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Influence of Hypoxia on Catecholamine Secretion Evoked by DMPP, McN-A-343, Excess K^+ and ACh from The Perfused Rat Adrenal Gland[#]

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ABSTRACT

It has been known that, during hypoxia, the adrenal medulla is activated to release catecholamines (CA) while hypoxia also inhibits high K^+-induced CA secretion in the cultured bovine adrenal chromaffin cells. The present study was attempted to examine the effect of hypoxia on CA secretion evoked by chlinergic stimulation and membrane-depolarization from the isolated perfused rat adrenal glands and also to clarify its mechanism of action. For this purpose, using the isolated rat adrenal glands, the effects of hypoxia on CA release evoked by nicotinic (N_1) and muscarinic (M_1) receptor agonists, membrane-depolarizing agent, Ca^{++}-channel activator, intracellular Ca^{++}-releaser and ACh were determined. Experiments were carried out, perfusing Krebs solution pre-equilibrated with a gas mixture of 95% N_2 and 5% CO_2. Hypoxia was maintained for 3~4 hours through the experiments. Hypoxia gradually caused a time-dependent reduction in CA secretion evoked by DMPP (100 uM), McN-A-343 (100 uM), ACh (5.32 mM), Bay-K-8644 (10 uM) and high K^+ (56 mM), respectively. However, it did not affect CA secretion evoked by cyclopiazonic acid (10 uM). Hypoxia itself also did fail to produce any influence on spontaneous secretory response of CA.

These experimental results suggest that hypoxia depresses CA release evoked by both cholinergic stimulation and membrane-depolarization from the isolated rat adrenal medulla, and that this inhibitory activity may be due to the result of the direct inhibition of Ca^{++} influx into the chromaffin cells without any effect on the calcium mobilization from the intracellular store.

Key Words: Hypoxia, Catecholamine secretion, Adrenal gland

INTRODUCTION

It has been generally accepted so far that systemic hypoxia increases concentration of catecholamines (CA) in the plasma (Cunningham *et al.*, 1965; Kotchen *et al.*, 1973; Sylvester *et al.*, 1979) and the excretion of CA into urine (Cunningham *et al.*, 1965; Claustre and Peyrin, 1982; Biesold *et al.*, 1989), and that in response to various stimuli that increase the secretion of CA from the adrenal medulla into plasma, the biosynthesis of CA in the adrenal gland increases to meet the increased demand for CA (Holland and Schumann, 1956; Bygdeman *et al.*, 1960; Dairman and Udenfriend, 1970; Kvetnansky *et al.*, 1971b). Moreover, hypoxia is known to cause adrenomedullary release of CA even in fetal lambs (Cohen *et al.*, 1984; Cheung, 1989; 1990), and in neonatal rats (Seidler and Slotkin; 1984; 1986). During anoxia or metabolic

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inhibition, there was a significant release of CA from the primary cultured bovine adrenal chromaffin cells (Dry *et al.*, 1991). More recently, it has been found that in vivo tyrosine hydroxylation, which is a rate-limiting step for CA synthesis, is enhanced under hypoxia in the rat adrenal gland, although availability of oxygen is reduced (Hayashi *et al.*, 1990), and that long-term hypoxia exerts a stimulatory influence on tyrosine hydroxylase protein in the carotid body and adrenal gland of the rat in addition to an elevation in dopamine and norepinephrine biosynthesis in the carotid body (Schmitt *et al.*, 1992).

However, in contrast to this fact that hypoxia causes CA release from adrenal glands in many species, hypoxia severely inhibited the overflow of [^{3}H] norepinephrine from the aortic strips prelabeled with [^{3}H] norepinephrine, but it had little effect on contractile responses to a high K^+ level, which is considered to reflect to contractility of the vascular smooth muscle (Lee *et al.*, 1988; 1989). From these results, it was concluded that one important mechanism by which hypoxia inhibited adrenergic neuronal signal transmission was the inhibition of norepinephrine from sympathetic nerve terminals in response to a given stimulus. Furthermore, Lee and his colleagues (1990) have suggested that in cultured bovine adrenal chromaffin cells hypoxia inhibits high K^+-induced CA release, and that this inhibitory effects is mainly the result of the inhibition of high K^+-induced increases in intracellular calcium subsequent to the inhibition of Ca^{++} influx through voltage-dependent Ca^{++} channels.

Therefore, in the present study, it was tried ① to investigate the effect of hypoxia on CA secretion from the isolated perfused rat adrenal gland and ② to clarify its mechanism of action and ③ to examine whether hypoxia-induced effects are associated with extracellulaar and intracellular alterations of calcium in adrenomedullary chromaffin cells.

