The Mode of Action of Pentazocine on Catecholamine Secretion from the Perfused Rat Adrenal Medulla[#]

Dong-Yoon Lim[*], Bong-Han Kim, Jae-Bong Heo, Cheo-Hee Choi,
Jin-Ho Kim, Young Jang and Jae-Joon Lee

Department of Pharmacology, College of Medicine, Chosun University, Kwang Joo 501-759, Korea

ABSTRACT

The present study was attempted to investigate whether pentazocine, which is known to possess both opioid agonistic and antagonistic properties, produces catecholamines (CA) secretion from the isolated perfused rat adrenal gland, and to establish the mechanism of its action, and also to compare its action with that of some opioids.

Pentazocine (30 to 300 ug) injected into an adrenal vein caused a dose-dependent secretory response of CA from the rat adrenal medulla. The pentazocine-evoked secretion of CA was remarkably diminished by the preloading with chlorisondamine (10^{-6} M), naloxone (1.22×10^{-7} M), morphine (1.7×10^{-5} M), met-enkephalin (9.68×10^{-6} M), nicardipine (10^{-6} M) and TMB-8 (10^{-5} M) while was not influenced by the pretreatment of pirenzepine (2×10^{-6} M). The perfusion of Ca^{++}-free Krebs solution for 30 min into the gland also led to the marked reduction in CA secretion evoked by pentazocine. Furthermore, the CA release evoked by ACh and/or DMPP was greatly inhibited by the pretreatment with pentazocine (1.75×10^{-4} M) for 20 min.

From these experimental results, it is thought that pentazocine causes markedly the increased secretion of CA from the isolated perfused rat adrenal medulla by a calcium-dependent exocytotic mechanism. The secretory effect of pentazocine appears to be mediated through activation of opioid receptors located on adrenal chromaffin cells, which may be also associated with stimulation of cholinergic nicotinic receptors.

Key Words: Pentazocine, Catecholamine secretion, Opiate receptors, Adrenal gland

INTRODUCTION

It has been found that pentazocine possesses both opioid agonistic action and weak antagonistic activity (Lange and Lasinki, 1986; Thompson, 1983) as a chemical structurally benzomorphinan derivative. Intravenous injection of pentazocine

and tripelennamine in combination has been reported to produce nausea, vomiting, headache, seizures, agitation, anxiety, muscle spasms, syncope and presyncope, and elevation of systolic and diastolic blood pressure (Polkis and Whyatt, 1988; DeBend and Jagger, 1981, Langer and Lasinki, 1986; Thompson, 1983). Pentazocine has been also shown to increase the plasma concentrations of both norepinephrine and epinephrine, accompanied by a rise in blood pressure and heart rate in man (Tammisto *et al.*, 1971; Manner *et al.*, 1987). In patients with coronary artery disease, intravenous pentazocine elevates mean aortic pressure, left ventricular end-diastolic pres-

---

[*] To whom correspondence should be addressed.

sure, and mean pulmonary artery pressure, and causes an increase in cardiac work (Alderman et al., 1972; Lee et al., 1976). It is also found that pentazcine reverses hemodynamic changes associated with anaphylactic shock in rats (Paciorek et al., 1985). In patients with myocardial infarction, it has been also shown to raise systemic vascular resistance, which may relate to its ability to increase serum catecholamine levels (Nagel and Pilcher, 1972; McGwier at al., 1992). Takki and his colleagues (1973) have reported that these effects of pentazocine seem to be exerted through the facilitation of sympathetic neurotransmission. However, it is not still established whether the cardiovascular effects of pentazocine are mediated via the central mechanism or the direct action on peripheral sympathoadrenal system. More recently, Fukumitsu and his coworkers (1991) have found that pentazocine directly acts on the adrenal medulla of the dog and induces CA efflux via a nonexocytotic mechanism which is not associated with opiate receptors. Nevertheless, Bender and Ardentova (1979) have shown in experiments on white rats that pentazocine reduces the epinephrine content in cardiac and hepatic tissues, insisting that the ascorbic pathway of carbohydrate oxygenation being predominant. Moreover, betaendorphin and morphine have been also shown to reduce secretion of CAs release to seventy-five percent (Kumakura et. al., 1980). Barron and Hexum (1986) found that opiates modulate the secretion of CA and met-enkephalin-immuno reactive materials from the perfused bovine adrenal gland.

Recently, it is also known 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) form 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, more recently, Lim and 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.

Therefore, in the present study it was sttempted to investigate whether pentazocine acts directly on the adrenal medulla of the rat to evoke CA se-cretion and to clarify the mechanism of its action.

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, 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 venal 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 a ISCO pump (WIZ Co.) at a rate of 0.4 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 ug/ml) and ascorbic acid (100 ug/ml) to prevent oxidation of CA.

Drug administration

Single injection of pentazocine (30~300 ug) or ACh (50 ug) in a volume of 0.05 ml were made into perfusion stream via a three way stopcock. In the preliminary experiments it was found that upon administration of the above drugs, secretory responses to Ach and pentazocine returned to preinjection level in about 4 min. Generally, the adrenal glands were perfused with normal Krebs solution for about one hour before the experimental protocols are initiated. The adrenal ferfusate was collected in chilled tubes.

Collection of perfusate

As a rule, prior to each stimulation with pentazocine or ACh samples were collected (4 min) to determine the spontaneous secretion of CA ("background sample"). Immediately after the collection of the "background sample", collection of the perfusate 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 min. The amounts secreted in the "background sample" have been subtracted from those secreted the "stimulated sample" to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effects of a test agent on the spontaneous and drug-evoked secretion, the adrenal gland was perfused with Krebs solution containing the agent for 20~30 min, then the perfusate was collected for a specific time period ("background sample"), and then the medium was changed to the one containing the test agent and the perfusate 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 Sayre (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981), using fluorospectrophotometer (Shimadzu Co., Japan). A volume of 0.2 ml of the perfusate was used for the reaction.

The CA content in the perfusate of stimulated glands by ACh or pentazocine was high enough to obtain readings several-fold 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 norpinephrine (base) equivalents.

Drugs and their sources

The following drugs were used: pentazocine was obtained from DaeWon Pharmaceutical Manuf., Co., Korea, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), methionine-enkephalin, norepinephrine bitartrate, nicardipine hydrochioride and 3. 4. 5-trimethoxy benzoic acid 8-(diethylamino) octylester (TMB-8) from Sigma Chemical Co., U.S.A..

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 except the case of pentazocine or ACh in ug.

Statistical analysis

The statistical significance between groups was determined by utilizing the Student's t-test. Data obtained from animals which served own control were analyzed for the significance using t-test for paired observation.

