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Influence of Glucocorticoids on Cholinergic Stimulation-Induced Catecholamine Secretion from the Rat Adrenal Medulla

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The present study was undertaken to examine the influence of glucocorticoids on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh), DMPP, McN-A-343, excess K^+ and Bay-K-8644 from the isolated perfused rat adrenal gland and to clarify the mechanism of its action. The perfusion of the synthetic glucocorticoid dexamethasone (10-100 μM) into an adrenal vein for 20 min produced a dose-dependent inhibition in CA secretion evoked by ACh (5.32 mM), excess K^+ (a membrane-depolarizor 56 mM), DMPP (a selective nicotinic receptor agonist, 100 μM for 2 min), McN-A-343 (a muscarinic receptor agonist, 100 μM for 4 min), Bay-K-8644 (a calcium channel activator, 10 μM for 4 min) and cyclopiazonic acid (a releaser of intracellular Ca^{2+}, 10 μM for 4 min). Similarly, the preperfusion of hydrocortisone (30 μM) for 20 min also attenuated significantly the secretory responses of CA evoked by nicotinic and muscarinic receptor stimulation as well as membrane-depolarization, Ca^{2+} channel activation and the release of intracellular Ca^{2+}. Furthermore, even in the presence of betamethasone (30μM), CA secretion evoked by ACh, excess K^+, DMPP and McN-A-343 was also markedly inhibited. Taken together, the present results suggest that glucocorticoids cause the marked inhibition of CA secretion evoked by both cholinergic nicotinic and muscarinic receptor stimulation from the isolated perfused rat adrenal gland, indicating strongly that this inhibitory effect may be mediated by inhibiting influx of extracellular calcium as well as the release of intracellular calcium in the rat adrenomedullary chromaffin cells.

Key Words: Glucocorticoids, Catecholamine Secretion, Rat Adrenomedullary Chromaffin Cells

INTRODUCTION

Glucocorticoids and CA, which are two major hormones being involved in the body's response to stress originate from the adrenal gland. Due to the anatomical association between the adrenal medulla and the adrenal cortex, the medulla is influenced by the high levels of glucocorticoids secreted by the cortex into the intra-adrenal portal system perfusing the medulla.

Epinephrine synthesis in the adrenal medulla is also subject to the biochemical influence of the adrenal cortex: the surrounding area of the adrenal gland. Because blood from the cortex passes through the medulla, most of the chromaffin cells are exposed to high levels of cortical steroids, the hormones released by the cortex (Carmichael & Winkler, 1985). When steroids are absent, the enzyme that catalyzed the conversion of norepinephrine into epinephrine is degraded at an unusually high rate and its cellular level declines. Stress is known to induce the secretion of hormones from the cortex as well as from the medulla. The cortical hormones apparently ensure that the synthesis of epinephrine is maintained (Carmichael & Winkler, 1985). Glucocorticoids have been shown to regulate the activity and/or mRNA of phenylethanolamine N-methyltransferase, the final enzyme in the CA biosynthetic pathway converting norepinephrine to epinephrine, both in vivo (Wurtman & Axelrod, 1965; 1966; Pohorecky & Wurtman, 1971; Ciaranello & Black, 1971; Stachowiak et al, 1988) and in vitro (Hersey & DisStefano, 1979;

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Kelner & Pollard, 1985; Wan & Livett, 1989; Stachowaik et al, 1990a; Ross et al, 1990; Betito et al, 1992).

Choi and his colleagues (1993) have reported that glucocorticoids enhance histamine-stimulated inositol phosphate accumulation in chromaffin cells. More recently, it is known that glucocorticoids may play a physiological role in modulating the responsiveness of chromaffin cells to histamine and other stimuli (Choi et al, 1995).

Dexamethasone is found to possess a selective effect on the response of the cells to histamine and causes an increase in histamine-evoked secretion from noradrenergic cells as well as secretion evoked by high K^+ (Choi et al, 1995). Dexamethasone appeared also to cause small but not significant increases in secretion evoked by dimethyl phenyl piperazinium, bradykinin, and angiotensin II (Choi et al, 1995).

