

.2006년 8월
박사학위논문

Inhibitory Mechanism of Ketamine on Catecholamine Secretion in the Perfused Rat Adrenal Medulla

조선대학교 대학원

의학과

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<국문 초록>

흰쥐 관류부신수질에서 카테콜아민 분비작용에 대한
Ketamine의 억제기전

정 용 훈

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본 연구는 해리성 마취제인 ketamine이 Sprague-Dawley종 흰쥐로부터 분리 적출한 부신의 관류모델에서 카테콜아민 (catecholamines, CA) 분비작용에 미치는 영향을 검색하여 그 작용기전을 규명하고, 초단시간 작용성 barbiturate계 마취제인 thiopental과 작용상의 차이유무를 비교 검색코자 본 연구를 시행하였다. Ketamine 이나 thiopental 자체는 기초 CA 분비량에 영향을 미치지 않았다. Ketamine (30~300 μ M)은 부신정맥 내로 60분간 관류 시 비교적 용량 및 시간 의존적으로 ACh (5.32 mM), 고칼륨 (56 mM, 막탈분극제), DMPP (100 μ M, 선택성 니코틴수용체 작용제), 및 McN-A-343 (100 μ M, 선택성 무스카린수용체 작용제)에 의한 CA 분비반응을 억제하였다. 또한, ketamine (100 μ M) 존재 하에서, veratridine (100 μ M, 전압 의존성 나트륨통로 활성화제), Bay-K-8644 (10 μ M, L형 칼슘통로 활성화제) 및 cyclopiazonic acid (10 μ M, 세포질에서 Ca^{2+} -ATPase 억제제)에 의한 CA 분비반응이 억제되었다.

흥미롭게도, thiopental (100 μ M) 존재 하에서 ACh, 고칼륨, DMPP, McN-A-343, veratridine, Bay-K-864 및 cyclopiazonic acid에 의한 CA분비작용도 시간 의존적으로 억제되었다.

이와 같은 연구결과를 종합하여 보면, 흰쥐 적출 관류 부신피질에서 ketamine 및 thiopental은 콜린성(니코틴 및 무스카린 수용체) 흥분작용 및 막탈분극에 의한 CA 분비작용에 대하여 억제작용을 나타내었다. 이러한 ketamine의 억제작용은 흰쥐 적출 부신피질에서 전압 의존성 칼슘통로를 통한 크롬친화세포 내로 칼슘유입 및 세포 내 칼슘저장고로부터 칼슘유리 억제 작용 뿐만 아니라 전압 의존성 나트륨통로를 통한 세포 내 나트륨 유입 억제 작용에 나타나며, 이작용은 적어도 니코틴수용체 차단작용과 관련성이 있는 것으로 생각된다. 또한 용량 및 작용기전으로 보아 ketamine은 흰쥐 관류 부신피질의 CA분비작용에서 thiopental과 유사한 억제작용을 일으키는 것으로 사료된다.

I. INTRODUCTION

Ketamine is an intravenous anesthetic and also used clinically to induce a dissociative anesthesia. It has also been known to produce analgesic, psychotropic and sympathomimetic effects (Domino et al., 1965; Traber et al., 1968). During the initial phase of anesthesia, ketamine elicits a pronounced rise in blood pressure and heart rate (Gemperle et al., 1973; Lanning and Harmel, 1975; Rust et al., 1978). Ketamine has also been shown to produce pressor effect (Corssen and Domino, 1966; Domino et al., 1965; Kreuscher and Gauch, 1967; Traber et al., 1968; Virtue et al., 1967) on the cardiovascular dynamics. The increase in systolic and diastolic pressure in animals, as well as in man, is mainly due to enhanced cardiac output (Domino et al., 1965; Virtue et al., 1967; Schwartz and Horwitz, 1975). Moreover, A number of investigators have reported that ketamine suppresses [^3H]NA uptake in various sympathetic nervous tissues (Taube et al., 1975; Azzaro and Smith, 1977; Garty et al., 1990; Graf et al., 1995) and the adrenal medulla (Hara et al., 1998; Hara et al., 2002). Local administration of ketamine was also found to induce an increased norepinephrine (NE) levels in dialysate in anesthetized cats (Kitagawa et al., 2002)