A P-value of less than 0.05 was considered to represent statistical significant changes unless specifically noted in the text. Values given in the text refer to means with standard errors of the mean (S.E.M.). The statistical analysis of the present experimental results was made by computer program of statistics described previously by Tallarida and Murray (1987).

RESULTS

The secretory effect of CA evoked by pentazocine

When the adrenal gland was perfused with oxygenated Krebs-bicarbonate solution for 60 min before experimental protocol is intitiated, the spontaneous CA secretion reached steady state. The releasing effects to the initial injection of a range of doses of pentazocine (30~300 ug) are shown in Figure 1. This range of doses of pentazocine produced a nearly complete dose-dependent curve.
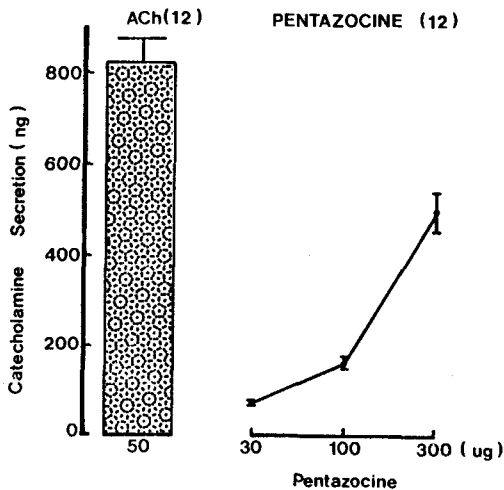
Fig. 1. Concentration-dependent increase of catecholamine secretion evoked by pentazocine from the isolated perfused rat adrenal glands. Secretion of catecholamines (CA) was evoked by introducing injections of pentazocine (30, 100 and 300 ug) into the perfusion stream at 30 min intervals after perfusion with normal Krebs solution for 60 min. Following the injection of drug, the perfusate was collected for 4 min. The column indicates ACh-induced CA secretion. Vertical bars indicate the standard error of the mean (SEM). Numeral in the parethesis denotes number of the experimental animals. Ordinate: the amounts of catecholamines secreted from the adrenal gland for 4 min. Abscissa: doses of pentazocine in ug.

Injection of 30 ug-pentazocine into the perfusion stream exerted significant output of CA over the background release, which was 57.9±7.6 ng for 4 min. A gradual increase in pentazocine concentration resulted in greater amounts of CA released in the perfusate. After administration of 100 ug and 300 ug pentazocine CA outputs were 165.6±14.2 ng and 506.9±40.3 ng for 4 min, respectively from 12 adrenal glands. These observations seem to be consistent with those reported previously in the perfused dog adrenals (Fukumitsu et al., 1991). In order to compare pentazocine's secretory effect with that of ACh, when ACh (50 ug) was given into the adrenal vein through the perfusion stream. CA output was increased to 823.9±52.1 ng
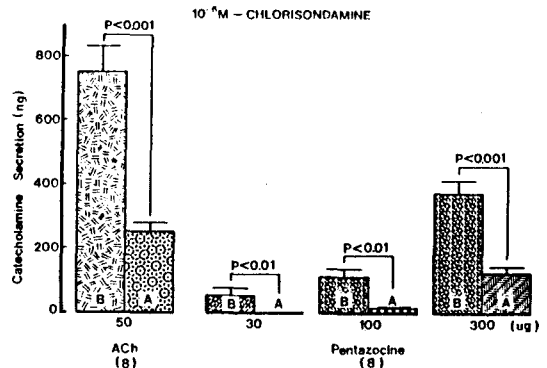

Fig. 2. The effect of chlorisondamine on ACh- and pentazocine-evoked CA secretion. Chlorisondamine (10⁻⁶ M) was perfused into the adrenal gland for 20 min before introducing the drugs. Other legends are the same as in Fig. 1.

for 4 min from the background level in 12 glands as shown in Figure 1.

Effect of chlorisondamine on pentazocine-evoked CA secretion

In order to certify the effect of chlorisondamine, a selective nicotinic receptor antagonist (Gilman et al., 1991) on pentazocine-induced CA release, the rat adrenal gland was preloaded with 10^{-6} M chlorisondamine for 20 min before pentazocine or ACh was introduced. In the presence of chlorisondamine effect, the CA outputs evoked by doses of 30, 100 and 300 ug of pentazocine were markedly inhibited to 0 (P < 0.01), 18.8±4.7 ng (P<0.01) and 127.5±20.6 ng (P< 0.001) for 4 min as compared with their corresponding control releases of 55.0±25.3 ng, 112.5± 28.5 ng and 275.0±43.4 ng for 4 min from 8 gland, respectively, In 8 glands, ACh-evoked CA secretion after preloading with chlorisondamine was also greatly attenuated to 251.3±32.4 ng/4 min (P <0.001) in comparison with its control of 750.0± 81.4 ng/4 min. Figure 2 illustrates the inhibitory effect of chlorisondamine on CA release evoked by pentazocine and ACh.

Effect of pirenzepine on pentazocine-evoked CA secretion

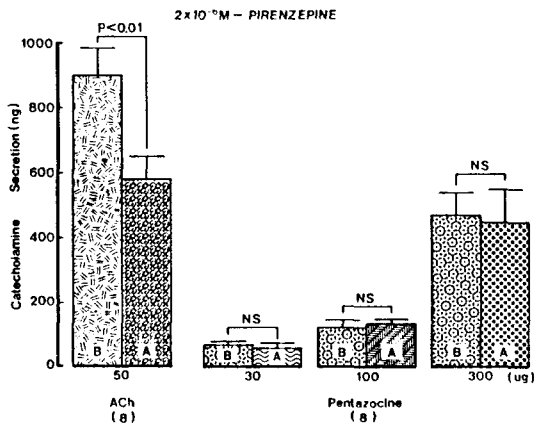It has been found that pirenzepine is a selective

Fig. 3. The effect of pirenzepine on CA secretion e-
voked by pentazocine. Pirenzepine (2×10^{-6} M)
was present 20 min before perfusion of
pentazocine or ACh. Other legends are the same
as in Fig. 1.