In an acute stress situation, there is a short-term exposure of the adrenal medulla to splanchnic nerve contents as well as to glucocorticoids. Secretion of CAs from the adrenal medulla is mostly in response to nicotinic receptor-mediated effect of ACh from splanchnic nerve (Douglas and Rubin, 1961; Douglas, 1966). Although adrenomedullary phenylethanolamine N-methyl-transferase activity is regulated predominantly by glucocorticoids, continuous exposure to cholinergic nicotinic agonists [18-24 hr (Evinger et al, 1988) or 6-18 hr (Stachowiak et al, 1990a)] has also shown to increase phenylethanolamine N-methyltransferase mRNA in bovine adrenal medullary cells. The present study was designed to investigate the effect of glucocorticoids on nicotinic and muscarinic receptor-mediated CA secretion from the isolated perfused rat adrenal gland and to establish the mechanism of its action.

METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180 to 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. 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 the 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 $37 \pm 1^{\circ}C$.

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 μg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of catecholamine.

Drug administration

The perfusions of DMPP (100 μM) and McN-A-343 (100 μM) for 2 minutes 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 via a three way stopcock, and Bay-K-8644 (10^{-5}M) and cyclopiazonic acid (10^{-5}M) were 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, cyclopiazonic acid and Bay-K-8644 returned to preinjection level in about 4 min, and the responses to DMPP in 8 min.

Collection of perfusate

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. Stimulated sample's 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 dexamethasone and its derivatives on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing dexamethasone for 20 min, and then the background and stimulated samples were collected as the same as the above. All samples were collected in chilled tubes.

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). An aliquat (0.2 ml) of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by secretagogues used in the present work was high enough to obtain readings several fold greater than the reading of control 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.

Statitical analysis

The statistical significance between groups was determined by utilizing the Student's t-test. 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: hydrocortisone (Upjohn Co., MI, U.S.A.), betamethasone sodium phosphate (Dongil, Co., Seoul Korea), dexamethasone disodium phosphate (Yuhan Corp. Seoul Korea), acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro methylphenyl)-pyridine-5-carboxylate (BAY-K-8644), metoclopramide hydrochloride (Sigma Chemical Co., U.S.A.), cyclopiazonic acid,(3-(m-cholro-phenyl-carbamoyl-oxy)-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. Bay-K-8644 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.

RESULTS

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

After the initial perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 24.3 ± 2.8 ng/2 min (n=8). The effects of dexamethasone on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion from perfused rat adrenal glands were examined, and secretagogues were given at 20 to 30 min-intervals. In the present study, dexamethasone itself did not produce any effect on basal CA output (data not shown). When ACh (5.32 mM) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 933 ± 104 ng for 4 min. However, in the presence of 10, 30 and 100 μM of dexamethasone for 20 min, ACh-stimulated CA secretory responses were significantly decreased 756 ± 32 ng ($P < 0.01$), 681 ± 62 ng ($P < 0.01$) and 620 ± 71 ng ($P < 0.01$), respectively, from 8 adrenal glands as shown in Fig. 1. As shown in Fig. 2, high KCl

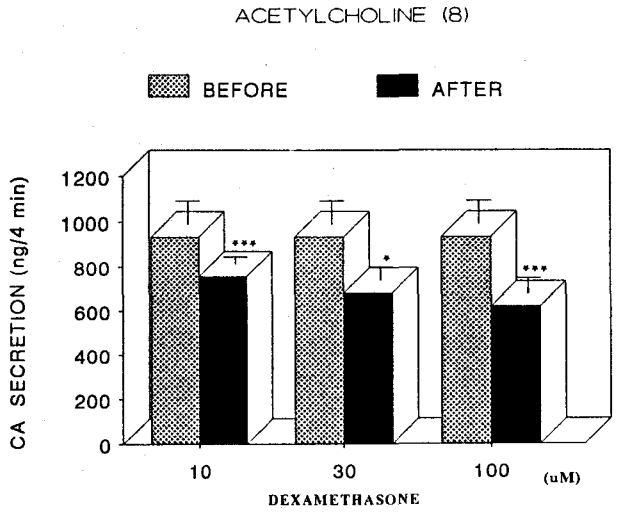

Fig. 1. Influence of dexamethasone on ACh-stimulated catecholamine (CA) secretion from the isolated perfused rat adrenal glands. CA secretion was induced by a single injection of ACh (5.32 mM) after perfusion with normal Krebs solution for one hour prior to initiation of the experimental protocol. "BEFORE" and "AFTER" denote CA secretion evoked by ACh before and after preloading with dexamethasone (10~100 μM) for 20 min, respectively. Numeral 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 by ACh from the adrenal gland in ng. Abscissa: concentrations of dexamethasone (10~100 μM). Statistical difference was obtained by comparing the control with the pretreated group. Each perfusate was collected for 4 minutes. ACh: acetylcholine. *: $P<0.05$, ***: $P<0.01$