In contrast, it has been known that ketamine produces depressor effect (Diaz et al., 1976; McGrath et al., 1975). The depressor effect has been ascribed to the direct action of ketamine on the heart (Diaz et al. 1976; Dowdy and Kaya, 1968; Goldberg et al., 1970; Schwartz and Horwitz et al., 1975; Traber et al., 1968) and blood vessels (Yamanaka and Dowdy, 1974). Several reports showed that ketamine inhibited the secretion of catecholamines caused by acetylcholine

receptor agonists from the isolated dog adrenals perfused with modified Locke's solution (Sumikawa et al., 1983), by nicotinic agonist at concentrations which did not alter secretion induced by elevated K^+ depolarization from cultured bovine adrenal chromaffin cells (Purifoy and Holz, 1984), and by acute cold exposure anesthesia in adult rhesus monkeys (Kolka et al., 1983). Ketamine also inhibited carbachol-induced ^{45}Ca influx and catecholamine secretion from cultured bovine adrenal medullary cells in a concentration-dependent manner (Takara et al., 1986).

On the other hand, some investigators have reported that ketamine causes biphasic effects (Dowdy and Kaya, 1968; McCarthy et al., 1965) on the cardiovascular dynamics. Okamoto and his co-workers (1996) demonstrated that ketamine depresses dopamine (DA) efflux related to membrane depolarization (K^+) from rat pheochromocytoma (PC-12) cells as a model of sympathetic nervous system but it promotes a number of spontaneous DA efflux. In dogs, no alteration of circulating catecholamines after injection of ketamine was found (Klose & Peter, 1973; Hensel et al., 1972).

Anyway, it seems that there are so many conflicting results concerning effects of ketamine on the cardiovascular system and its neural regulatory systems. Therefore, the present study was designed to re-investigate the effects of ketamine on secretion of catecholamines (CA) by cholinergic receptor stimulation and membrane depolarization from the perfused model of the isolated rat adrenal gland, and to clarify the mechanism of action.

II. MATERIALS AND 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 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 gauze pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

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

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; CaCl₂, 2.5; MgCl₂, 1.18; NaHCO₃, 25; KH₂PO₄, 1.2; glucose, 11.7. The solution was constantly bubbled with 95 % O₂ + 5 % CO₂ 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), veratridine (10⁻⁴ M) and McN-A-343 (10⁻⁴ M) for 4 minutes and/or a single injection of ACh (5.32 x 10⁻³ M) and KCl (5.6 x 10⁻² M) in a volume of 0.05 ml were made into perfusion stream via a three-way stopcock, respectively. 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

perfusate was collected for 4 to 8 min. The CA 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 ketamine on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing ketamine for 60 min, 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 ketamine, 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 and Sayre, 1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981) using fluorospectrophotometer (Kontron Co., Milano, Italy).

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

Statistical analysis

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

Drugs and their sources

The following drugs were used: ketamine hydrochloride (Yuhan Corporation, Seoul, Korea), acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), veratridine, norepinephrine bitartrate, methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (BAY-K-8644) (Sigma Chemical Co., U.S.A.), cyclopiazonic acid, (3-(*m*-chloro-phenyl-carbamoyl -oxy)-2-butynyltrimethyl ammonium chloride [McN-A-343] (RBI, U.S.A.), thiopental sodium (Choongwae Pharmaceutical Corporation, Seoul, Korea). 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.

III. RESULTS

Effect of ketamine on CA secretion evoked by ACh, excess 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 22 ± 2.1 ng/2 min (n=8). Since ketamine was found to suppress [³H]NA uptake in the adrenal medulla (Hara et al., 1998; Hara et al., 2002), it was attempted initially to examine the effects of ketamine itself on CA secretion from the perfused model of the rat adrenal glands. However, in the present study, ketamine ($3 \times 10^{-5} \sim 3 \times 10^{-4}$ M) itself did not produce any effect on basal CA output from perfused rat adrenal glands (data not shown). Therefore, it was decided to investigate the effects of ketamine on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15~20 min-intervals. Ketamine was perfused for 60 min.

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 1058 ± 49 ng for 4 min. However, the pretreatment with ketamine in the range of $3 \times 10^{-5} \sim 3 \times 10^{-4}$ M for 60 min concentration- and time-dependently inhibited ACh-stimulated CA secretion. As shown in Fig. 2 and Table 1~3, in the presence of ketamine, CA releasing responses were inhibited by 55% of the corresponding control release (100%). Also, it has been found that the depolarizing agent, KCl (5.6×10^{-2} M) stimulates markedly CA secretion (605 ± 27 ng for 0-4 min). As shown in Fig. 3 and Table 1~3, following the pretreatment with ketamine (3×10^{-5} M $\sim 3 \times 10^{-4}$ M), excess K⁺

