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Effect of Opioid on Nicotinic Receptor-Mediated Catecholamine Secretion in the Rat Adrenal Gland

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ABSTRACT

The present study was conducted to investigate the effect of opioids on catecholamine (CA) secretion evoked by a selective cholinergic nicotinic agonist, 1,1-dimethyl-4-phenyl piperazinium (DMPP) and acetylcholine from the retrogradely perfused rat adrenal glands. Methionine-enkephalin (9.68×10^{-6} M) caused a significant inhibition of CA secretion evoked by DMPP (100 uM) and ACh (50 ug), but had no effect on the spontaneous (basal) CA release. Morphine (1.73×10^{-5} M) attenuated considerablely the increase in CA release induced by DMPP and ACh. Morphine itself also did not affect the basal CA output. A 20 to 65% reduction of the DMPP- and ACh-evoked increase in CA release was observed after the pretreatment with methionine-enkephalin or morphine.

The increase in CA release evoked by DMPP and ACh was reduced markedly by preloading with an opiate antagonist naloxone (1.22×10^{-7} M) while basal CA output was not affected by naloxone.

These present experimental results suggest that the nicotinic stimulation-evoked CA release from the perfused rat adrenal gland is inhibited by endogenously released opioid peptides through activation of opiate receptors located in the adrenal gland.

Key Words: Met-Enkephalin, Naloxone, Morphine, Catecholamine-Secretion, Opiate receptors

INTRODUCTION

Generally, chromaffin cells in the adrenal medulla synthesize the catecholamines (CA) norepinephrine and epinephrine and secrete these substances in response to acetylcholine (ACh) which is released from the splanchnic nerve terminals. Catecholamine release from the adrenal medulla is partly under the control of the splanchnic nerve. In addition to CA, adrenal medullary cells produce a variety of regulatory peptides, including methionine-enkephalin [Met-Enkephalin] (Pelto-Huikko *et al.*, 1985; Kuramoto, *et al.*, 1985; Terenghi *et al.*, 1983). It has been shown that opioid peptides are co-released with CA from the isolated bovine adrenal chromaffin cells in primary cultrue (Kumakura *et al.*, 1980; Stine *et al.*, 1980; Livett *et al.*, 1981; Rossier *et al.*, 1981)and from adrenal glands in vitro (Viveros *et al.*, 1979; Kilpatric *et al.*, 1980; Corder *et al.*, 1982; Chaminade *et al.*, 1984; Barron and Hexum, 1985) and vivo (Hexum *et al.*, 1980; Govonic *et al.*, 1981; Kimura *et al.*, 1988; Jarry *et al.*, 1989).

Kumakura and his co-workers have reported that beta-endorphin and morphine reduced CA secretion induced by nicotine by as much as fifty percent whereas [Met^5]-enkephalin decrease CA release by seventy-five percent. In subsequent experiments, it has been found that a stereoselective opiate receptor exists on chromaffin cell membranes that may modulate the function of the nicotinic receptor in releasing CA from the adrenal medulla. Barron and Hexum (1986) demonstrated that opiates modulate the secretion of CA and met-enkephalin-immunoreactive materials from the perfused bovine adrenal gland. Recently, it is

※ Author for corres pondence

also known that splanchnic nerve stimulation-induced CA release was markedly attenuated by opiate agonist (opioid peptides or morphine) and also enhanced by an opiate antagonists (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 this result, more recently, Jarry and his colleagues (1989) have shown that neuropeptides endogenous to the adrenal gland not only reduce the amplitude of CA secretion in response to an ACh stimulus, but, in addition, modulate the duration of CA secretion.

However, it has been reported that opioid antagonist, including naloxone, inhibit the nicotine-induced CA release from the adrenal chromaffin cells in culture (Lemaire *et al.*, 1981; Dean *et al.*, 1982; Marley *et al.*, 1986a). Dean *et al.* (1982) showed that narotic antagonists naloxone, naltrexone and levallorphan did not reverse the inhibition produced by either the narcotic analgesics (e.g morphine) or the opioid peptides (e.g. dynorphine), and that these antagonists themselves inhibited the nicotine-mediated release of [^{3}H]-NE from the bovine adrenal chromaffin cells in culture. Marley and Livett (1987) also demonstrated that adrenal opioid peptides probably do not act on adrenal opioid binding sites characterized from ligand binding studies to prevent the nicotinic response from desensitizing from cultured bovine adrenal chromaffin cells. In addition, more studies support that endogenous opiate do not modulate sympathoadrenal response to hypoxemia (Lewis and Sedeghi, 1991).