Fig. 4. The effect of naloxone on pentazocine-induced
CA secretory response. Naloxone (1.22×10^{-7} M)
was administered for 30 min prior to perfusion
of pentazocine or ACh. Other legends are the
same as in Fig. 1.

muscarinic M_1-receptor antagonist (Doods et al.,
1987; hammer et al., 1988). Thus, it would be inter-
esting to examine the effect of pirenzepine on CA
release evoked by pentazocine and ACh. In the
present work, the CA output induced by
pentazocine or ACh was not affected in the rat
adrenal gland preloaded with 2×10^{-6} M-pir-
enzepine for 30 min. In 8 rat adrenal glands, pen-
tazocine-evoked CA releases at doses of 30, 100
and 300 ug after pretreatment with pirenzepine
were 59.6 ± 11.8 ng, 135.9 ± 15.0 ng and 458.5 ± 95.7
ng for 4 min, respectively as compared with their
control secretions of 70.4 ± 10.3 ng, 127.5 ± 21.8 ng
and 472.5 ± 75.7 ng, respectively as shown in figure
2. There was no statistical significance in differ-
ence between the control responses and
pirenzepine-treated responses in pentazocine-in-
duced CA secretion. However, ACh-induced CA
secretion was considerably inhibited to $586.5 \pm$
71.3 ng (P<0.001) by comparing with its control
release of 905.8 ± 76.5 ng/4 min (Fig. 3).

**The effect of naloxone on pentazocine-evoked CA
secretion**

Since it is known that opiate antagonists facili-
tate the splanchnic nerve stimulation-induced CA
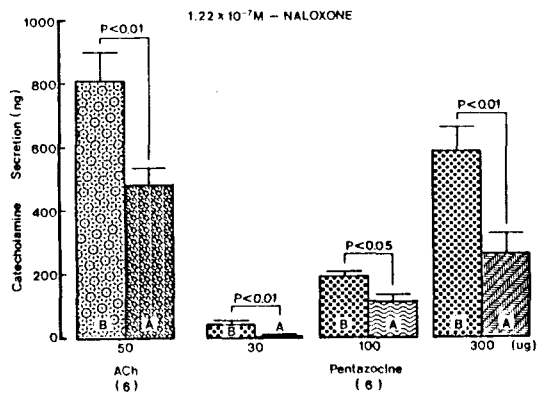secretion from the adrenal gland of anesthetized

dogs (Costa et al., 1983; Kimura et al., 1988) and re-
verse the inhibition of the DMPP-stimulated CA
secretion evoked by etorphine, an opiate agonist
from the bovine adrenal gland (Barron and
Hexum, 1986) while it has been reported that nal-
oxone, an opiate antagonist, inhibited markedly
nicotinic agonist-induced CA release from the
perfused rat adrenal gland (Lim et al., 1992), it is
exicting to test the influence of naloxone on
pentazocine-evoked CA outputs from the per-
fused rat adrenal medulla. After naloxone ($1.22 \times$
10^{-7} M) was preloaded into the adrenal gland for
30 min, CA releases at doses of 30, 100 and 300 ug
of pentazocine were markedly attenuated to $7.5 \pm$
3.4 ng (P<0.01), 114.0 ± 22.5 ng (P<0.05) and 262.5
± 61.5 ng (P<0.01) as compared to their corre-
sponding control releases of 45.0 ± 13.4 ng, $192.0 \pm$
15.3 ng and 592.5 ± 72.7 ng/4min from 6 glands,
respectively (Fig. 4). ACh(50 ug)-induced CA re-
lease in the absence of naloxone amounted to 812.
5 ± 90.3 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 greatly reduced to $485.5 \pm$
51.8 ng (P<0.01)/4 min, which was 60% of the cor-
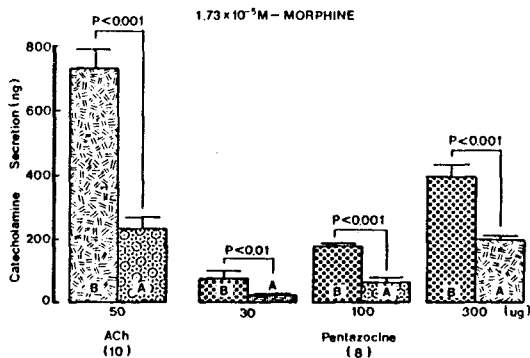responding control release as illustrated in Figure
4.

Fig. 5. The effect of morphine on CA secretion evoked by pentazocine. Morphine (1.73×10^{-5} M) was perfused in to the adrenal gland 30 min before introducing pentazocine of ACh. Other legends are the same as in Fig. 1.

Fig. 6. The effect of enkephalin on pentazocine-evoked CA secretion. Enkephalin (9.68×10^{-6} M) was introduced into the adrenal gland for 30 min before pentazocine of ACh was injected. Other legends are the same as in Fig. 1.

The effect of morphine on pentazocine-evoked CA secretion

It was tried to investigate the influence of morphine on pentazocine-induced CA release from the perfused rat adrenal gland since it has been known that several opioid agonists inhibit the nicotinic receptor-mediated CA release from 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., 1986 a, b; Barron and Hexum, 1986; Kimura et al., 1988; Jary et al., 1989; Lim et al., 1992). Under the presence of morphine (1.73×10^{-5} M) which was preloaded for 30 min, pentazocine-evoked CA secretions at doses of 30, 100 and 300 ug were significantly attenuated to 20.6 ± 3.5 ng ($P < 0.01$), 67.5 ± 14.2 ng ($P < 0.001$) and 206.3 ± 12.3 ng ($P < 0.001$) for 4 min from 8 glands as compared with their corresponding control releases of 79.4 ± 20.3 ng, 180.0 ± 16.4 ng for 4 min, respectively. ACh 50 ug) −induced CA output was considerably weakened to 234.0 ± 38.0 ng ($P < 0.001$) for 4 min which was 32% of the control(735.0 ± 56.7 ng/4 min). Figure 5 illustrates the inhibitory responses of morphine on pentazocine- and ACh-induced CA secretions.
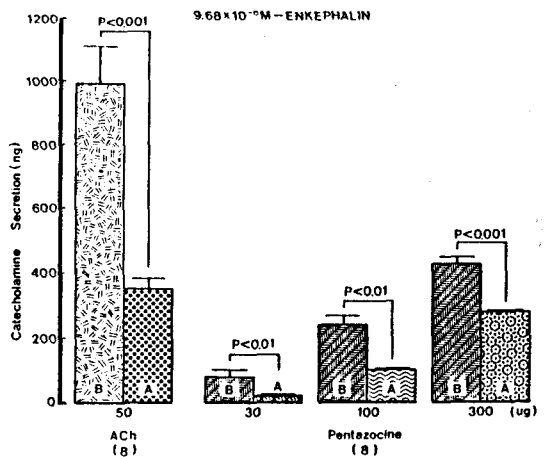
The effect of met-enkephalin on pentazocine-evoked CA secretion