(5.6×10^{-2} M)-stimulated CA secretion was significantly inhibited to 54% of the control release (100%). When perfused through the rat adrenal gland, DMPP (10^{-4} M), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (1071 ± 34 ng for 0-8 min). However, as shown in Fig. 4, DMPP-stimulated CA secretion after pretreatment with ketamine was greatly reduced to 53% of the corresponding control release (Table 1~3). McN-A-343 (10^{-4} M), which is a selective muscarinic M_1 -agonist (Hammer and Giachetti, 1982), perfused into an adrenal gland for 4 min caused an increased CA secretion (486 ± 20 ng for 0-4 min). However, McN-A-343-stimulated CA secretion in the presence of ketamine was markedly depressed to 56% of the corresponding control secretion as depicted in Fig. 5 and Table 1~3.

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

Since Bay-K-8644 is known to be a calcium channel activator which enhances basal Ca^{2+} uptake (Garcia et al., 1984) and CA release (Lim et al., 1992), it was of interest to determine the effects of ketamine 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 ketamine was inhibited to 72% of the corresponding control release (461 ± 21 ng for 0-4 min) from 10 glands as shown in Fig. 6 and Table 4.

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been

described as a highly selective inhibitor of Ca^{2+} -ATPase in skeletal muscle sarcoplasmic reticulum (Goeger and Riley, 1989; Seidler et al., 1989). The cyclopiazonic acid (10^{-5} M)-evoked CA secretory response prior to treatment with ketamine was 435 ± 21 ng for 0-4 min. As shown in Fig. 7 and Table 4, however, in the presence of ketamine (10^{-4} M) in 10 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was depressed to 71% of the control response (100%).

It has been known that veratridine-induced Na^{+} influx mediated through Na^{+} channels increased Ca^{2+} influx via activation of voltage-dependent Ca^{2+} channels and produced the exocytotic secretion of CA in cultured bovine adrenal medullary cells (Wada et al., 1985a). As shown in Fig. 8 and table 4, veratridine greatly produced CA secretion (1254 ± 63 ng for 0-4 min). Ketamine (100 μM) also attenuated veratridine-induced CA secretion by 53% of the corresponding control release in a time-dependent manner.

Effect of thiopental on CA secretion evoked by ACh, excess K^{+} , DMPP and McN-A-343 from the perfused rat adrenal glands

It has been reported that thiopentone blocks the stimulus-secretion coupling in the chromaffin cells as a result of inhibiting Ca^{2+} uptake through nicotinic receptor-linked channels in cultured bovine adrenal chromaffin cells (Matsumoto et al., 1986). Therefore, in order to compare the effect of thiopental with that of ketamine, it was likely of interest to examine effect of thiopental on CA secretion evoked by ACh, high K^{+} and DMPP from the isolated perfused rat adrenal glands. In order to test the effect of thiopental on cholinergic receptor-stimulated CA

secretion as well as membrane depolarization-mediated secretion, concentration of 10^{-4} M thiopental was loaded into the adrenal medulla. In the present experiment, ACh (5.32 mM)-evoked CA release before perfusion with thiopental (10^{-4} M) was 1440 ± 125 ng (0-4 min) from 8 rat adrenal glands. However, in the presence of thiopental (10^{-4} M) for 60 min, it was significantly attenuated to 71% of the control release (Fig. 9 and Table 5). High K^+ (56 mM)-evoked CA release under the presence of thiopental (10^{-4} M) was also reduced to 71% of the corresponding control secretion (614 ± 32 ng, 0-4 min) from 10 glands, as shown in Fig. 10 and Table 5. In 6 rat adrenal glands, DMPP (10^{-4} M) perfused into the adrenal gland produced great CA secretion (1024 ± 47 ng, 0-8 min) prior to loading with thiopental. Following perfusion with thiopental (10^{-4} M) it was diminished to 75% of the corresponding control release (Fig. 11 and Table 5). Moreover, in the presence of thiopental (10^{-4} M), McN-A-343-evoked CA secretory responses was also time-dependently inhibited by 72% of the control secretion (461 ± 21 ng, 0-4 min) from 10 glands, as shown in Fig. 12 and Table 5.