MATERIALS AND METHODS

Experimental Procedure

Mature male Sprague-Dawley rats, weighing 180~250 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. A small slit was made into the adrenal cortex just opposite to 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℃.

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 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+0.05. The solution contained disodium EDTA (10 ug/ml) and ascorbic acid (100 ug/ml) to prevent oxidation of CA.

Induction of hypoxia

Hypoxia was induced by perfusing Krebs-bicarbonate solution pre-equilibrated with a gas mixture of 95% N_2 and 5% CO_2 (Shinil Gas Co., Korea). Hypoxia was maintained for 3~4 hours through the experiments. After hypoxic experiments, adrenal glands were reperfused with normal Krebs solution saturated with 95% O_2+5% CO_2.

Drug administration

The perfusions of DMPP (100 uM) for 1 min

and McN-A-343 (100 uM) for 2 min and/or a single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml were made into perfusion stream, and Bay-K-8644 (10 uM) was also perfused for 4 min.

In the preliminary experiments it was found that upon administration of the above drugs, secretory responses to ACh, KCl, McN-A-343 and Bay-K-8644 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 rule, prior to each stimulation with cholinergic agonists or excess K^+, perfusate was collected for 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 stimulated 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 value of CA, which is shown in all of the figures.

To study the effects of hypoxa on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution bubbled with a gas mixture of 95% N_2 and 5% CO_2 for 3~4 hours through the experiments, then the perfusate was collected for a specific time period (beckground 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 flurometric 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 secretogogues used in the present work was high enough to obtain readings several fold greater than the reading to 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

All data are presented as means with their standard errors, and the significance of differences was analyzed by Student's paired t-test using the computer system as previously described (Tallarida and Murray, 1987).

Drugs and their sources

The following drugs were used: acetylcholine chloride, 1,1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, methyl-1,4-dihydor-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay-K-8644), (Sigma Chemical Co., U.S.A.), cyclopiazonic acid, (3-(m-chlorophenyl-carbamoyloxy)-2-butynyl trimethyl ammonium chloride [McN-A-343] (RBI, U.S.A.). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644 and cyclopiazonic acid. Bay-K-8644 was dissolved in 95% ethanol, and cyclopiazonic acid in dimethylsulfoxide (DMSO) and diluted appropriately (final concentration of alcohol or DMSO was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

The effects of hypoxia on acetylcholine-evoked CA release from the perfused rat adrenal glands

In general, when the oxygenated normal Krebs-bicarbonate solution was perfused into an adrenal vein for one hour before induction of stimulation, spontaneous CA secretion gets to the steady-state. The basal CA release from the perfused rat adrenal medulla amounted to 21.5+2.8 ng for 2 min from 8 experiments. In order to examine the effects of hypoxia on the evoked CA release, hypoxia was induced by bubbling a gas mixture of 95% N_2 and 5% CO_2 into the Krebs solution.

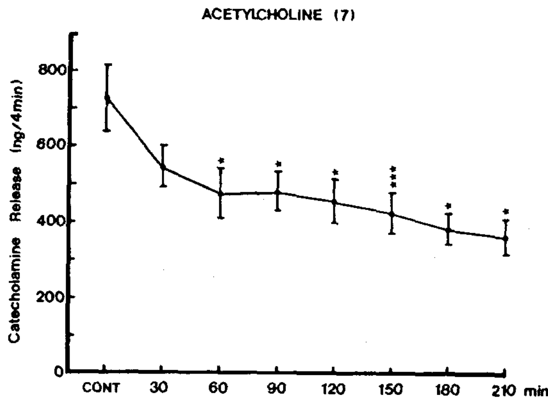

Fig. 1. Effects of hypoxia on the time course of ACh-stimulated catecholamine (CA) secretion from the isolated perfused rat adrenal glands. CA secretion was induced by a single injection of ACh (5.32 mM) after perfusion with normal Krebs solution for one hour prior to initiation of the experimental protocol. ACh was given at every 30 mim-intervals during the hypoxia. Numbers in the parenthesis indicate number of experimental rat adrenal glands. Vertical bars represent the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from the adrenal gland in ng. Abscissa: sampling time (min) during the hypoxia. Statistical difference was obtained by comparing the control with the hypoxia group at each period. Each perfusate was collected for 4 minutes. ACh: acetylcholine.
*: P<0.05, ***: P<0.01