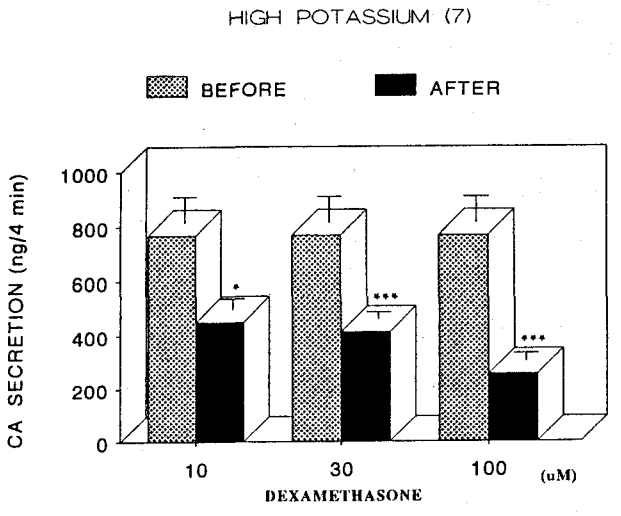

Fig. 2. Influence of dexamethasone on excess K^+-stimulated CA secretory responses from the rat adrenal glands. Other legends are the same as in Fig. 1. *: $P<0.05$, ***: $P<0.01$

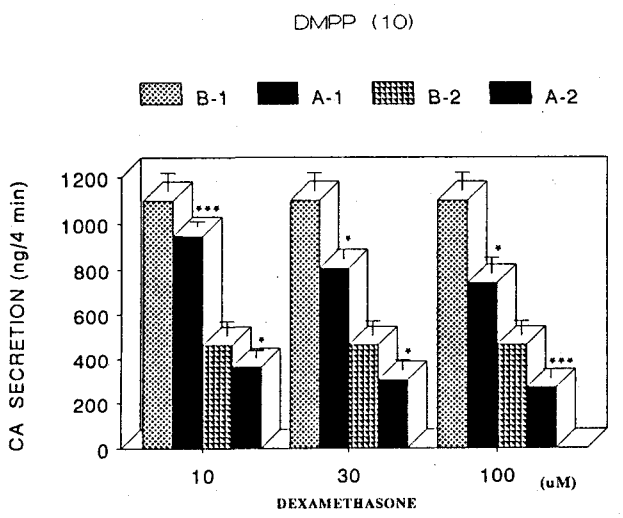

Fig. 3. Influence of dexamethasone on DMPP-stimulated CA secretory responses. DMPP (100 μM) was perfused into an adrenal vein for 2 min before and after preloading with dexamethasone (10~100 μM) for 20 min, respectively. DMPP-induced perfusates was collected twice successively for each 4 minutes. B-1(0~4 min) and B-2 (4~8 min) indicate amounts of CA secreted by DMPP before the perfusion of dexamethasone, while A-1 (0~4 min) and A-2 (4~8 min) denote amounts of CA by it after preloading with dexamethasone. Other legends are the same as in Fig. 1. *: $P<0.05$, ***: $P<0.01$

(56 mM)-stimulated CA secretion was attenuated significantly after the pretreatment with dexamethasone (10~100 μM) for 20 min. In the presence of dexamethasone, its CA secretion was concentration-dependently decreased from 7 glands, which was significantly lower than the control secretion (768± 94 ng/4 min).