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

In the presence of thiopental (10^{-4} M), the secretory responses evoked by Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) given into the adrenal gland for 4 min were greatly depressed to 69% and 71% of their the corresponding control responses (410 ± 17 ng for 4 min and 435 ± 21 ng for 4 min), respectively (Fig. 13-14 and Table 6). The CA secretion evoked by veratridine (10^{-4} M), an

activator of Na⁺ channels, was greatly increased to 1203±44 ng for 0-4 min before loading of ketamine. However, in the presence of thiopental, it was inhibited to 53% of the corresponding control secretion, as shown in Fig. 15 and table 6.

IV. DISCUSSION

The experimental results obtained from the present study demonstrate that ketamine dose- and time-dependently inhibits the CA secretion evoked by the stimulation of cholinergic (both nicotinic and muscarinic) receptors and direct membrane depolarization in concentration- and time-dependent manners from the isolated perfused rat adrenal gland. It seems likely that the inhibitory effect of ketamine is mediated by blocking both the calcium influx into the rat adrenal medullary chromaffin cells and Ca^{2+} release from the cytoplasmic calcium store through the blockade of voltage-dependent Ca^{2+} channels as well as voltage-dependent Na^{+} channels on the rat adrenal medullary chromaffin cells, which are relevant to the blockade of cholinergic receptors.

The present results are in agreement with the findings by Purifoy and Holz (1984) that ketamine inhibited the CA secretion evoked by a nicotinic agonist DMPP in cultured bovine adrenal chromaffin cells in a noncompetitive fashion. In the present work, the in vitro concentrations of ketamine for inhibiting the effect of various secretagogues used in the rat perfused adrenal medulla are not much different from those attained in vivo in laboratory animals and humans (Dowdy and Kaya, 1968; Traber et al., 1968; Goldberg et al., 1970; Yamanaka and Dowdy, 1974; Schwartz and Horwitz et al., 1975; McGrath et al., 1975; Diaz et al. 1976; Kolka et al., 1983; Sumikawa et al., 1983; Purifoy and Holz, 1984; Takara et al., 1986).

In the present work, ketamine concentration-dependently suppressed the CA secretory responses evoked by ACh and DMPP. These results suggest that

ketamine inhibits the nicotinic receptor stimulation-induced CA secretion by interfering with the influx of Ca^{2+} . In this experiment, ketamine as well as thiopental also depressed the CA secretion induced by Bay-K-8644, which is found to enhance the CA release by increasing Ca^{2+} influx through L-type Ca^{2+} channels in chromaffin cells (Garcia et al., 1984). These findings that ketamine inhibited the CA secretion evoked by high K^+ and also by Bay-K-8644 suggest that ketamine directly inhibits the voltage-dependent Ca^{2+} channels through nicotinic receptor stimulation, just like Ca^{2+} channel blockers (Cena et al., 1983), which have direct actions on voltage-dependent Ca^{2+} channels. In the bovine chromaffin cells, stimulation of nicotinic, but not muscarinic ACh receptors is known to cause CA secretion by increasing Ca^{2+} influx largely through voltage-dependent Ca^{2+} channels (Burgoyne, 1984; Oka et al., 1979). Some previous reports also showed that Bay-K-8644 selectively potentiates the CA secretory responses mediated through the activation of voltage-sensitive Ca^{2+} channels; during nicotine or high-K stimulation (Ladona et al, 1987; Uceda et al, 1992). Therefore, it seems that ketamine inhibits DMPP-evoked CA secretion through inhibition of Ca^{2+} influx through voltage-dependent Ca^{2+} channels activated by nicotinic ACh receptors. However, in the present study, thiopental also inhibited the secretion of CA from the perfused rat adrenal glands induced by Bay-K-8644 as well as high potassium, suggesting that both ketamine and thiopental suppress influx of Ca^{2+} induced by an activator of L-type voltage-sensitive Ca^{2+} channels such as Bay-K-8644, which is thought to pass through voltage-sensitive Ca^{2+} channels and to stimulate CA secretion.

In support of this idea, it has been shown that ketamine also inhibited the carbachol-induced influx of $^{45}\text{Ca}^{2+}$ with a concentration-inhibition curve similar to

that for the CA secretion (Takara et al., 1986). This finding suggests that ketamine inhibited the carbachol-induced secretion of CA by interfering with the influx of Ca^{2+} . However, it is not likely that ketamine suppressed directly the voltage-dependent Ca^{2+} channels themselves since the influx of $^{45}\text{Ca}^{2+}$ due to high K^+ was not affected by ketamine. This result is not in agreement with that of the present work. This difference is not clearly find out, but seems to be difference between concentrations, preparations and the experimental methods used in studies. It has been reported that the impairment of the righting reflex in rats anesthetized with ketamine was reversed when the free plasma concentration of the anesthetic decreased to 17 μM (Cohen et al., 1973). However, a much higher concentration of ketamine in plasma (60 μM) was also demonstrated in patients 5 min after the intravenous injection of 2 mg/kg (Idvall et al., 1979). In humans, the free plasma concentration of ketamine 5 min after intravenous injection of 2.5 mg/kg was approximately 103 μM (Wieber et al., 1975). Concentrations of ketamine between 20 μM and 100 μM are attained during anesthesia (Domino et al., 1982).