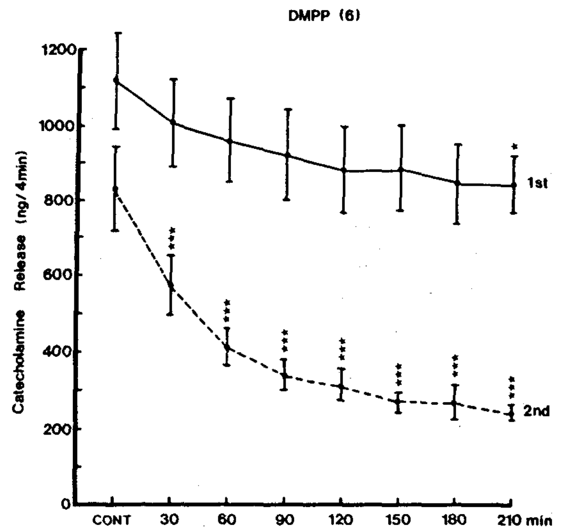

Fig. 2. Time course of DMPP-stimulated CA secretory response during the hypoxia. DMPP (100 uM) was perfused into an adrenal vein for 2 min at evry 30 min-intervals during the hypoxia. DMPP-induced perfusates were collected twice successively for each 4 minutes. Other legends are the same as in FIg. 1. ***: P<0.01

During hypoxia, ACh (5.32 mM) was administered at 30 min-intervals. As shown in figure 2, prior to introduction of hypoxic condition, ACh (5.32 mM) given in a volume of 0.05 ml into the rat adrenal gland produced an increased CA release of 724.3±86.4 ng for 4 min from 7 rat adrenal glands. However, under the hypoxic condition, ACh (5.32 mM)−evoked CA releases at 30 min-intervals were 542.5±58.8 ng (20~34 min), 477.3±68.2 ng (60~64 min, P<0.05), 482.7±53.5 ng (90~94 min, P<0.05), 456.1±61.8 ng (120~124 min, P<0.05), 425.1 ± 54.8 ng (150 ~ 154 min, P < 0.01), 383.1±42.2 ng (180~184 min, P<0.05) and 363.6±47.2 ng (210~214 min, P<0.05), respectively as compared with the corresponding control release. Statistical differences were compared between groups of control and hypoxia. However, hypoxia itself did not modify spontaneous CA release through the experiments (data not shown).

The effects of hypoxia on DMPP-evoked CA release from the perfused rat adrenal glands

DMPP (dimethylphenylpiperazinium) is a synthetic quaternary ammonium compound that is more selective for ganglionic receptors as a typical autonomic ganglionic stimulant (Rang & Dale, 1987). It also causes the hypertensive responses mediated by nicotinic receptors in cats, dogs and rats, which disappear after the blockade of adrenergic receptors and the automic ganglia (Chen *et al.*, 1951).

DMPP (100 uM) perfused into the adrenal gland for 2 min before induction of hypoxia caused a significant recrease in CA secretion of 1117.3±128.1 ng (0~4 min) and 829.3±112.2 ng (4~8 min) from 6 glands, respectively. In the presence of hypoxic condition, DMPP (100 uM) given

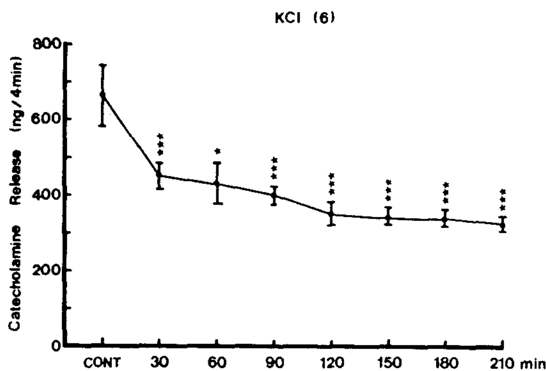

Fig. 3. Effects of hypoxia on the time course of excess K^+-stimulated CA secretory response from the rat adrenal glands. KCl (56 mM) was injected into an adrenal vein at 30 min-intervals during the hypoxia after obtaining the control response. Other legends are as in Fig. 1 and 2. *: $P<0.05$, ***: $P<0.01$