When perfused through the rat adrenal gland, DMPP (100 μM for 2 min), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion. As shown in Fig. 3, DMPP-stimulated CA secretion before preloading with dexamethasone was 1099±78 ng (0~4 min) and 462±66 ng (4~8 min), while after pretreatment with dexamethasone (10~100 μM) for 20 min they were greatly reduced from 10 rat adrenal glands. As illustrated in Fig. 4,

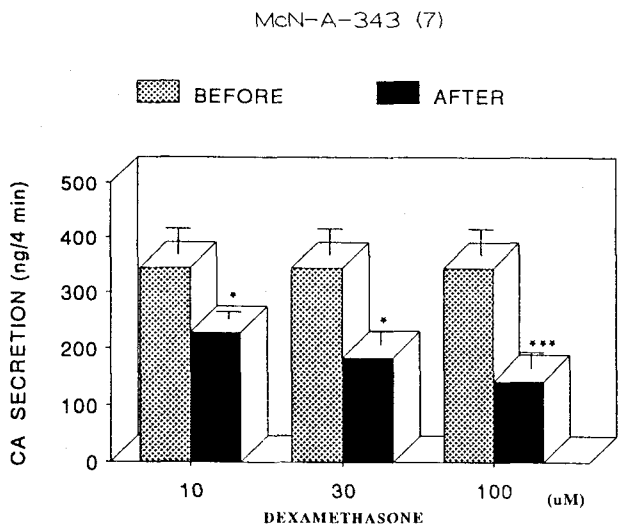

Fig. 4. Influence of dexamethasone on McN-A-343-stimulated CA secretory responses. McN-A-343 (100 μM) was perfused into an adrenal vein for 2 min before and after preloading with dexamethasone (10~100 μM) for 20 min, respectively. McN-A-343-induced perfusates was collected for 4 min. Other legends are the same as in Fig. 1. *: $P<0.05$, ***: $P<0.01$

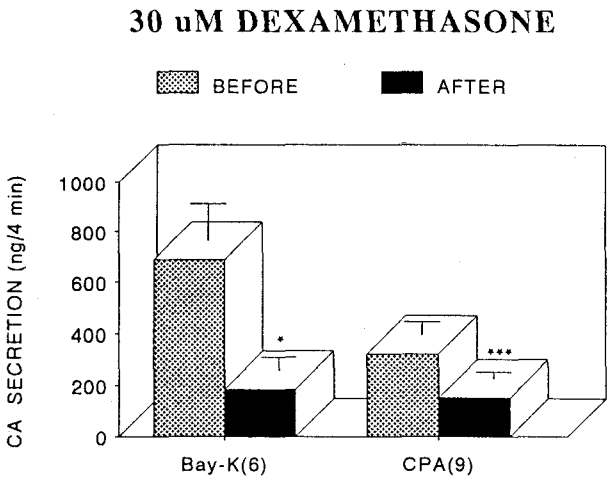

Fig. 5. Influence of 30 μM dexamethasone on CA secretion evoked by Bay-K-8644 (Bay-K) and cyclopiazonic acid (CPA). Bay-K-8644 (10 μM) and cyclopiazonic acid were perfused into an adrenal vein for 4 min before and after the preloading with 30 μM dexamethasone for 20 min. Ordinate: the amounts of CA secreted from the adrenal gland in ng. Abscissa: Secretagogues. Statistical difference was obtained by comparing the control with the pretreated group. Its pefusate was collected for 4 min. Other legends are the same as in Fig. 1. ***: $P<0.01$

McN-A-343 (100 μM), which is a selective muscarinic M_1- agonist (Hammer & Giachetti, 1982), perfused into an adrenal gland for 2 min caused an increased CA secretion to 344±48 ng for 4 min from 7 experiments. However, McN-A-343-stimulated CA secretory responses in the presence of 10, 30 and 100 μM of dexamethasone were markedly inhibited to 228 ±13 ng ($P<0.05$), 184±24 ng ($P<0.05$) and 143± 28 ng ($P<0.01$) for 4 min, respectively, as compared to corresponding control secretion.

Effect of 30 μM dexamethasone on CA secretion evoked by Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