In the light of these facts that pentazocine-induced CA release was significantly inhibited by the pretreatment with morphine as in Figure 5, and that met-enkephalin also blocked ACh- and DMPP-induced CA secretory responses from the perfused rat adrenal glands (Lim et al., 1992), and that beta-endorphine and morphine reduced CA secretion induced by nicotine by as much as fifty percent whereas [Met[5]]-enkephalin diminishes CA release by seventy five percent (Kumakura et al., 1980), it was of interest to examine the influence of met-enkephalin on pentazocine-induced CA secretion in the perfused rat adrenal gland. In the presence of met-enkephalin (9.68×10^{-6} M) which was preloaded into the gland for 30 min, the secretory responses of pentazocine at doses of 30, 100 and 300 ug were significantly attenuated to 25.4 ± 5.7 ng ($P < 0.01$), 108.8 ± 2.5 ng ($P < 0.01$) and 277.5 ± 4.9 ng ($P < 0.001$) for 4 min from 8 glands as compared with their corresponding control releases of 83.6 ± 22.6 ng, 240.0 ± 25.7 ng and 525.0 ± 17.5 ng, respectively. ACh (50 ug)-induced
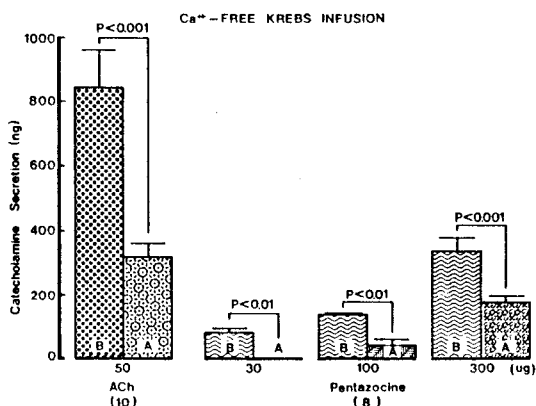
Fig. 7. The effect of perfusion of calcium-free Krebs
solution on secretory response of CA evoked
by pentazocine. Calcium-free Krebs solution
was infused for 30 min prior to introduction of
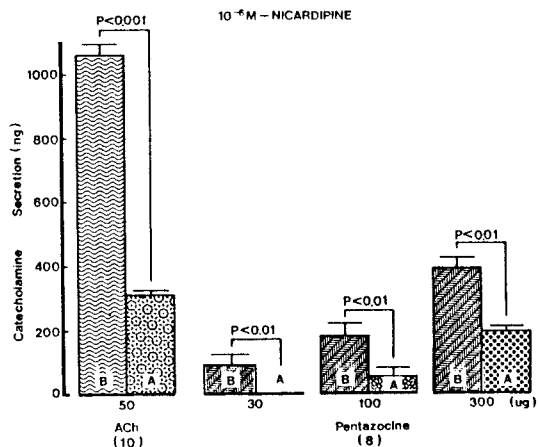pentazocine or ACh. Other legends are as in
Fig. 1.

Fig. 8. The effect of nicardipine on pentazocine-e-
voked CA secretory responses. Nicardipine
(10^{-6} M) was perfused for 30 min before intro-
ducing pentazocine or ACh. Other legends are
the same as in Fig. 1.

CA secretion following the pretreatment with
met-enkephalin was greatly inhibited to 25.4 ± 5.7
ng (P<0.001, n=8)/4 min by comparing with its
control of 997.5 ± 118.2 ng. Figure 6 shows the in-
hibitory effect of met-enkephalin on pentazocine-
and ACh-evoked CA responses.

**The effect of perfusion with calcium-free Krebs on
pentazocine-evoked CA secretion**

Since it is found that the physiological release
of CA and dopamine-beta-hydroxylase from the
perfused cat adrenal gland is dependent on the
extracellular calcium concentration (Dixon, Gar-
cia and Kirpeka, 1975), it is of particular interest
to test whether the secretory effect induced by
pentazocine is also related to extracellular calci-
um ions. Thus, the adrenal gland was preperfused
with calcium-free Krebs solution for 30 min prior
to introduction of pentazocine or ACh. In the
absence of extracellular calcium, CA releases of
pentazocine at doses of 30, 100 and 300 ug were
significantly blocked to o ng (P<0.01, n=8), $47.1 \pm$
17.8 ng (P<0.01, n = 8) and 180.0 ± 19.2 ng (P<
0.001, n=8) for 4 min as compared with their cor-
responding control responses of 84.7 ± 11.5 ng,
139.3 ± 7.1 ng and 341.3 ± 40.2 ng as shown in Fig-

ure 7. ACh (50 ug)-induced CA release following
preloading with calcium-free Krebs solution was
also markedly reduced to 321.0 ± 45.1 ng (P <
0.001, n=10), which was 38% of its control ($846.0 \pm$
119.6 ng) as depicted in Figure 7.

**The effect of nicardipine on pentazocine-evoked
CA secretion**

In order to investigate the effect of nicardipine,
a dihydropyridine derivative and L-type Ca^{++}
channel blocker (Gilman et al., 1991) on pen-
tazocine-evoked CA secretion, nicardipine (10^{-6}
M) was preloaded into the adrenal gland for 30
min. In the presence of nicardipine effect, CA re-
leases induced by pentazocine at the indicated
doses (30, 100 and 30 ug) were greatly depressed
to 0 ng (P<0.01) 56.3 ± 24.5 ng(P<0.01) and $198.8 \pm$
16.7 ng (P<0.01) for 4 min from 8 adrenal glands
in comparison with their corresponding control
values of 95.0 ± 25.8 ng, 183.8 ± 37.7 ng and $397.5 \pm$
24.5 ng for 4 min, respectively. ACh-induced CA
release was also decreased to 315.0 ± 13.8 ng/4 min
(P<0.001) as compared to its control of $1059.0 \pm$
40.2 ng/4 min from 10 glands. Figure 8 illustrates
that nicardipine inhibits CA secretory responses
evoked by pentazocine and ACh.