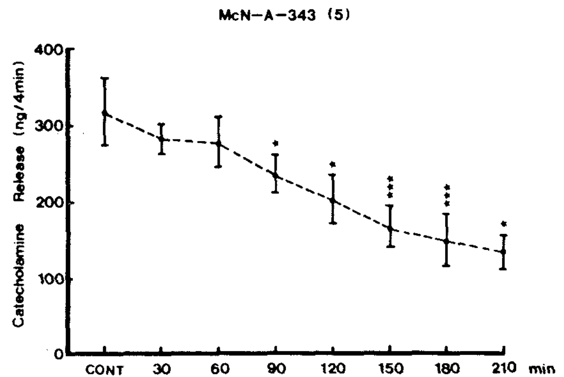

Fig. 4. Time course of adrenomedullary CA secretion evoked by McN-A-343 during the hypoxia. McN-A-343 (100 uM) was perfused into an adrenal vein for 2 min at 30 min-intervals after obtaining the control response. Other legend are as in Fig. 1 and 2. *: $P<0.05$, ***: $P<0.01$

at 30 min-intervals evoked CA secretory responses of 1004.9 ± 116.8 ng (0~4 min, ns) and 573.5 ± 78.3 ng (4~8 min, $P<0.01$) at 30 min-period, 961.0 ± 113.2 ng (0~4 min, ns) and 413.3 ± 50.9 ng (4~8 min, $P<0.01$) at 60 min-period, 922.3 ± 121.7 ng (0~4 min, ns) and 341.0 ± 39.1 ng (4~8 min, $P<0.01$) at 90 min-period, 883.5 ± 117.9 ng (0~4 min, $P<0.05$) and 317.8 ± 41.8 ng (4~8 min, $P<0.01$) at 120 min-period, 886.6 ± 115.9 ng (0~4 min, $P<0.05$) and 272.8 ± 25.1 ng (4~8 min, $P<0.01$) at 150 min-period, 847.3 ± 110.5 ng (0~4 min, $P<0.05$) and 271.3 ± 45.4 ng (4~8 min, $P<0.01$) at 180 min-period, 842.2 ± 80.2 ng (0~4 min, $P<0.05$) and 245.2 ± 20.7 ng (4~8 min, $P<0.01$) at 210 min-period, respectively as compared to the control secretory response, as shown in figure 3.

The effects of hypoxia on excess K^+-evoked CA release from the perfused rat adrenal glands

Since hypoxia is found to inhibit high K^+-induced CA release in cultured bovine adrenal chromaffin cells (Lee et al., 1990), it is of interest to test the influence of hypoxia on excess K^+-induced CA secretion from the perfused rat adrenal glands.

As shown in figure 4, in the absence of hypoxic condition, the excess K^+ (56 mM)-evoked CA release amounted to 663.1 ± 81.5 ng for 4 min from 6 experiments. However, in the presence of hypoxia, high K^+ (56 mM)-induced CA secretory responses, when given at 30 min-intervlas, were time-dependently reduced to 451.4 ± 35.7 ng (30~34 min, $P<0.01$), 431.2 ± 53.8 ng (60~64 min, $P<0.05$), 403.0 ± 25.9 ng (90~94 min, $P<0.01$), 351.3 ± 31.4 ng (120~124 min, $P<0.01$), 346.2 ± 24.2 ng (150~154 min, $P<0.01$), 341.0 ± 21.9 (180~184 min, $P<0.01$) and 326.4 ± 22.5 ng (210~214 min, $P<0.01$), respectively as compared to its control response.

The effects of hypoxia on CA release evoked by McN-A-343 from the perfused rat adrenal glands

It has been known that McN-A-343 increases the blood pressure and heart rate, both responses being readily blocked by atropine (Roszkowsk, 1961; Fozard & Muscholl, 1972) and that McN-A-343 as well as DMPP causes greatly secretion of CA from the isolated perfused rat adrenal glands by a calcium-dependent exocytotic mechanism (Lim and Hwang, 1991).

Therefore, an attempt was made to test the effect of hypoxia on CA secretory response of McN-A-343. McN-A-343 (100 uM) perfused into the adrenal gland for 2 min before induction of hypoxia caused CA secretion of 318.6 ± 43.1 ng for

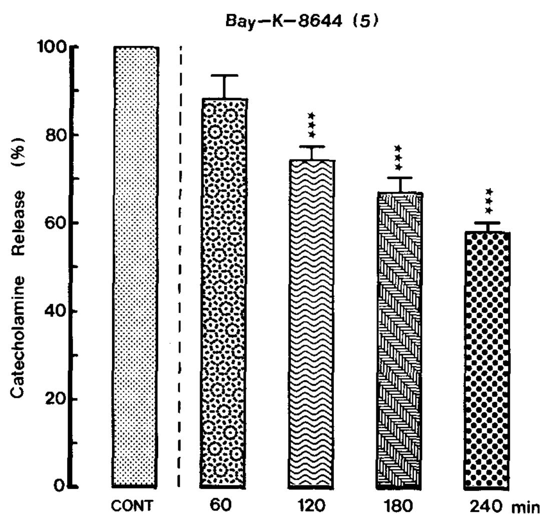

Fig. 5. Effects of hyoxia on the time course of CA secretion evoked by Bay-K-8644 from the perfused rat adrenal medulla. Bay-K-8644 (10 uM) was perufsed into an adrenal vein for 4 min at one hour-interval after obtaining the control response, Its pefusate was collected for 4 min. Other legends are the same as in Fig. 1 and 2. ***: P<0.01