Since Bay-K-8644 is known to be a calcium channel activator which causes positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm et al, 1982; Wada et al, 1985) and enhances basal Ca^{++} uptake (Garcia et al, 1984) and CA release (Lim et al, 1992), it was of interest to determine the effects of 30 μM dexamethasone on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Fig. 5 illustrates the inhibitory effect of 30 μM dexamethasone on Bay-K-8644-evoked CA secretion. Bay-K-8644 (10 μM) given into the perfusion stream for 4min increased CA secretion to 690±148 ng from 6 rat adrenal glands. However, under the effect of 30 μM dexamethasone which was preloaded for 20 min before Bay-K-8644 was introduced, Bay-K-8644 -stimulated CA secretion was strikingly depressed to 185±50 ng ($P<0.05$) for 4 min as compared to the corresponding control release; thus, the release was reduced to 27% of the control secretion. Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{++}-ATPase in skeletal muscle sarcoplasmic reticulum (Georger & Riley, 1989; Seidler et al, 1989). It may be extremely valuable pharmacological tool for investigating intracellular Ca^{++} mobilization and ionic current regulated by intracellular calcium (Suzuki et al, 1992). Fig. 5 shows the inhibitory action of dexamethasone on cyclopiazonic acid-evoked CA secretory response. Under the influence of dexamethasone (30 μM) in 9 rat adrenal glands, cyclopiazonic acid (10 μM)-evoked CA secretion was reduced to 153±26 ng ($P<0.01$) from the control response (323±50 ng).

Effect of 30μM hydrocortisone on CA secretion evoked by ACh, excess K+, DMPP and McN-A-343 from the isolated rat adrenal glands

In the previous results as shown in Fig. 1∼4, it was found that dexamethasone showed a dose-dependent inhibition in CA secretory responses. Moreover, it has been known that hydrocortisone (cortisol) is a natural glucocorticoid which has the same structural formula as cortisone except that OH is in position 11. Therefore, it is likely of very interest to examine the effect of hydrocortisone on CA secretion evoked by various secretagogues.

CA release evoked by ACh (5.32 mM) and excess K^+ (56 mM) after preloading with 30 uM hydrocortisone for 20 min was reduced to 374±51 ng ($P<0.05$, n=8) and 348±78 ng ($P<0.05$, n=6) for 4 min, respectively from the control secretion of 752 ±64 ng and 865±238 ng (Fig. 6).

DMPP (100 μM)- and McN-A-343 (100 μM)-stimulated CA releases in the absence of 30 μM hydrocortisone were 1390±103 ng (0∼4 min) and 242± 10 ng (4∼8 min), and 270±45 ng (0∼4 min), respectively. However, after preloading with 30 μM hydrocortisone for 20 min they were significantly reduced to 1093±48 ng (0∼4 min, $P<0.01$, n=12) and 139±33 ng (4∼8 min, $P<0.05$, n=8), and 49± 10 (0∼4 min, $P<0.01$, n=8), respectively (Fig. 7).

Figure 8 illustrates the inhibitory effects of 30 μM hydrocortisone on CA release evoked by Bay-K-8644 and cyclopiazonic acid. Under the 30 μM hydrocortisone, Bay-K-8644 (10 uM) and cyclopiazonic acid (10 μM) perfused into the adrenal gland for 4 min exerted the marked reduction in CA secretory responses of 189±27 ng ($P<0.01$, n=10) and 75±26 ng ($P<0.01$, n=8), respectively from the corresponding control releases of 558±82 ng and 221±

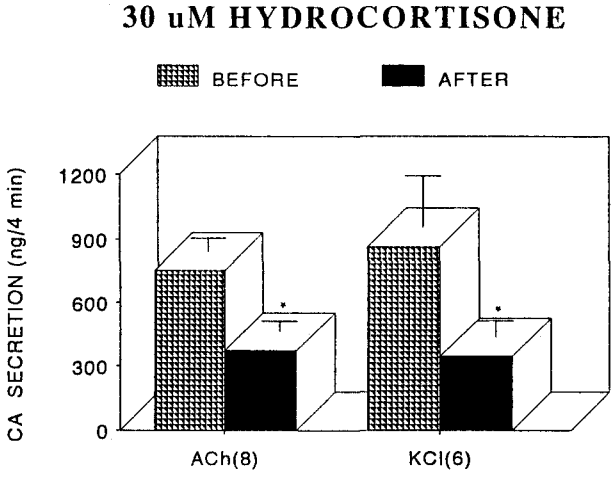

Fig. 6. Influence of 30 μM hydrocortisone on ACh- and excess K^+-stimulated CA secretory responses from the isolated rat adrenal glands. Other legends are the same as in Fig. 1 and 5. *: $P<0.05$, ***: $P<0.01$