The purpose of the present study is to attempt to examine whether opiate agonists, met-enkephalin and morphine, and antagonist naloxone modify the CA release evoked by ACh and DMPP from the isolated perfused rat adrenal glands and to elucidate the mode of functional role in releasing adrenal medullary CA.

MATERIALS AND METHODS

Experimental animals

Mature male Sprague-Dawley rats, weighing 180-300 grams, were anesthetized with ether, 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 placing 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 (A). was inserted into the distal end of the renal vien after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparine (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±1℃ (B)

Perfusion of adrenal gland

The adrenal glands were perfused by means of a ISCO pump (WIZ Co.) at a rate of 0.3 ml/min. The perfusion was carried out with Kerbs-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±0.05. The solution contained disodium EDTA (10 ug/ml) and ascorbic acid (100 ug/ml) to prevent oxidation of catecholamine.

Drug administration

The perfusion of DMPP (100 uM) for 2 minutes and/or a single injection of ACh (5.32 mM) and in a voulme of 0.05 ml were made into perfusion stream via a three way stopcock (Fig. 1).

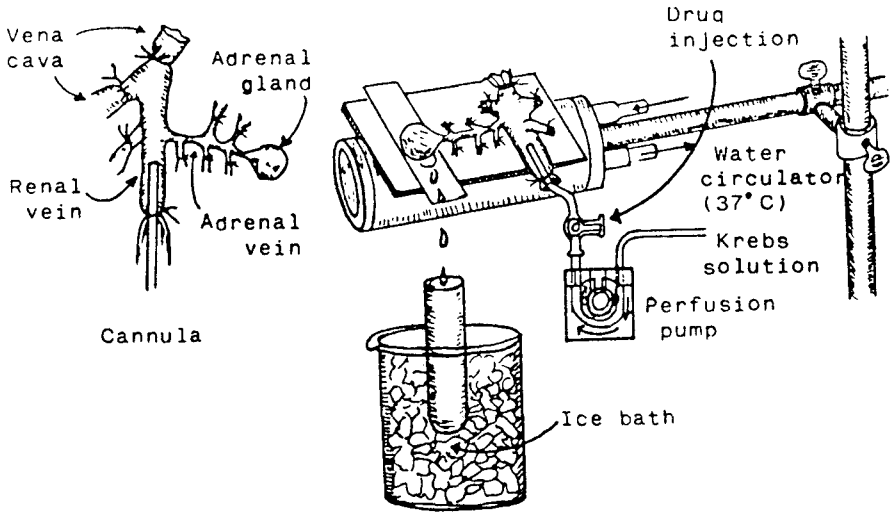

Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamines in the isolated perfused adrenal gland of the rat.

In the preliminary experiments it was found that upon administration of the above drugs, secretory responses to ACh returned to preinjection level in about 4 min, but the responses to DMPP in 8 min. Generally, the adrenal glands perfusate was collected in chilled tubes.

Collection of perfusate

As a rule, prior to each stimulation with ACh and DMPP perfusate samples were collected (4 min) to determine the spontaneous secretion of CA ("background sample"). Immediately after the collection of the "backgound sample", collection of the perfusates was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Each perfusate was collected for 4 to 8 min. The amounts secreted in the "background sample" have been subtracted from those secrected from the "stimulated sample" to obtain the net secretion valuc of CA, which is shown in all of the figures.

To study the effects of opiate agonist and antagonist on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing opiate agonist or antagonist for 30 min, then the perfusate was collected for a specific time period ("background sample"), and then the medium was changed to the one containing the stimulating agent and the perfusates were collected for the same period as that for the "background sample".

Measurement of catecholamines

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayer (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981), using spectrofluorophotometer (Shima dzu Co.).

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 fold greater than the reading of control smples (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 expressed in terms of norepinephrine (base) equivalents.

All data are presented as means with their standard errors, and the significance of differences were analyzed by student's t-test using the computer system as previously described (Murray

and Tallarida, 1987).

Drugs and their sources

The following drugs were used: acetylcholine chloride, l.l-dimethyl-4-phenyl piperazinium iodide (DMPP), methionine-enkephalin, norepinephrine bitarterate (Sigma Chemical Co., U.S.A.), morphine sulfate and naloxone hydrochloride (Reyon Pharmaceutical Co., R.O.K.).

Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required. Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

Effect of met-enkephalin on ACh-and DMPP-evoked CA output

Generally, when the perfusion of oxygenated Krebs-bicarbonate solution for on hour, basal CA release reaches steady state. In order to examine the effect of met-enkephalin on nicotinic receptor stimulation-mediated CA release, met-enkephalin (9.68×10^{-6} M) was introduced 30 min before DMPP or ACh was given. In the present work, perfusion of met-enkephalin itself did not affect spontaneous CA release (Data not shown). When ACh (50 ug) in a volume of 0.05 ml was administered into the perfusion stream via a three way stopcock, CA release amounted to 545.2 ± 40 ng for 4 min from 12 rat adrenal glands. However, in the presence of met-enkephalin, ACh-induced CA output was greatly reduced to 325.0 ± 11.3 ($p<0.001$, $n=12$) ng for 4 min as compared to its corresponding control CA release. Figure 2 shows the inhibitory effect of met-enkephalin on ACh-induced CA secretion from the perfurat rat adrenal glands. When perfused through the rat adrenal gland, DMPP (100 uM for 2 min), which is known to be a selective nicotinic receptor agonist in autonomic sympathetic ganglia, produced a sharp and rapid increase in CA secretion. As illustrated in Fig. 3, DMPP-induced CA release in the absence of met-enkephalin was 567.5 ± 29.5 (0~4 min) ng and 130.8 ± 18.9 (4~8 min) ng while in the presence of met-enkephalin, which was perfused 30 min before perfusion of DMPP, it was

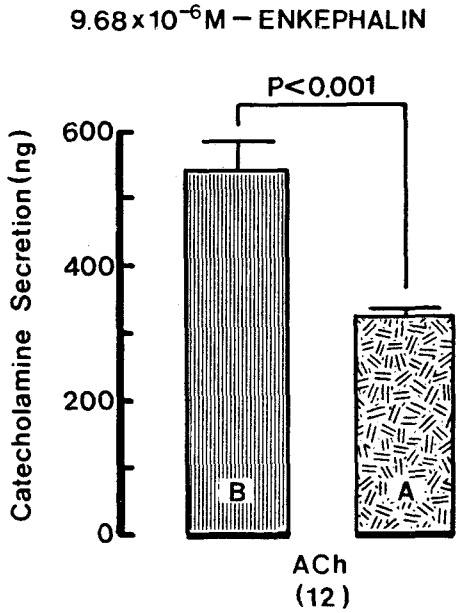

Fig. 2. Influence of met-enkephalin on ACh-induced CA secretion from the isolated perfused rat adrenal glands. CA secretion was evoked by a single injection of ACh(50 ug) after perfusion with normal Krebs solution for one hour before the experimental protocol was initiated. "B" and "A" indicate CA secretion evoked by ACh before(B) and after(A) preloading with 9.68×10^{-6} M met-enkep-halin for 30 min, respectively. Numeral in the bracket denotes number of experimental rat adrenal glands. Vertical bars represent the standard error of mean(S.E.M.). ordinate: the amounts of CA secreted from the adrenal glands in ng. Abscissa: secretogogue. Statistical difference was calculated by comparing the control with the preloading group. Perfusate was collected for 4 min. ACh: acetylcholine.

markedly reduced to 450.8 ± 28.0 (0~4 min, $p<0.001$) ng and 45.0 ± 16.5 (4~8 min, $p<0.01$) ng, respectively as compared with their corresponding control values from 12 rat adrenal glands.

Effect of naloxone on ACh-and DMPP-evoked CA output

Since it has been found that opiate antagonists faciliate the splanchnic nerve stimulation-induced CA release from the adrenal gland of anes-