The mechanism by which the stimulation of ACh receptors activates voltage-dependent Ca^{2+} channels in adrenal medullary cells is well understood. It has also been shown that ACh depolarizes chromaffin cell membranes and that this is dependent on the inward movement of Na^+ into the cells (Douglas et al., 1967). Kidokoro and his co-workers (1982) demonstrated that ACh generates Na^+ -dependent action potentials and that these are mediated by nicotinic (but not muscarinic) ACh receptors. Taking these previous observations into account, it has been suggested that the influx of Na^+ via nicotine receptor-associated ionic channels leads to the activation of voltage-dependent Ca^{2+} channels by altering

the membrane potentials (Wada et al., 1985b). In the present study, ketamine suppressed the veratridine-evoked CA secretory response. This result suggests that the inhibition by ketamine of the veratridine-evoked CA secretion as well as by ACh and DMPP is responsible for the inhibition of Ca^{2+} influx and the CA secretion. Ketamine was also found to inhibit the carbachol-induced influx of $^{22}\text{Na}^+$ at the same concentrations as it inhibited carbachol-induced $^{45}\text{Ca}^{2+}$ influx and the CA secretion (Takara et al., 1986). It has also been reported to depress synaptic transmission in the neuromuscular junction by interacting with the ionic channels of nicotine receptors (Maleque et al., 1981; Volle et al., 1982). Therefore, it seems likely that the predominant site of action of ketamine is nicotinic receptor-gated ionic channels in the rat adrenomedullary chromaffin cells.

Takara and his colleagues (1986) also found that ketamine, at higher concentrations, reduced the veratridine-induced influx of $^{22}\text{Na}^+$, $^{45}\text{Ca}^{2+}$ and the secretion of CA with a similar potency (IC_{50} 260 μM). Veratridine-induced influx of Na^+ is a requisite for triggering Ca^{2+} influx and the CA secretion (Wada et al., 1985a; 1985b). Therefore, the inhibition by ketamine of voltage-dependent Na^+ channels is responsible for the inhibition of Ca^{2+} influx and the CA secretion. Voltage-dependent Na^+ channels are indispensable for axonal conduction in central and peripheral neurons. However, based on the present results, since ketamine depressed the veratridine-evoked CA secretion at concentrations (30–100 μM) used clinically, it seems likely that the inhibition of voltage-dependent Na^+ channels might be produced during clinical anesthesia.

In *in vivo* (Clanachan and McGrath, 1976) and *in vitro* (Juang et al., 1980; Mahmoodi et al., 1980) experiments, ketamine has been shown to depress

synaptic transmission at peripheral sympathetic ganglia. In cultured adrenal medullary cells, which are devoid of preganglionic innervation, the effects of ketamine are confined to the postsynaptic action of the anesthetic. In this study, it looks likely that, in the isolated perfused adrenal gland, ketamine acts on the postsynaptic membrane and could selectively inhibit nicotine receptor-associated ionic channels. The present results are consistent with those of electrophysiological experiments (Gallagher et al., 1976): i.e. ketamine suppressed the postganglionic action potentials elicited by iontophoretically applied ACh, while ketamine had little effect on preganglionic axonal conduction.

In the present study, both ketamine and thiopental also suppressed the CA secretion evoked by McN-A-343, a selective muscarinic M_1 -receptor agonist. Generally, it has been shown that muscarinic stimulation generates a depolarizing signal which triggers the firing of action potentials, resulting in the increased CA release in the rat chromaffin cells (Akaike et al, 1990; Lim & Hwang, 1991). The elevation of intracellular Ca^{2+} mobilized from intracellular storage sites is thought to contribute to the muscarinic receptor-mediated secretion of adrenal CA (Harish et al., 1987; Misbahuddin et al., 1985; Nakazato et al., 1988). Furthermore, it has been shown that muscarinic receptor activation depolarizes the adrenal chromaffin cells of chickens (Knight and Baker, 1986), rats (Akaike et al., 1990), and guinea pigs (Inoue and Kuriyama, 1991). In terms of these findings, in this study, the inhibitory effect of ketamine on the muscarinic receptor-mediated secretion of CA can be explained in the same manner as for the nicotinic receptor-mediated secretion.