The effect of TMB-8 on pentazocine-evoked CA release

Since it has been reported that muscarinic, but not nicotinic activation causes CA secretion independent of extracellular calcium in the perfused adrenal glands of the cat (Nakazato et al., 1988), suggesting that the presence of an intracellular calcium pool linked to a muscarinic receptors, and that TMB-8, an intracellular calcium antagonist, inhibits both nicotinic and muscaric stimulation-induced CA release in the rat adrenal glands (Lim et al., 1992), an attempt was made to test the TMB-8 on pentazocine-evoked CA secretion. In 4 rat adrenal glands, CA secretions evoked by pentazocine at doses of 30, 100 and 300 ug after preloading with TMB-8 (10^{-10} M) for 30 min were clearly blocked to 7.5 ± 2.3 ng ($P<0.05$), 30.0 ± 1.5 ng ($P<0.01$) and 172.5 ± 12.9 ng ($P<0.05$) for 4 min in comparison with their control releases of $72,5 \pm 4.3$ ng, 202.5 ± 30.3 ng and 510.0 ± 103.9 ng for 4 min, respectively as shown in figure 9. ACh (50 ug)-induced CA output following pretreatment with TMB-8 (10^{-5} M) was also greatly diminished to 150.0 ± 34.6 ng ($P<0.01$)/4 min as compared with its control response of 607.5 ± 77.5 ng/4 min from 4 rat adrenal glands (Fig. 9).

The effect of pentazocine infusion on DMPP- and ACh-evoked releases

In terms of the facts that pentazocine-induced CA release was markedly inhibited by the pretreatment of chlorisondamine as in Figure 2 and that met-enkephalin and morphine also depressed ACh- and DMPP-induced secretory responses in the perfused rat adrenal gland (Lim et al., 1992), it is of interest to examine the influence of pentazocine perfusion on DMPP- and ACh-induced CA secretion. The adrenal gland was preloaded with 1.75×10^{-4} M pentazocine for 30 min before DMPP and ACh were introduced. In the presence fo pentazocine effect, ACh-induced CA output was greatly attenuated to 306.3 ± 25.6 ng ($P<0.01$, n=12) for 4 min as compared to its corresponding CA secretion of 608.8 ± 64.3 ng/4 min prior to perfusion with pentazocine. In the present work, in the absence of pentazocine, DMPP (10^{-4} M)- evoked CA secretion was $846.8 \pm$
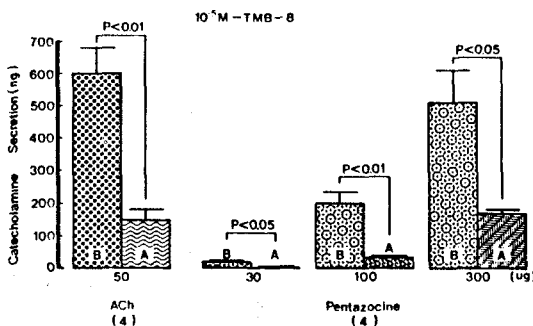

Fig. 9. The effect of TMB-8 on CA secretion evoked by pentazocine. TMB-8 (10^{-5} M) was given into the perfusion stream for 30 min after obtaining the corresponding control responses of pentazocine or ACh. Other legends are as in Fig. 1.
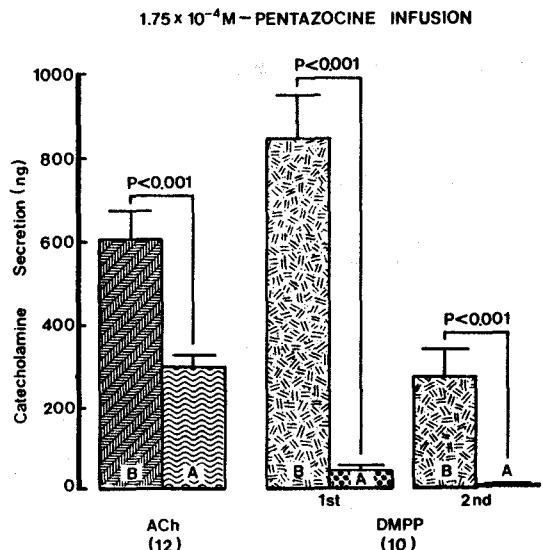
Fig. 10. The effect of pentazocine infusion on CA secretory responses evoked by ACh and DMPP. Pentazocine (1.75×10^{-4} M) was perfused for 20 min after obtaining the control response of DMPP- and ACh-evoked CA secretory responses. Other legends are the same as in Fig. 1.

105.1 ng (0~4 min) ng and 275.8±63.7 (4~8 min) ng while in the presence of pentazocine (1.75×10^{-4} M) which was preperfused into the giand, DMPP-evoked CA secretion was prominently reduced to 43.6±12.2 ng (0~4 min, P<0.01) and 9.2±2.7 ng (4~8 min, P<0.01) from 10 adrenal glands, respectively. Figure 10 illustrates the inhibitory effect of pentazocine perfusion on the secretory responses induced by DMPP and ACh.

DISCUSSION

In the present study, it has been found that pentazocine causes a dose-related increase in CA secretion from the isolated perfused rat adrenal medulla by a calcium-dependent exocytotic mechanism, and that this releasing effect of CA seems to be mediated through activation of opioid receptors located on adrenomedullary chromaffin cells, which may be also associated with stimulation of cholinergic nicotinic receptors. In terms of the fact that pentazocine produces CA secretion in the perfused rat adrenal gland, the present experimental result appears to be similar to the case of the perfused dog adrenals (Fukumitsu et al., 1991) but the mechanism of its secretory action is much different from each other. Fukumitsu and his colleagues (1991) have shown that pentazocine directly acts on the dog perfused adrenal medulla to induce CA efflux via a non-exocytotic mechanism, and that opioid receptors do not play a role in this action. On the other hand, Bender and Ardentova (1979) have reported in experiments on white rats that pentazocine (3 mg/kg) reduces the adrenaline content in cardiac and hepatic tissues, the ascorbic pathway of carbohydrate oxygenation being predominant. However, the present experimental data demonstrate that pentazocine-induced CA secretory effect is surely exerted through the activation of opiate receptors existed on adrenomedullary chromaffin cells of the rat. Thus, pentazocine-evoked CA secretory activity is also surely due to peripheral sympathetic stimulation, not to the direct of central action. In support of this idea, Takki and his co-workers (1973) have reported that epidural blockade prevents the pressor response to pentazocine in man but fails to cause any significant differ-

ence in plasma CA levels. Although they insisted the site of pentazocine-evoked sympathomimetic stimulation was central, it has not been verified. Sellevold and his researchers (1985) reported a case of phaeochromocytoma in which pentazocine caused a marked increase in blood pressure in spite of the presence of spinal blockade. In this case, the pressor response to pentazocine seemed to be mediated not by a central action but by a direct action on tumor cells to induce CA efflux, because the spinal anesthesia would block sympathetic discharge, and furthermore, tumor cells ordinarily have no sympathetic innervation (Winkler and Smith, 1968). In the present work, the fact that pentazocine-induced CA release was greatly inhibited by naloxone-pretreatment illustrates that pentazocine causes CA secretion through the opiate receptors. Furthermore, this idea could be supported by the findings that pentazocine-evoked CA secretion was also depressed by preloading with morphine and met-enkephalin which are known to be opiate agonists.