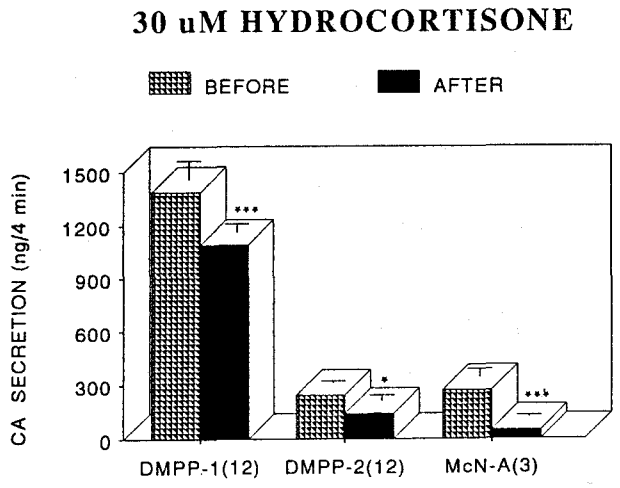

Fig. 7. Influence of 30 μM hydrocortisone on nicotinic and muscarinic stimulated CA secretory responses in the isolated rat adrenal glands. Other legends are the same as in Fig. 1 and 5. *: $P<0.05$, ***: $P<0.01$

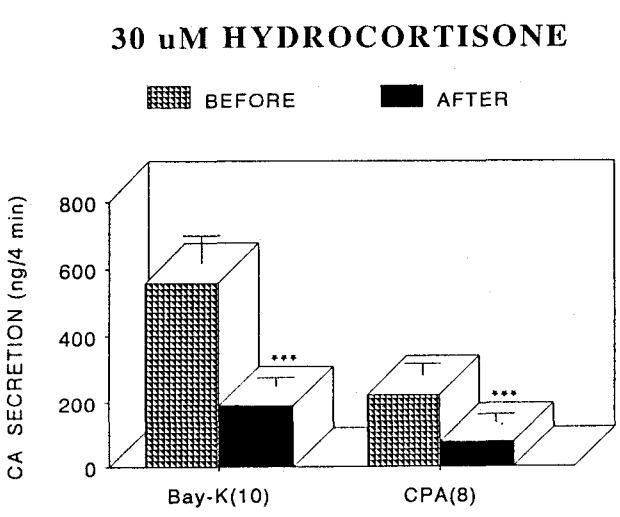

Fig. 8. Influence of 30 μM hydrocortisone on CA secretion evoked by Bay-K-8644 (Bay-K) and cyclopiazonic acid (CPA). Bay-K-8644 (10 μM) and cyclopiazonic acid were perfused into an adrenal vein for 4 min before and after the preloading with 30 μM hydrocortisone for 20 min. Its pefusate was collected for 4 min. Other legends are the same as in Fig. 1 and 5. *: $P<0.05$, ***: $P<0.01$

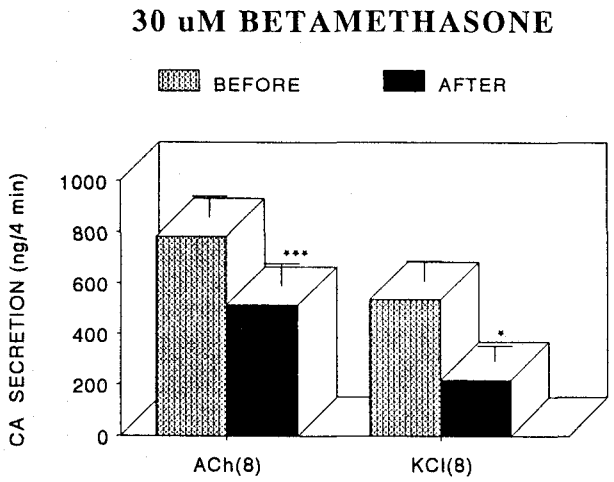

Fig. 9. Influence of 30 μM betamethasone on ACh- and excess K^+-stimulated CA secretion from the rat adrenal glands. ACh (5.32 mM) and excess KCl (56 mM) were given into an adrenal vein before and after the perfusion with 30 μM betamethasone for 20 min, respectively. Other legends are the same as in Fig. 1 and 5. *: $P<0.05$, ***: $P<0.01$