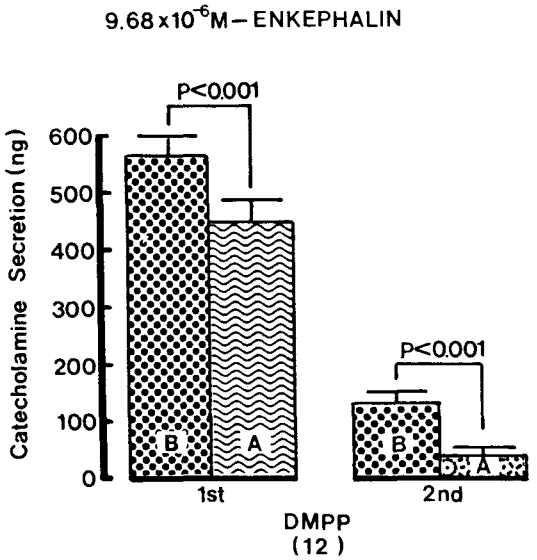

Fig. 3. Influence of met-enkephalin on DMPP-induced CA secretion from the isolated perfused rat adrenal glands. DMPP(100 uM) was perfused into an adrenal vein for 2 min before and after pretreatment with 9.68×10^{-6} M met-enkephalin for 20 min respectively and its perfusate was collected twice successively for each 4 min. Other legends and methods are as in Fig. 2.

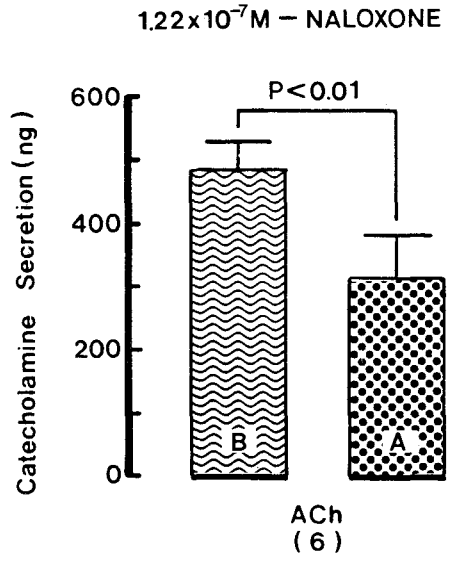

Fig. 4. Influence of naloxone on ACh-induced CA secretion from the isolated perfused rat adrenal glands. ACh was introduced before and after pretreatment with 1.22×10^{-7} M naloxone for 30 min, respectively. Other legends and methods are as in Fig. 2.

thetized dogs (Costa *et al.*, 1983; Kimura *et al.*, 1988) and reverse the inhibition of the DMPP-stimulated CA release caused by etorphine, an opiate agonist from the bovine adrenal gland (Barron and Hexum, 1986), it was of interest to examine the influence of naloxone on ACh-and DMPP-evoked CA secretion from the perfused rat adrenal glands. ACh (50 ug)-induced CA release in the absence of naloxone amounted to 487.5 ± 39.6 ng for 4 min from 6 rat adrenal glands, but following the preloading with naloxone (1.22×10^{-7} M) for 30 min, it was significantly reduced to 317.5 ± 66.7 ($p<0.01$, $n=6$) ng/4 min, which was 65 % of the corresponding control release, as shown in Fig. 4. In 6 rat adrenal glands, DMPP (100 uM)-induced CA release was 700.0 ± 44.9 (0~4 min) ng and 147.5 ± 24.0 (4~8 min) ng before pretreatment with naloxone but in the presence of naloxone DMPP-induced CA release was significantly diminished to 47.5 ± 18.7 (0~4 min, $p<0.001$) ng and 10.0 ± 3.2 (4~8 min, $p<0.001$) ng, respectively as compared to their control release. Figure 5 represents the marked inhibition by naloxone of DMPP-induced CA release from rat adrenal glands.

Effect of morphine on ACh-and DMPP-evoked CA output

It was attempted to demonstrate the effect of morphine on the ACh-and DMPP-evoked CA secretion from the perfused rat adrenal gland since it has been reported that several opiate agonists inhibit the nicotinic receptor-mediated CA secretion in adrenal chromaffin cells and perfused adrenal glands (Kumakura *et al.*, 1980; Saiani and Guidotti, 1982; Dean *et al.*, 1982; Costa *et al.*, 1983; Marley *et al.*, 1986a,b; Barron and Hexum, 1986; Kimura *et al.*, 1988; Jarry *et al.*, 1989).