It has been also reported that opioid antagonists, including naltrexone and naloxone, inhibit the nicotine-induced CA secretion from the adrenal chromaffin cells in culture (Lemaire et al., 1981; Dean et al., 1982; Marley et al., 1986a), and that naloxone also depresses ACh- and DMPP-evoked CA secretion in the perfused rat adrenal glands (Lim et al., 1992). In terms of these findings, the present experimental results that naloxone markedly inhibits pentazocine-evoked CA release in the perfused rat adrenal gland suggest strongly that naloxone markedly inhibits pentazocine-evoked CA release in the perfused rat adrenal gland suggest strongly that the inhibitory effect of nicotinic stimulation-evoked CA secretion by naloxone may be exerted through the other unknown mechanism in addition to the opiate antagonism. Opioid receptors have been shown to exist in the chromaffin cells and to play a significant role in modulating CA release (Saiani and Guidotti, 1982). Most opioid receptors in adrenal medullary cells are k-receptors (Castanas et al., 1983; 1985a; 1985b). Pentazocine is a benzomorphan derivative and is considered to possess k-and δ-agonistic activities and weak μ-receptor antagonistic action (Martin, 1983). The present findings demonstrate that the actions of pentazocine, i.e. both the stimu-

latory effect on CA secretion and the inhibitory effects on DMPP-and ACh-evoked CA release appear to be mediated through the activation of opioid receptors, because naloxone inhibit pentazocine-evoked CA secretory activity, suggesting that pentazocine may activate k- or δ-opioid receptors.

In the present investigation, pentazocine- as well as ACh-induced CA release was greatly depressed by prior treatment with chlorisondamine, an autonomic ganglionic blocking agent. This finding indicates strongly that pentazocine-evoked CA secretion is associated with stimulation of cholinergic nicotinic receptors in adrenal medulla. Moreover, the fact that ACh- and DMPP-evoked CA secretion was prominently attenuated by prior perfusion of pentazocine for 20 min confirms that pentazocine-induced CA release may be surely associated with cholinergic nicotinic receptors similar to that of ACh or DMPP. Pretreatment with pirenzepine, a selective muscarinic M_1-receptor antagonist (Doods et al., 1987; Hammer et al., 1988) did not affect the CA secretion of pentazocine while did inhibit greatly ACh-induced secretory effect of CA. This finding indicates that pentazocine-evoked CA release is not related to muscarinic M_1-receptor activation.

Generally, the indispensible role of calcium in the neurosecretory process has been well established. Yet, according to the assumptions of Baker and Knight (1978; 1980), the relationship between the concentration of intracellular calcium and the transmitter release has not been determined in nerve terminals. As mentioned above, calcium plays the crucial role in process of depolarization-neurotransmitter release coupling in many types of secretory cells (Douglas, 1968; Schultz and Stolze, 1980; Williams, 1980). In the present work, removal of extracellular Ca^{++} inhibited greatly CA secretion evoked by pentazocine or ACh. The secretory effect of CA by pentazocine is apparently dependent on extracellular calcium.

However, in the present experiment, the reason for considerable response to pentazocine is not clear. It may be that chromaffin cells of the rat adrenal gland contain an intracellular store of calcium which participates in the secretion of CA as shown in the bovine adrenal gland (Baker and Knight, 1978). Such a store may not be easily depleted by mere removal of extracellular calcium.

Some investigators (Boxler, 1968; Ohashi et al., 1974; Casteels and Raeymeakers, 1979; Malagodi and Chiou, 1974; Takahara et al., 1990) reported that intracellular stores of calcium have been shown to play some role in contraction of smooth muscle produced by noradrenaline of ACh in Ca^{++}-free medium.

Moreover, in the present study, the finding that considerable response to pentazocine still remained in the presence of a Ca^{++}-channel blocker nicardipine although the secretory effect of CA evoked by pentazocine is significantly diminished by pretreatment with Ca^{++}-entry blocker may support above the results.

In addition, in terms of the fact that prior administration of TMB-8 inhibited clearly CA secretion evoked by pentazocine as well as that by ACh in the perfused rat adrenal gland, it is felt that pentazocine-evoked CA release may be exerted at least partly through mobilization of calcium from the intracellular store located with in chromaffin cells. TMB-8 [3. 4. 5-trimethoxybenzoate 8-(N.N-diethylamino) octylester], a benzoic acid derivative, is known well to act by preventing mobilization of calcium from intracellular stores without altering Ca^{++} influx into stores (Charo et al., 1976; Chiou and Malagodi, 1975; Rubin et al., 1980; Smith and Idea, 1979; Wiedenkeller and Sharp, 1984). In support of the present results, Yamada and his colleagues (1988) have found that the secretory effect of CA evoked by caffeine is inhibited by TMB-8 from perfused cat adrenal glands in the absence fo extracellular calcium. The similar results were also obtained from the rat adrenal gland (Lim et al., 1991; Lim et al., 1992). Moreover, it is known that ACh and pilocaprpine cause a partial increased CA release from both guinea pig adrenal glands (Nakazato et al., 1984) and perfused cat adrenal glands (Nakazato et al., 1988) with Ca^{++}-free Lock solution.

At least a part of the pentazocine-evoked CA secretion is induced by a sustained intracellular calcium rise due to the continuous Ca^{++} influx through Ca^{++} channels. These channels are sensitive to dihydropyridine, dut do not appear to be identical to the L-type voltage-sensitive Ca^{++} channel. Anyway, from the present experimental results, CA secretion evoked by pentazocine is exerted through stimulation of opioid receptors and

still remains considerably even after removal of Ca^{++} from the Krebs solution, at least partly being due to the liberation of Ca^{++} from the internal stores.