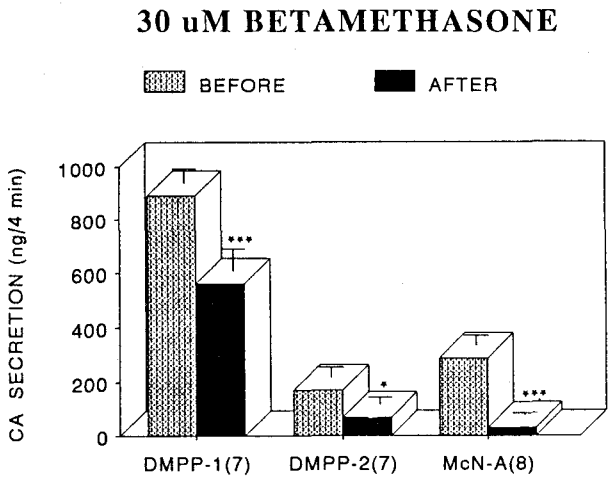

Fig. 10. Influence of 30 μM betamethasone on nicotinic and muscarinic stimulated CA secretory responses. Other legends are the same as in Fig. 1 and 5. *: $P<0.05$, ***: $P<0.01$

36 ng.

The effects of 30 μM betamethasone on CA release evoked by ACh, excess K^+, DMPP and McN-A-343 from the isolated rat adrenal glands

Since it has been found that betamethasone is the same as dexamethasone except that the CH_3 in position 16 is a β substitution instead of α (9α -fluoro-16β-methylprednisolone), it was tried to determine the effect of betamethasone on CA secretion evoked by various secretagogues from the isolated rat adrenal glands. Betamethasone (30 μM, 20 min) depressed the ACh (5.32 mM)- and excess K^+ (56 mM)-induced CA releases to 514±89 ng ($P<0.01$, n=8) and 216±59 ng ($P<0.05$, n=8), respectively from the control secretory responses of 783±82 ng and 534±78 ng (Fig. 9). On the other hand, betamethasone failed to alter the basal CA secretory response (data not shown). As illustrated in Fig. 10, DMPP (100 μM)- and McN-A-343 (100 μM)-induced CA secretions under the existence of 30 μM betamethasone were also greatly attenuated to 561±82 ng (0~4 min, $P<0.01$, n=7), 69±27 ng (4~8 min, $P<0.05$, n=7) and 30±7 ng (0~4 min, $P<0.01$, n=6), respectively from their control secretory responses of 891±55 ng (0~4 min) and 171±38 ng (4~8 min), and 289±36 (0~4 min).

DISCUSSION

The present data suggest that glucocorticoids cause the marked inhibition of CA secretion evoked by both cholinergic nicotinic and muscarinic receptor stimulation from the perfused rat adrenal medulla, indicating strongly that this inhibitory effect may be mediated by inhibiting influx of extracellular Ca^{2+} as well as the release of intracellular Ca^{2+} in the rat adrenomedullary chromaffin cells. However, in the previous studies Choi and his coworkers (1995) have shown that the synthetic glucocorticoid dexamethasone enhance histamine-evoked CA secretion from cultured bovine chromaffin cells. Moreover, glucocorticoids are known to regulate the synthesis and release of neurohormones and neurotransmitters (McEwen et al, 1986). Wurtman & Axelrod (1965) showed that hypophysectomy reduced the levels of adrenal medullary phenylethanolamine N-methyltransferase (PNMT), an enzyme catalyzing the conversion of norepinephrine to epinephrine. The effects of hypophysectomy were reversed by administration of the synthetic glucocorticoid, dexamethasone, suggesting that glucocorticoids regulate levels of PNMT. Later experiment with cultures of isolated bovine adrenal medullary cells showed that long-term (18~48 hr) exposure of medullary cells to glucocorticoids resulted in an increase in PNMT activity (Hersey & Distefano, 1979; Kelner & Pollard, 1985; Betito et al, 1992). Recently, Betito, Diorio & Boksa (1993) have found

that cortisol pulses (10^{-4} and 10^{-5} M), as short as 15min, increased PNMT activity measured 2 days following cortisol exposure with a required lag period of 18 h or more in bovine adrenal medullary cells.