Under the presence of morphine (1.73×1010^{-5} M) which was perfused 30 min before the injection of ACh (50 ug), ACh-induced CA output was significantly attenuated to 345.0 ± 16.9 ($p<0.05$, n

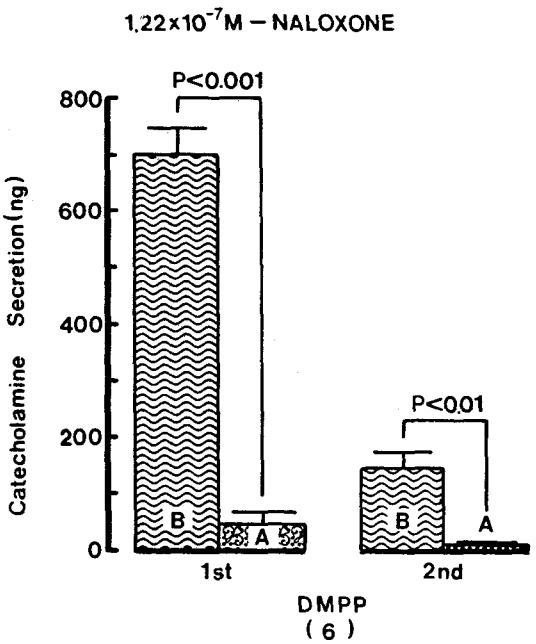

Fig. 5. Influence of naloxone on DMPP-induced CA secretion from the isolated rat adrenal glands. Other legends and methods are the same as in Fig. 2, 3 and 4.

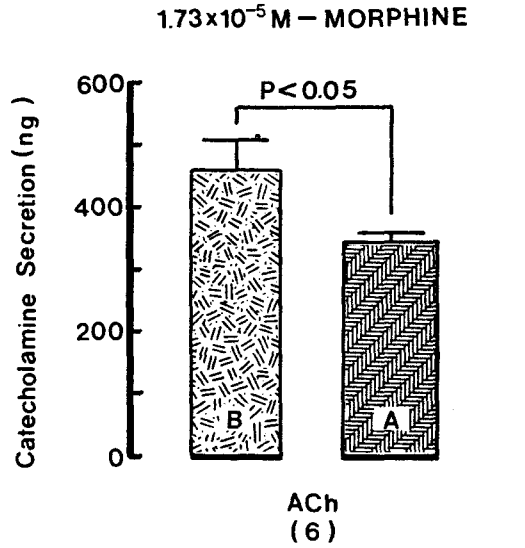

Fig. 6. Influence of morpine on ACh-induced CA secretion from the isolated rat adrenal glands. ACh was introduced in the presence of 1.73×10^{-5}M morphine and in the absence of it, respectively, Other legends and methods are the same as in Fig. 2.

=6) ng/4min as compared to its corresponding CA release of 460.0±47.8 ng/4 min prior to preloading with morphine as shown in Fig. 6. Figure 7 shows the inhibitory response of morphine on DMPP-evoked CA secretion from the isolated perfused rat adrenal glands. In the present work, in the adsence of morphine, DMPP (100 uM)-evoked CA release was 667.5±39.8 (0~4 min) ng and 165.6±14.5 (4~8 min) ng while in the presence of morphine which was preloaded 30 min before stimulation DMPP-induced CA release was prominently diminished to 528.8±32.4 (0~4 min, $p<0.01$) ng and 102.5±30.8 (4~8 min, $p<0.05$) ng, respectively from 8 rat adrenal glands.

DISCUSSION

The present experimental data demonstrate that met-enkephalin and morphine inhibit ACh- and DMPP-evoked CA release through activation

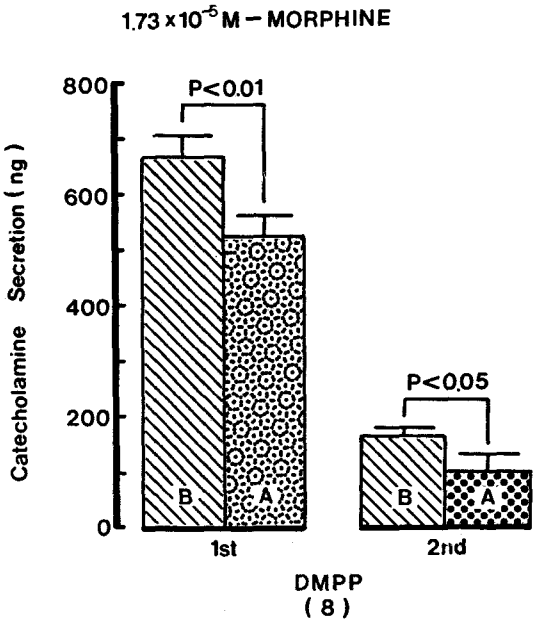

Fig. 7. Influence of morphine on DMPP-induced CA secretion from the isolated rat adrenal glands. Other legends and methods are the same as in Fig. 2, 4 and 6.