REFERENCES

Alderman EL, Barry WH, Graham AF, Harrison DC: Hemodynamic effects of morphine and pentazocine differ in cardiac patients. N Engl J Med 287: 623-627, 1972

Anton AH and Sayre DF: A study of the factors affecting the aluminum oxidetrihydroxylindole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 138: 360-375, 1962

Baker PF and Knight DE: Calcium-dependent exocytosis in bovine adrenal medullary cells with leaky plasma membrane. Nature 276: 620-622, 1978

Baker PF and Knight DE: The relation between ionized cacium and cortical granule exocytosis in eggs of the sea urchin Echinus esculentus. Pro R Soc London Ser 207: 149-161, 1980

Barron BA and Hexum TD: Modulation of bovine adrenal gland secretion by etorphine and diprenorphine. Life Sci 38: 935-940, 1986

Bender KI and Ardentova NN: Effect of pentazocine on the catecholamine content, phosphorylase activity and carbohydrate metabolism in tissue. Farmakol-Toksikol 42(6): 655-658, 1979

Boxler E: Role of calcium in inhibition of activity of smooth muscle. Am J Physiol 216: 671-674, 1968

Castanas E, Bourhim N, Giraud P, Boudouresque F, Cantau P and Oliver C: Interaction of opiates with opioid binding sites in the bovine adrenal medulla: I. Interaction with sigma and u sites. J Neurochem 45: 677-687, 1985a

Castanas, E, Bourhim N, Giraud P, Boudouresque F, Cantau P and Oliver C: Interaction of opiates with opioid binding sites in the bovine adrenal medulla: II. Interaction with sites. J Neurochem 45: 688-693, 1985b

Castanas E, Giraud P, Audigier Y, Drissi R, Boudouresque F, ConteDevolx B, Oliver C: Opiate binding sites spectrum on bovine adrenal medullas and six human pheochromocytomas. Life Sci 33: 295-298, 1983

Casteels R and Raeymaekers L: The action of acetylcholine and catecholamines on an intracellular calcium store in the smooth muscle cells of the guinea-pig taenia cells. J Physiol 294: 51-68, 1979

Charo IF, Feinnman and Detwiler TC; Inhibition of platelet secretion by and antagonist of intracellular cal-

cium. Biochim Biophys Res Commun 72: 1462-1467, 1976

Chiou CY and Malagodi MH: Studies on the mechanism of action of a new Ca^{++} antagonist-(N, N-diethylamino-octyl-3,4,5-trimethoxy benzoate hydrochloride in smooth muscles, Br J Pharmacol 53: 279-285, 1975

Costa E, Guidotti A, Hanbauer L and Saiani L: Modulation of nicotinic receptor function by opiate recognition sites highly selective for Met^6-enkephalin (Arg^6 Phe^7). Fed Proc 42: 2946-2952, 1983

Dean DM, Lemaire S and Livett BG: Evidence that inhibition of nicotine-mediated catecholamine secretion from adrenal chromaffin cells by enkephalin, beta-endorphin, dynorphin(1~13) and opiates is not mediated via specific opiate receptors. J Neurochem 38: 606-614, 1982

DeBand ML, Jagger JA T's and B's: Midwestern heroin substitute. Clin Toxical 18: 1117-1123, 1981

Dixon Wr, Garcia AG and Kirkekar SM: Release of catecholamines and dopamine-beta-hydroxylase from the rat adrenal gland of the cat. J Physiol 244: 805-824, 1975

Doods HN, Mathy MJ, Davesko K, Van Charldorp KJ, Dejong A and Ven Zwieten PA: Selectivity of muscarinic agonists in radioligand and in vivo experiments for the putative M_1, M_2 and M_3 receptors. J Pharmacol Exp Ther 242: 257-262, 1987

Douglas WW: Stimulus-secretion coupling: The concept and clues from chromaffin and other cells. Br J Pharmacol 34: 451-474, 1968

Fukumitsu K, Sumikawa K, Hayash Y, Kinouchi K and Yoshiya I: Pentazocine-induced catecholamine efflux from the dog perfused adrenal. J Pharm Pharmacol 43: 331-336, 1991

Gilman AG, Rall TW, Nies AS and Taylor P: The pharmacological basis of Therapeutics. 8th Ed., Maxwll Macmillan International edition, New York, p181, p764, p794, 1991

Hammer R, Monferini E, Deconti L, Giraldo E, Schiavi GB and Landsky H: Ditinct muscarinic receptor subtypes in the heart and in exocrine glands. In cellular and molecular basis of cholinergic function. Ed. by Dowall MJ and Hawhorne JN, New, Ellishorwood, pp56-63, 1988

Jarry H, Dietrich M, Barthel A, Giesler A and Wuttke W: In vivo demonstration of a paracrine, inhibitory action of met-enkephalin on adrenomedullary catecholamine release in the rat. Endocrinology 125(2): 624-629, 1989

Kimura T, Katoh M and Satoh S: Inhibition by opioid agonists and enhancement by antagonists of the release of catecholamines from the dog adrenal gland in response to splanchnic nerve stimulation: Evidence for the functional role of opioid receptors. J Pharmacol Exp Ther

244(3): 1098-1102, 1988

Kumakura K, Karoum F, Guidotti A and Costa E: *Modulation of nicotinic receptors by opiate receptor agonists in cultured adrenal chromaffin cells. Nature (Lond) 283: 489-492, 1980*

Lange WR, Lasinki DR: *The clinical pharmacology of pentazocine and tripelennamine (T's and B's). Adv Alcohol Subst Abuse 5: 71-83, 1986*

Lee G, Demaria AN, Amsterdam EA, Realyvasquez F, Angel J, Morrisoa S, Mason DT: *Comparative effects of morphine, meperidine and pentazocine on cardiovasculartory dynamics in patients with acute myocardial infarction. Am J Med 60: 949-955, 1976*

Lemaire S, Livett B, Tseng R, Mercier P and Lemaire I: *Studies in the inhibitory action of opiate compounds in isolated bovine and adrenal chromaffin cells: non-involvement of stereospecific opiate binding sites. J Neurochem 36: 886-892, 1981*

Lim DY, Lee JJ and Choi CH: *Effect of opioid on nicotinic receptor-mediated catecholamine secretion in the rat adrenal gland. Korean J Pharmacol 28(2): 181-190, 1992*

Lim DY, Lee JH, Kim WS, Lee EH, Kim SP, Lee BJ and Koh ST: *Studies on secretion of catecholamines evoked by caffeine from the isolated perfused rat adrenal glands. Arch Pharmac Res 14(1): 55-67, 1991*

Malagodi MH and Chiou CY: *Pharmacological evaluation of a new Ca^{++} antagonist, 8-(N,N-diethylamino)-octyl-3, 4, 6-trimethoxybenzoate hydrochloride) [TMB-8]: Studies in smooth muscle. Eur J Pharmacol 27: 25-33, 1974*

Manner T, Kanto J, Schcinin H, Schcinin M: *Meptazinol and pentazocine: plasma catecholamines and other effects in healthy volunteers. Br J Clin Pharmacol 24: 689-697, 1987*

Marley PD, Mitchelhill KI and Livett BG: *Effects of opioid peptides containing the sequence of met^5-enkephalin or leu^5-enkephalin on nicotine-induced secretion from bovine adrenal chromaffin cells. J Neurochem 46: 1-11, 1986a*