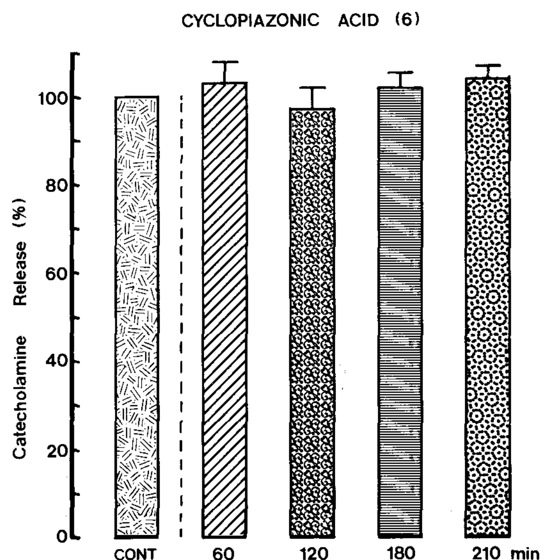

Fig. 6. Time course of cyclopiazonic acid-induced CA secretory response during the hypoxia from the isolated rat adrenal glands. Cyclopiazonic acid (10 uM) was perfused into an adrenal veing for 4 min at 60 min-intervals obtaining the control response. There was no significant differences between both groups of control and hypoxia. Other legends are as in Fig. 1 and 2.

4 min from 5 adrenal glands. During hypoxia, McN-A-343 (100 uM)-evoked CA secretory responses at every 30 min-intervals were inhibited to 282.5±19.7 ng (30~34 min, ns), 279.0±32.7 ng (60~64 min, ns), 237.6±24.3 ng (90~94 min, P<0.05), 204.9 ± 30.9 ng (120 ~ 124 min, P < 0.05), 168.8 ± 27.4 ng (150~154 min, P<0.01), 150.4±34.5 ng (180~184 min, P<0.01) and 135.2±23.8 ng (210~214 min, P<0.05), respectively as compared to the control response. Figure 5 shows time course of adrenomedullary CA secretion evoked by McN-A-343 during hypoxia.

The effects of hypoxia on CA release evoked by Bay-K-8644 from the perfused rat adrenal glands

Since Bay-K-8644 is known to be a calcium channel activator and to cause positive inotropy and vasoconstriction in isolated tissue and intact animals (Schramm *et al.*, 1982; Wada *et al.*, 1985) and to enhance basal Ca^{++} uptake (Garcia *et al.*, 1984) and CA release (Lim *et al.*, 1992), it was of interest to determine the effect of hypoxia on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands.

Figure 6 illustrates inhibitory effects of hypoxia on CA release evoked by Bay-K-8644. Under hypoxia condition, Bay-K-8644 (10 uM) perfused into the adrenal gland for 4 min at 60 min-intervals exerted the marked reduction in CA secretory responses of 88.4±4.8% (60~64 min, ns), 74.9± 3.0% (120~124 min, P<0.01), 67.8±3.1% (180~184 min, P<0.01) and 58.9±1.6% (240~244 min, P< 0.01), respectively as compared with the corresponding control release (100%) from 2 rat adrenal glands.

The effects of hypoxia on cyclopiazonic acid-evoked CA release from the perfused rat adrenal glands

Since cyclopiazonic acid, a mycotoxin from Aspergillus and Penicillium, has been described as a highly selective inhibitor of Ca^{++}-ATPase in skel-

etal muscle sarcoplasmic reticulum (Georger & Riley, 1989; Seidler et al., 1989) and it may be extremely valuable pharmacological tool for investigating intracellular Ca^{++} mobilization and ionic current regulated by intracellular calcium (Suzuki *et al.,* 1992), it is of particular interest to test the effect of hypoxia on cyclopiazonic acid-induced CA secretory responses.

Figure 7 shows time course of cyclopiazonic acid-evoked CA secretory response during the hypoxia from the isolated rat adrenal glands. Cyclopiazonic acid (10 uM) given into the adrenal gland for 4 min at 60 min-intervals caused the CA secretory responses of 103.3±4.8% (60~64 min, ns), 97.5±4.6% (120~124 min, ns), 102.8±2.9% (180~184 min, ns) and 104.2±2.8% (240~244 min, ns), respectively as compared to the corresponding control response (100%) from 6 experiments.

