contribution of the Na^+ channel. Previous studies have shown that CA release in PC12 cells stimulated by carbamylcholine or K^+-depolarization is a transient phenomenon, which ceases after about 15 min (Ritchie, 1979; Dendorfer & Dominiak, 1995). Based on these findings, the present results that naltrexone inhibits the CA secretory responses evoked by DMPP and McN-A-343 as well as high potassium suggested that naltrexone has the activity directly to block both nicotinic and muscarinic receptors located on the rat adrenomedulary chromaffin cells. Thus, naltrexone seems to block the cholinergic stimulation that can induce the opening of Na^+, and Ca^{2+} channels, causing the firing of action potentials and the entry of Ca^{2+} through L-type Ca^{2+} channels, and then increase in $[Ca^{2+}]_i$, triggering exocytosis from the rat adrenomedullary chromaffin granules (secretion of CA). In the present investigation, naltrexone greatly attenuated CA secretions evoked by high potassium, a direct membrane-depolarizing agent. This result strongly suggests that naltrexone can block voltage-sensitive Ca^{2+} channels. In support of this finding, in the present study, naltrexone greatly attenuated CA secretion evoked by Bay-K-8644, an activator of L-type Ca^{2+} channels. This result indicates that naltrexone may act as a Ca^{2+}channel antagonist in the rat adrenal medulla. Bay-K-8644 is found to potentiate the release of CA by increasing Ca^{2+} influx through L-type Ca^{2+} channels in cultured bovine chromaffin cells (Garcia et al, 1984). Moreover, previous studies on primary cultures of bovine chromaffin cells have shown that dihydropyridines can partially inhibit CA secretion induced by depolarization with ACh, nicotine or K^+. The degree of inhibition varied between studies depending on the dihydropyridine used, its concentration, and the concentration of agonist (Cena et al, 1983; Boarder et al, 1987; Owen et al, 1989). However, at high concentration ($\geq 1\,\mu M$), dihydropyridines block the nicotinic receptor ion channels, and at these concentrations they inhibit calcium uptake and CA secretion induced by nicotinic agonists without comparable effects on K^+-evoked responses (Lopez et al, 1993). Nitrendipine at a concentration of $1\,\mu M$ was found to be sufficient to reduce 30 mM potassium-induced contraction of pig coronary artery rings mounted organ baths (O'Farrell et al, 1997). The most plausible explanation of this finding is, that naltrexone has a direct blocking effect on the Ca^{2+} channels. Therefore, the present experimental results imply that naltrexone itself blocks Ca^{2+} entry into the adrenomedullary chromaffin cells by inhibiting voltage-dependent Ca^{2+} channels and as consequence, it inhibits Ca^{2+}-dependent release of CAs evoked by cholinergic stimulation as well as membrane-depolarization.

Moreover, the present study has also shown that naltrexone inhibits the CA secretion evoked by cyclopiazonic acid. Cyclopiazonic acid is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Geoger & Riley, 1989; Seidler et al, 1989) and a valuable pharmacological tool for investigating intracellular Ca^{2+} mobilization and ionic currents regulated by intracellular Ca^{2+} (Suzuki et al, 1992). Therefore, it is felt that the inhibitory effect of naltrexone on CA secretion evoked by cholinergic stimulation as well as by membrane-depolarization may be associated with the mobilization of intracellular Ca^{2+} from the cytoplasmic calcium store. This indicates that the naltrexone 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. 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 & 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. 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 naltrexone on Ca^{2+} movement from intracellular pools is due to its direct effect on the PI response or the indirect effects.

In the present study, met-enkephalin also caused very similar inhibitory effects with naltrexone on CA release evoked by cholinergic stimulation and membrane depolarization, strongly suggesting that naltrexone-induced inhibition of CA secretion is not relevant to the involvement of opioid receptors. In support of this idea, methionine-enkephalin and ATP in the secretory vesicles of adrenal chromaffin cells (Winkler & Westhead, 1980) are released on exocytosis together with CA (Livett et al, 1981). It has been shown that these substances affect the activity of voltage-dependent calcium channels (VDCCs). The application of a soluble vesicular lysate of bovine chromaffin cells causes a rapid inhibition of currents through VDCCs, which is recovered by antagonists of opioid receptors and purino-

ceptors (Albillos et al, 1996). It has been reported that exogenously applied opioids suppress VDCC activity in bovine chromaffin cells (Kleppisch et al, 1992; Twitchell & Rane, 1993; Albillos et al, 1996), and inhibit CA secretion evoked by nicotine (Kumakura et al, 1980) and ACh (Saiani & Guidotti, 1982). Therefore, it is hypothesized that opioids co-released with CA regulate negative feedback control for the CA secretion through the suppression of VDCC activity. Moreover, beta-endorphin and morphine have been also shown to reduce CA secretion induced by nicotine to as much as fifty percent whereas [Met^5]-enkephalin decreased CA release to seventy-five percent (Kumakura et al, 1980). Barron & Hexum (1986) found that opiates modulate the secretion of CA and met-enkephalin-immunoreactive materials from the perfused bovine adrenal gland. It has also been shown that splanchnic nerve stimulation-induced CA output was markedly reduced by opiate agonist (opioid peptides or morphine) and also enhanced by an opiate antagonist (naloxone or naltrexone) from the dog adrenal gland in vivo, and that these effects are clearly associated with opiate receptor located in the adrenal gland (Kimura et al, 1988). In support of these hypotheses, Lim & his coworkers (1992) have demonstrated that both met-enkephalin and morphine decrease greatly CA secretion evoked by DMPP and ACh in the perfused rat adrenal gland. In terms of these results, it seems that naltrexone-induced inhibition of the CA release is not mediated through the opioid receptors, suggesting that the effect of naltrexone might be due to the direct blockade of cholinergic receptor itself.

Collectively, these experimental results demonstrate that naltrexone inhibits greatly CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as that by membrane depolarization. It seems that this inhibitory effect of naltrexone does not involve opioid receptors, but might be mediated by the blockade of both the calcium influx into the rat adrenal medullary chromaffin cells and the uptake of Ca^{2+} into the cytoplasmic calcium store, which are at least partly relevant to the direct interaction with the nicotinic receptor itself.

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