In this study, both ketamine and thiopental also inhibited the CA secretory response evoked by cyclopiazonic acid, which is known to be a highly selective

inhibitor of Ca^{2+} -ATPase in skeletal muscle sarcoplasmic reticulum (Goeger & Riley, 1989; Seidler et al., 1989) and a valuable pharmacological tool for investigating intracellular Ca^{2+} mobilization and ionic currents regulated by intracellular Ca^{2+} (Suzuki et al., 1992). Therefore, this result suggests that the inhibitory effect of ketamine on the CA secretion evoked by cholinergic muscarinic stimulation might be associated with the mobilization of intracellular Ca^{2+} in the rat adrenal chromaffin cells. This indicates that ketamine 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 CA secretion. 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 cyclopiazonic acid (Uyama et al., 1992). Suzuki and his coworkers (1992) have shown that cyclopiazonic acid easily penetrates into the cytoplasm through the plasma membrane and reduces Ca^{2+} -ATPase activity in sarcoplasmic/endoplasmic reticulum, resulting in increase in the subsequent Ca^{2+} release from those storage sites. Moreover, in bovine adrenal chromaffin cells, stimulation of muscarinic ACh receptors is also proposed to cause activation of phosphoinositide metabolism, resulting in the formation of inositol 1,4,5-trisphosphate, which induces the mobilization of Ca^{2+} from the intracellular pools (Cheek et al., 1989; Challis et al., 1991). However, in the present study, it is uncertain whether the inhibitory effect of ketamine on Ca^{2+} mobilization from

intracellular pools is due to its direct effect on the PI response or an indirect effect.

In conclusion, these experimental results demonstrate that ketamine inhibits the CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors and the membrane depolarization from the isolated perfused rat adrenal gland. It seems likely that the inhibitory effect of ketamine is mediated by blocking the calcium influx through voltage-dependent Ca^{2+} channels into the rat adrenal medullary chromaffin cells as well as Ca^{2+} release from the cytoplasmic calcium store partly through the blockade of voltage-dependent Na^{+} channels on the rat adrenal medullary chromaffin cells, which are at least relevant to the blockade of cholinergic receptors.

V. SUMMARY

The present study was attempted to examine the effects of ketamine, a dissociative anesthetics, on CA (CA) secretion evoked by cholinergic stimulation and membrane-depolarization from the isolated perfused rat adrenal gland, and to establish its mechanism of action, and to compare ketamine effect with that of thiopental sodium, which is one of intravenous barbiturate anesthetics.

Ketamine (30~300 μM) perfused into an adrenal vein for 60 min dose- and time-dependently inhibited the CA secretory responses evoked by ACh (5.32 mM), high K^+ (a direct membrane-depolarizer, 56 mM), DMPP (a selective neuronal nicotinic N_n receptor agonist, 100 μM) and McN-A-343 (a selective muscarinic M_1 receptor agonist, 100 μM). Ketamine or thiopental itself did not affect basal CA secretion (data not shown). Also, in the presence of ketamine (100 μM), the secretory responses of CA evoked by veratridine (a voltage-dependent Na^+ channel activator, 100 μM), Bay-K-8644 (an L-type dihydropyridine Ca^{2+} channel activator, 10 μM), and cyclopiazonic acid (a cytoplasmic Ca^{2+} -ATPase inhibitor, 10 μM) were significantly reduced, respectively. Interestingly, thiopental-sodium (100 μM) also caused the inhibitory effects on the CA secretory responses evoked by ACh, high K^+ , DMPP, McN-A-343, veratridine, Bay-K-8644, and cyclopiazonic acid.

Collectively, these experimental results demonstrate that ketamine greatly inhibits the CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors and the membrane depolarization from the isolated perfused rat adrenal gland. It seems likely that the inhibitory effect of ketamine is

mediated by blocking both the calcium influx through voltage-dependent Ca^{2+} channels into the rat adrenal medullary chromaffin cells and Ca^{2+} release from the cytoplasmic calcium store as well as by the blockade of voltage-dependent Na^{+} channels on the rat adrenal medullary chromaffin cells, which are relevant to the blockade of cholinergic receptors. It is also thought that, on the basis of concentrations, ketamine causes similar inhibitory effect with thiopental in the CA secretion from the perfused rat adrenal medulla.

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