DISCUSSION

In the present study, cholinergic (nicotinic and muscarinic) receptor-stimulated and membrane depolarization-mediated CA secretory responses as well as Bay-K-8644-evoked CA release were significantly inhibited by hypoxia. However, hypoxia did fail to affect cyclopiazonic acid-induced CA secretion. Taken together, these experimental results suggest strongly that hypoxia depresses CA secretory responses evoked by nicotinic and muscarinic receptor stimulation and also that by excess K^+-induced membrane depolarization, and that this inhibitory effect may be mediated through direct inhibition of calcium influx into adrenomedullary chromaffin cells without any effects on intracellular calcium store.

In support of this idea, it has been reported that hypoxia inhibits electrical stimulation-induced outflow of [^{3}H] norepinephrine from isolated rabbit aortic strips (Lee *et al.,* 1988) and also thoracic aortic strips prelabeled with [^{3}N] norepinephrine (Lee *et al.,* 1989). These results have suggested that inhibition of adrenergic transmission under hypoxic conditions is mainly the result of a decrease in the stimulus-evoked release of norepinephrine and of a decrease in the affinity of α-adrenoceptor for norepinephrine and/or inhibition of signal transduction mechanisms, althtough hypoxia also causes a slight decrease in the contractility of vascular smooth muscle.

Moreover, previous investigators who examined the direct effects of hypoxia on peripheral sympathetic neurons and their effectors were exclusively concerned with the contractile responses of isolated vascular smooth muscle to exogenous norepinephrine or high K^+. They found that the contractile response to only one or two concentrations of norepinephrine (or epinephrine) or to high K^+ were considerable decreased under hypoxic conditions, and they concluded that this decrease was mainly a result of the direct inhibitory effects of hypoxia on the contractile machinery of the vascular smooth muscle (Coret and Hughes 1964; Shibata and Briggs, 1967; Detar and Bohr 1968; Hughes and Coret, 1969; Detar and Gellai, 1971; Namm and Zucker, 1973; Vanhotte, 1976; coburn *et al.,* 1979; Ebeigbe *et al.,* 1980). These results are in good agreement with the present experimental results which hypoxia depresses CA secretory responses evoked by DMPP, a selective nicotinic receptor agonist and McN-A-343, a selective muscarinic M_1-agonist as well as that by excess K^+, a membrane depolarizing agent.

Dart and his coworkers (1984) have shown that in severe ischemia there is a depression of the norepinephrine release produced by stimulation of cardiac sympathetic nerve. In addition, hypoxia is also known to inhibit high K^+-induced CA release and ^{45}Ca uptake in cultured bovine adrenal chromaffin cells (Lee *et al.,* 1990). More recently, Kass and his coworkers (1994) have also demonstrated that anoxia attenuates depolarization-induced Ca^{++} uptake. They found that veratridine-induced depolarization caused a large increase in Ca^{++} uptake in rat hippocampal slice (30.2 VS. 9.0 nM/mg dry weight). This uptake was reduced to 18.4 nM/mg when veratridine was combined with anoxia. These results could be supported by the electrophysiologic studies of Krnjevic, Cherubini and BenAri (1989) who found that the slow calcium currents were inhibited during anoxia. Based on these findings, the present experimental results demonstrate strongly that hypoxia inhibits secretory responses of CA evoked by cholinergic receptor-stimulation and membrane-depolarization from the isolated perfused rat adrenal medulla.

However, in contrast to the present data, previ-

ous investigators have reported that the adrenal medulla of such as rats (Seidler and Slotkin, 1985; 1986; Biesold *et al.*, 1989), sheep (Cohen *et al.*, 1984; Cheung, 1989; 1990) and even cultured bovine chromaffin cells (Dry *et al.*, 1991) are responded directly to hypoxic conditions by releasing CA, since the pioneering discovery by Cannon and Hoskins (1911) and Czubalski (1913), who were the first to demonstrate independently that acute asphyxia increased adrenal medullary hormone secretion into the blood.

Moreover, Hayashi and his colleagues (1990) have shown that invo tyrosine hydroxylation in the rat adrenal gland is enhanced under hypoxic conditions mainly via the following mechanisms: ① Tyrosine hydroxylase sensitive to an increase in tissue levels of tyrosine, and ② tissue levels of tyrosine are increased. More recently, it has been also reported that long-term hypoxia elicits a time-dependent increase in tyrosine hydroxylase protein content in the rat carotid body and adrenal gland. Thus, hypoxia exerts a stimulatory influence on the tyrosine hydroxylase expressing cells, which leads to an elevation of dopamine and norepinephrine biosynthesis (Schmitt *et al.*, 1992). In addition to hypoxic effect on adreno-medullary CA secretion, studies carried out with isolated perfused rat hearts suggest that carrier-mediated efflux of norepinephrine is a significant mode of release during prolonged stop-flow ischemia of more than 10 minutes (Dart *et al.*, 1984; 1987; Schoming *et al.*, 1984).