of opiate receptors present in the rat adernal glands. However, this inhibitroy effect of them was not by pretreatment with naloxone. The findings that opiate agonists inhibit nicotinic receptor stimulation-mediate CA secretion in the present work confirm that the opiate receptors are exist in the rat adrenal gland. In support of this idea, it was found that a number of opioid agonists could inhibit the nicotinic receptor-mediated release of CA from adrenal chromaffin cells in vitro (Kumakura *et al.*, 1980; Saiani and Guidotti, 1982; Dean *et al.*, 1982; Costa *et al.*, 1983; Marley *et al.*, 1986a,b) as well as in vivo adrenal glands such as in cows (Stine *et al.*, 1980; Kilpatric *et al.*, 1980; Barron and Hexum, 1985; 1986), cats (Hexum *et al.*, 1980; Corder *et al.*, 1982, Chaminade *et al.*, 1984), dogs (Govoni *et al.*, 1981; Kimura *et al.*, 1988) and rats (Jarry *et al.*, 1989). However, there is no informations so far, showing that opiate agonists are capable of inhibiting the physiological release of CA in the perfused rat adrenal glands in vitro. In the present experiments, both met-enkephalin and morphine significantly depressed the increase in CA output induced by ACh and/or DMPP without affecting spontaneous basal CA release. What of the enkephalins? They are certainly the most abundant of the neuropeptieds in the adrenal medulla and splanchnic nerve (Livett *et al.*, 1982; Viveros and Wilson 1983; Uden friend and Kilpatrick 1983). Livett and his coworkers (1983) showed that the inhibition of the nicotinic response produced in vitro by opiates and by Leu-and Met enkephalin was probably not via conventional high affinity streospecific opiate receptors since it required relatively high concentrations of the opioids, was not reversed by naloxone and naltrexone, and showed no preference for levorphanol over dextrorphan (or of iodo-Tyr-beta-endorphin over beta-endorphine). However, it is now apparent that a unique opiate receptor with a relatively low affinity for morphine (and for the penta peptide enkephalins), but a much higher affinity for the large enkephalin congener (e.g. the heptapeptide, Met^5-enkephalin-Arg^6-Phe^7, MEAP) may be involved. Saiani and Guidotti (1982) have reported that MEAP and etorphine binding to adrenal chromaffin cell membranes were displaceable by low concentrations of diprenorphine that also reversed the etorphine inhibition of nicotine-evoked release of CA. If endogenous release opioid peptides inhibit the release of CA concurrently with their release by the injection of ACh of DMPP, it would be expected that the CA secretion is potentiatled by opioids antagonists as a result of the cancellation of the opioid-mediated inhibition, However, in the present study, it was found that the preloading with naloxone did not enhance CA secretion evoked by DMPP or ACh, but rather did markedly inhibit CA release by them.

In support of this results, it has been reported that opioids antagonists, including naloxone and naltrexone, inhibit the nicotine-induced release of CA from the adrenal chromaffin cells in culture (Lemaire *et al.*, 1981; Dean *et al.*, 1982; Marley *et al.*, 1986a). More recently, Lewis and Sadeghi (1991) found that in fetal lamb neither dose of naloxone significantly altered plasma norepinephrine or epinephrine concentrations. Therefore, it was concluded that endogenous opiates do not modulate the sympathoadrenal response to moderately long periods of hypoxemia unaccompanied by acidemia. In addition it known that prophylatic administration of naltrexone has no effect on hemodynamic parameters during staged hemorrhage and that the concurrent adrenal secretion of CA and met-enkephalin is not modulate by actions on opiate receptors in the halothane-anesthetized cat (Gaumann *et al.*, 1989).

Thus, the present experimental results that naloxone markedly depresses CA secretion evoked by ACh and DMPP in the perfused rat adrenal gland suggest strongly that naloxone has inhibitory effect of nicotinic stimulation-evoked CA release via the other unkown mechanism in addition to opiate antagonism. Otherwise, it is felt that concentration of naloxone used in the present study may be too high to increase CA release in the rat adrenal gland.