McGwier BW, Alpert MA, Panayiotou H and Lambert CR: *Acute myocardial infarction associated with intravenous injection of pentazocine and tripelennamine. Chest 101: 1730-1732, 1992*

Nagle RE, Pilcher J: *Respiratory and circulatory effect of pentazocine: reviewing analgesics often used in myocardial infarction. Br Heart J 34: 244-251, 1972*

Nakazato Y, Yamada Y, Tomita U and Ohga A: *Muscarinic agonists release adrenal catecholamines by mobilizing intracellular Ca^{2+}. Proc Jap Acad 60: 314-317, 1984*

Nakazato Y, Ohga A, Oleshansky M, Tomita U and Yamada Y: *Voltage-independent catecholamine release mediated by the activation of muscarinic receptors in*

guinea-pig adrenal glands. Br J pharmacol 93: 101-109, 1988

Ohashi H, Takewaki T and Okada T: *Calcium and the contractile effect of carbachol in the depolarized guinea pig taenia caecum. Jap J Pharmacol 24: 601-611,1974*

Paciorek PM, Todd MH, Wateriall JF: *The effects of meptazinol in comparison with pentazocine, morphine and naloxone in a rat model of anaphylactic shock. Br J Pharmacol 84: 469-475, 1985*

Polkis A, Whyatt PL: *Current trends in the abuse of pentazocine and tripelennamine: The metropolitan St. Louis experience J Forensic Sci 25: 72-78, 1988*

Rubin RP, Shen JC and Laychock SG: *Evidence for the mobilization fo cellular calcium by prostacyclin in cat adrenocortical cells. The effect of TMB-8. Cell Calcium 1: 391-400, 1980*

Saiani L and Guidotti A: *Opiate receptor-mediated inhibition of catecholamine release in primary cultures of bovine adrenal chromaffin cells. J Neurochem 39: 1669-1676, 1982*

Schulz I and Stolze HH: *The exocrine pancreas: The role of secretogogues cyclic nucleotides and calcium in enzyme secretion. Ann Rev Physiol 42: 127-156, 1980*

Sellevold OFM, Raeder J, Stenseth R: *Undiagnosed phaeochromocytoma th the perioperative period. Acta Anaesthesiol Scand 29: 474-479, 1985*

Smith RJ and Idea SS: *Phorbol myristate acetate-induced release of granul enzymes from human neutrophils inhibition by the calcium antagonist, 8-(N, N-diethylamino)-octyl-3, 4, 5-trimethoxybenzoate hydrochloride. Biochem Biophys Res Commun 91: 263-271, 1979*

Takahara A, Suzuki-Husaba M, Hisa H and Satoh S: *Effects of a novel Ca^{2+} entry blocker, CD-349, and TMB-8 on renal vasoconstriction induced by angiotensin II and vasopressin in dogs. J Cardiovasc Pharmacol 16: 966-970, 1990*

Takki S, Nikki, Tammisto T, Jaatela A: *Effect of epidural blockade on the pentazocine-induced increase in plasma catecholamines and blood pressure. Br J Anaesth 45: 376-380, 1973*

Tallarida RJ, Murray RE: *Mannual of pharmacologic calculations with computer programs. 2nd ed Springer-Verlag, New Uork, p132, 1987*

Tammisto T, Jaatela A, Nikki P, Takki S: *Effect of pentazocine and pethidine on plasma catecholamine levels. Ann Clin Res 3: 22-29, 1971*

Rhompson EB: *Cardiovascular effects of pentazocine and tripelennamine in combination in conscious rats. Res Comm Subst Abuse 4: 201-214, 1983*

Wakade AR: *Studies on secretion of catecholamines evoked by acetylcholine of transmural stimulation of the rat adrenal gland. J Physiol 313: 463-480, 1981*

Wiedenkeller DE and Sharp GWG: *Unexpected potentia-*

tion of insulin release by the calcium store bloker TMB-8. Endocrinology 114: 116-119, 1984

Williams JA: *Regulation of pancreatic acinal cell function by intracellular calcium. Science 177: 1104-1105, 1980*

Wihkler H, Smith AD: *Catecholamines in phaeo-chromocytoma. Normal storage but abnormal release?*

Lancet 1: 793-795, 1968

Yamada Y, TeraokaH, Nakazato Y and Ohga a: *Intra-cellular Ca²⁺ antagonist TMB-8 blocks catecholamine secretion evoked by caffeine and acetylcholine from per-fused cat adrenal glands in the absence of extracellular Ca²⁺. Neuroscience Let 90: 338-342, 1988*

=국문초록=

흰쥐 관류부신에서 Pentazocine의 카테콜아민 분비작용의 기전

조선대학교 의과대학 약리학교실

임동윤 · 김봉한 · 허재봉 · 최철희 · 김진호 · 장 영 · 이재준

Pentazocine은 opioid 수용체에 대한 흥분작용과 길항작용을 겸유한 opioid계 약물로 알려져 있다. 본 연구에서 흰쥐 적출 관류부신으로 부터 pentazocine의 catecholamine (CA) 분비작용을 관찰하여 그 기전을 규명하고 또한 다른 opioid의 작용과 비교하여 얻어진 결과는 다음과 같다.

Pentazocine (30~300 ug)을 부신정맥내에 주사하였으때 현저한 용량의 존성의 CA 분비작용을 나타내었다. Pentazocine의 이러한 CA 분비작용은 chlorisondamine (10^{-6} M), naloxone (1.22×10^{-7} M), morphine (1.73×10^{-5} M), enkephalin (9.68×10^{-6} M), nicardipine (10^{-6} M) 및 TMB-8 (10^{-5} M)등의 전처치로 뚜렷이 억제되었으나 pirenzepine (2과10^{-6} M)의 전처치에 의해서는 영향을 받지 않았다. Ca⁺⁺-free Krebs 용액으로 30분간 관류한 후에 pentazocine의 CA 분비작용은 현저한 감소를 나타냈었다. Pentazocine (1.75×10^{-4} M)을 20분간 관류시킨 후에 ACh (5.32×10^{-3} M)과 DMPP (10^{-4} M)에 의한 CA 분비작용이 의의 있게 감약되었다.

이상과 같은 연구결과를 종합하면, pentazocine은 흰쥐 적출 관류부신에 투여시 현저한 CA 분비작용을 일으키고 이는 칼슘의존성 exocytotic mechanism에 의한 것으로 생각되며, 이러한 pentazocine의 CA 분비작용은 부신 chromaffin cell에 있는 opioid 수용체의 활성화를 통하여 나타나며, 또한 부신의 nicotine 수용체의 흥분작용과도 관련성이 있는 것으로 사료된다.