In general, nicotine-evoked CA release from chromaffin cells in strictly calcium-dependent; in contrast, anoxia-induced CA release from bovine adrenal chromaffin cells was not inhibited in Ca^{++}-free medium, nor was it blocked by verapamil (Dry *et al.*, 1991). Although this lack of Ca^{++} dependence suggest a release mechanism that differs from that involved in nicotine stimulation, the lack of inhibition by desipramine and (+)-oxaprotiline suggests that metabolic inhibitors do not evoke CA release via the uptakel carrier. In support of this suggestion, Schöming and his co-workers (1987) have indicate that nonexocytotic CA release is a two-step process induced by energy deficiency in the sympathetic varicosity. In a first step, norepinephrine is lost from storage vesicles, resulting in increasing axoplasmic concentrations. The second step is the rate-limiting transport of intracellular norepinephrine across the cell membrane by the $uptake_1$ carrier that has reversed its normal net transport direction.

However, in the present investigation, hypoxia depressed the secretory effect of CA evoked by Bay-K-8644, which is known to be a Ca^{++}-channel activator and to cause positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm *et al.*, 1982; Wada *et al*, 1985) and to enhance basal Ca^{++}-uptake (Garcia *et al.*, 1984) and CA release (Lim *et al.*, 1992). This finding that hypoxia inhibited CA secretory responses by Bay-K-8644 as well as cholinergic receptor-stimulation and membrane depolarization demonstrates that the inhibitory effect of hypoxia is mediated through the blockade of Ca^{++} entry into the chromaffin cells.

In supprot of these idea, there are now sizeable literatures demonstrating a key role of Ca^{++} influx through voltage-sensitive Ca^{++} channels as a physiological pathway for activation of adrenal CA (Douglas, 1975; Aguirre *et al.*, 1977; Schneider *et al.*, 1977; 1981; Holz *et al.*, 1982; Kao and Schneider, 1986). Moreover, it is found that the activation of nicotinic receptors stimuated CA secretion by increasing Ca^{++} entry through receptor-linked and/or voltage-dependent Ca^{++} channels in both perfused rat adrenal glands (Wakade and Wakade, 1983) and isolated bovine adrenal chromaffin cells (Kilpatric *et al.*, 1981; 1982; Knight and Kesteven, 1983), and that the muscarinic receptor activation causes an increase in adrenal CA secretion independent of extracellular Ca^{++} in various species (Nakazato *et al.*, 1988; Wakade *et al.*, 1986; Harish *et al.*, 1987) and in cytosolic free Ca^{++} in bovine isolated adrenal chromaffin cells without associated CA secretion (Cheek and Burgoyne, 1985; Kao and Schneider, 1985; 1986; Misbahuddin *et al.*, 1985). However, recently, Lim and Hwang (1992) have reported that removal of extracellular Ca^{++} depresses CA release evoked by DMPP or McN-A-343.

High K^+-induced CA release from adrenal chromaffin cells in now found to consist of the following processes: depolarization of membrane, Ca^{++} influx through voltage-dependent Ca^{++} channels, elevation of intracellular Ca^{++} and activation of the machinery of CA release by the elevated intracellular Ca^{++} (Lee *et al.*, 1990). In the light of these previous findings, the present

results that hypoxia suppresses CA secretory responses induced by membrane-depolarization and cholinergic stimulation as well as by Ca^{++}-channel activation strongly suggest that this inhibitory effect of hypoxia may be exerted through the direct inhibition of voltage-dependent and/or receptor-linked Ca^{++} channels, resulting in blockade of Ca^{++} entry into the adrenomedullary chromaffin cells. Because the high K^{+}-induced CA release from adrenal chromaffin cells in considered to be regulated by the increased intracellular Ca^{++} (Knight and Kesteven, 1983; Kao and Schneider, 1986).