In contrast with these results, diprenorphine, an opioid antagonist, is reported to faciliate the splanchnic nerve stimulation-induced release of CA from the anesthetized dog adrenal gland (Costa *et al.*, 1983) and to reverse the inhibition of the DMPP-stimulated release caused by etorphine an opiate agonist as well as to enhance DMPP-induced CA release (Barron and Hexum, 1986). Recently, Kimura and his co-workers (1988) have

shown that both naloxone and naltrexone markedly enhance the increase in epinephrine and norepinephrine output induced by 1 and 3Hz of splanchnic nerve stimulation. Furthermore, the increase in CA output is significantly attenuated by either leu-enkephalin or morphine during splanchnic nerve stimulation over the same range of stimulus frequencies. Jarry *et al.*, (1989) also showed in vivo a paracrine or autocrine action of met-enkephalin on ACh stimulated CA release by applying the peptide directly into the adrenal gland via a microdialysis system. Intraadrenal application of met-enkephalin in the rat in vivo reduced ACh-stimulated epinephrine, but not norepinephrine, secretion significantly; application of naloxone did not affect ACh-stimulated CA secretion in the initial fraction after ACh injection, but significantly prolonged amine secretion after the cholinergic stimulus. Application of naloxone followed by compined administration of met enkephalin and naloxone was without an effect on the amount of CA released in the initial fraction after ACh injection compared to that in the control group. Thus, naloxone prevented the inhibitory effect of met-enkephalin on ACh stimulated CA release. It has been known that met-enkephalin has greater affinity for delta receptors than for other types and that morphine has greater affinity for mu receptors. Adrenal medulla possesses opioid binging sites of mu, delta and kappa types, and several opioid peptides present in the medullary chromaffin cells have high affinity for delta and mu receptors (Castanas *et al.*, 1985a, b). Thus, it seems likely that endogenously released opioid peptides partially occupied delta and mu receptors. Exogenous administered opioid peptide (met-enkephalin) and morphine would further inhibit the DMPP- and ACh-evoked CA secretion by activating remaining opioid receptors. However, in the present study, opiate agonists did not completely abolish the response of the gland to DMPP- and ACh-mediated CA secretory action, despinte the fact that naloxone greatly depressed CA secretory effect evoked by them. This further supports the role of receptors other than opiate receptors as being involved in inhibition of cholinergically stimulated CA secretion in the perfused rat adrenal medulla.

Anyway, the results presented here support the findings of others wherein the opiate inhibition of cholinergically and mediated secretion from the isolated perfused rat adrenal gland and also they agree with findings reported previously[i.e.,opiate inhibition of release (Saiani *et al.*, 1982; Kumakura *et al.*, 1980). Opiate receptor binding sites on chromaffin cell membranes (Chavkin *et al.*, 1979; Saiani *et al.*, 1982); Dumont and Lemaire, 1984; Castanas *et al.*, 1984) and endogenous opioid peptides present in the chromaffin cells and splanchnic nerve terminals (Schultzberg *et al.*, 1978; Hexum *et al.*, 1980) strengthens the conculsion that the endogenous opioids peptides can act as modulators of cholinergically-mediated CA secretion in the rat adrenal glands.

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= 국문초록 =

흰쥐 부신에서 Opioid가 니코틴 수용체를 통한 카테콜아민 분비작용에 미치는 영향

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흰쥐 적출관류 부신에서 선택적인 nicotine 수용체 효능약인 DMPP(1,1-dimethyl-4-phenylpiperazinium)와 acetylcholine(ACh)의 카테콜아민(CA) 분비작용에 대한 opioids의 영향을 연구하고자 시행하여 얻어진 연구결과는 다음과 같다.

Methionine-enkephalin(9.68×10^{-6} M)으로 전처치시 DMPP(100 uM)과 ACh(50 μg)에 의한 CA 유리작용이 현저히 억제되었으며 basal CA release는 영향을 받지 않았다. Morphine(1.73×10^{-5} M)으로 전처치시 DMPP 및 excess K^+의 CA 분비작용은 뚜렷이 약화되었다. Morphine 역시 그자체는 basal CA release에는 영향을 미치지 않았다. Opiate 수용체 길항제인 naloxone(1.22×10^{-7} M)은 DMPP 및 ACh에 의한 CA 분비작용을 현저히 차단 하였으나 basal CA release에는 영향을 미치지 못하였다.

이와 같은 연구결과로 보아, 흰쥐 관류 부신에서 니코틴 수용체에 의한 CA 분비작용은 내인성 opioid peptide에 의해서 억제되며, 이는 부신에 존재하는 opiate 수용체 흥분작용에 기인되는 것으로 사료된다.