As for the mechanism for the inhibition of Ca^{++} influx by hypoxia through receptor-linked and/or voltage-dependent Ca^{++} channels, the following three possibilities must be considered. Firstly, the inhibition of Ca^{++} influx may be due to a decreased Ca^{++} concentration gradient across the cell membrane subsequent to an increased intracellular Ca^{++}. Secondly, hypoxia may alter molecular structure of Ca^{++} channels themselves, which cause a decreased conductance of Ca^{++} channels, or modify the sensitivity of Ca^{++} channels to depolarization by excess K^{+} and/or cholinergic receptor stimulation. Thirdly, hypoxia may cause the functional disturbance of Ca^{++} channels, resulting in a reduced magnitude of depolarization evoked by excess K^{+} and/or cholinergic receptor (both nicotinic and muscarinic) stimulation. However, the present experimental data suggest that Ca^{++} channels are functionally disturbed by hypoxia in the isolated perfused rat adrenal gland, although the exact mechanism for the inhibition of Ca^{++} influx through voltage-dependent and/or receptor-linked Ca^{++} channels remains to be resolved.

Also, in the present study, hypoxia did fail to affect CA secretory effect evoked by cyclopiazonic acid, which is known to be a highly selective inhibitor of Ca^{++}-ATPase in skeletal muscle sarcoplasmic reticulum (Geoger and Riley, 1989; Seidler *et al.*, 1989) and a valuable pharmacological tool for investigating intracellular Ca^{++} mobilization and ionic currents regulated by intracellular Ca^{++} (Suzuki *et al.*, 1992). These results illustrate that inhibitory effects of hypoxia on CA release evoked by cholinergic stimulation and/or membrane-depolarization may be not associated with intracellular Ca^{++} mobilization. It is shown that Ca^{++}-uptake into intracellular storage sites susceptible to caffeine (Iino, 1989) was almost completely abolished by treatment with cyclopiazonic acid during the preceding Ca^{++} load (Suzuki et al., 1992). This finding is consistent with the results revealed in skinned smooth muscle fibers of the longitudinal layer of the guinea-pig ileum, where Ca^{++}-uptake was also inhibited by cyclopiazonic acid (Uyama, *et al.*, 1992). Suzuki and his coworkers (1992) have suggested that cyclopiazonic acid easily penetrates into the cytoplasm through the plasma membrane, reduces Ca^{++}-ATPase activity in sarcoplasmic/endoplasmic reticulum, decreases the subsequent Ca^{++} release from those storage sites and, thereby reduces Ca^{++}-dependent K^{+}-current.

Based upon these results, the present experimental data demonstrate strongly that the inhibitory effect of hypoxia on the evoked CA release may be mediated through direct inhibition of Ca^{++} influx into the chromaffin cells without intracellular Ca^{++} mobilization from those storage sites.

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=국문초록=

저산소증이 흰쥐 관류부신에서 DMPP, McN-A-343, Excess K^+ 및 ACh의 카테콜아민 분비작용에 미치는 영향

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저산소 상태에서는 부신수질로부터 카테콜아민 (CA) 유리작용이 활성화되지만 반면에 소의 배양 chromaffin cell에서는 고농도의 K^+에 의한 CA 분비작용이 억제된다고 알려져 있다. 본 연구에서는 적출 흰쥐 관류부신에서 콜린성 자극과 막탈분극에 의한 CA 분비작용에 대한 저산소증의 영향을 검색하고 그 작용기전을 규명코자 하였다. 본 연구목적을 위하여, 적출 흰쥐 관류부신을 이용, 저산소증이 니코틴 (N_1), 무스카린 (M_1) 수용체 흥분약, 막탈분극 약물, 칼슘채널 활성화 약물, 세포내 칼슘유리 약물 및 ACh에 의한 CA 분비에 미치는 영향을 연구하였으며, 저산소증은 95% 질소 및 5% 이산화탄소 혼합가스를 Krebs액에 주입하여 유발시켰으며, 3~4시간동안 유지하였다.

저산소증 유발시, DMPP (100 uM), McN-A-343 (100 uM), ACh (5.32 mM), Bay-K-8644 (10 uM) 및 high K^+ (56 mM)에 의한 CA 분비작용을 시간의존적으로 점차 유의성인 감소를 나타내었다. 그러나, cyclopiazonic acid (10 uM)에 의한 CA 분비반응에는 하등의 영향을 일으키지 못하였다. 또한 저산소증 자체가 CA의 기초분비 작용에는 영향을 미치지 않았다.

이와같은 실험결과로 보아, 저산소증시 콜린성 자극 및 막탈분극에 의한 CA 분비 작용이 억제되며, 이러한 억제작용은 chromaffin cell내로 Ca^{++} 유입을 직접적으로 억제시키는 결과에 기인되며, 세포내 칼슘저장고로부터 칼슘유리작용과는 관계없는 것으로 사료된다.