In contrast to the previous findings, the present results showed that dexamethasone attenuated significantly the CA secretory responses evoked by cholinergic nicotinic (DMPP) and muscarinic (McN-A-343) receptor stimulation along with membrane-depolarization (high K^+) from the isolated perfused rat adrenal medulla. From these results, it could be felt that there may be a species difference between animal models, the cultured bovine chromaffin cells and the rat adrenal medullary cells. It is also thought that the difference may be due to the dose used in the experiment. However, in terms of the fact that cortisol pulses (10^{-4} and 10^{-5} M) increase PNMT activity in the bovine adrenal medullary cells (Betito et al, 1993), it could be ruled out that the concentration ($10^{-6}\sim10^{-4}$ M) used in the present study is not so high. Basal plasma levels of glucocorticoids reaching most tissues (other than the adrenal) are in the range 10^{-8} to 10^{-7} M, and these may rise to 10^{-6} M during a stressful event (Zumoff et al, 1974; Schoneshofer & Wagner, 1977; Dallman et al, 1987). In many of the tissues, nanomolar concentrations of glucocorticoids have been shown to translocate glucocorticoid receptors (McEwen et al, 1986). By contrast, levels of glucocorticoids measured in the adrenal vein of the anesthetized rat are between 6×10^{-5} and 10^{-4} M (Jones et al, 1977), indicating that the adrenal medulla can be exposed to concentrations of glucocorticoids at least as high as 10^{-4} M during stressful events.

In support of this finding, Choi and his coworkers (1995) found that dexamethasone possesses a selective effect on the response of the cells to histamine. Thus, although dexamethasone caused a threefold increase in histamine-evoked secretion from noradrenergic cells, it produced only a 30~35% increase in secretion evoked by high K^+. Dexamethasone appeared to cause a small but not significant increase in secretion evoked by DMPP, bradykinin, and angiotensin II (Choi et al, 1995). In the present investigation, glucocorticoids including dexamethasone, hydrocortisone and betamethasone inhibited the secretory responses evoked by DMPP, McN-A-343, high K^+ and ACh from the perfused rat adrenal medulla.

Wan & Livett (1989) showed that cortisol, prednisolone and 11-deoxy -17-hydroxycorticosterone were all effective in stimulating PNMT mRNA expression while cortisone, progesterone and β-estradiol with no effect. These results indicate that the effects are mediated by specific glucocorticoid receptor activation and exhibited a strict structural requirement for the ability of glucocorticoids to induce PNMT mRNA expression. Moreover, the pituitary-adrenocortical axis is known to be involved in the regulation of the steady-state levels of tyrosine hydroxylase and PNMT mRNAs. This regulation involves : ① induction of tyrosine hydroxylase mRNA contents in the adrenal medulla and superior cervical ganglia by increased plasma glucocorticoid levels and ② maintenance of the steady-state levels of PNMT mRNA in the adrenal medulla by glucocorticoid-dependent mechanisms (Stachowiak et al, 1988).

Betito and his coworkers (1992) have shown that bovine adrenal medullary cells contain classical glucocorticoids receptors that are translocated in response to nanomolar concentrations of glucocorticoids, as has been found in other tissues. Further translocation occurs in reponse to higher glucocorticoids concentrations, which may be encountered in the adrenal gland during stress, and this may be accompanied by further increases in PNMT activity. Glucocorticoid receptor has been purified, and monoclonal antibodies against glucocorticoid receptor have been produced (Okret et al, 1984) and used to analyze the distribution of the glucocorticoid receptor in the rat central nervous system with immunohistochemistry (Fuxe et al, 1985; Gustafsson et al, 1987). Chromaffin cells are known to contain the glucocorticoid, or type II, corticosteroid receptor (Kelner & Pollard, 1985; Betito et al, 1992). Therefore, it could be thought that glucocorticoids may cause inhibition of the CA secretory responses evoked by cholinergic muscarinic and nicotinic stimulation as well as membrane-depolarization by mediation of the glucocorticoid receptors.

In the present investigation, dexamethasone as well as hydrocortisone 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 glucocorticoids inhibited CA secretory responses by Bay-K-8644 as well as cholinergic receptor -stimul-

ation and membrane depolarization demonstrates that the inhibitory effect of glucocorticoids is mediated through the blockade of Ca^{++} entry into the chromaffin cells.

In support of these idea, there are many reports demonstrating a key role of Ca^{++} influx through voltage-sensitive Ca^{++} channels as a physiological pathway for the activation of adrenal CA secretion (Douglas, 1975; Aguirre et al, 1977; Schneider et al, 1977; 1981; Holz et al, 1982; Kao & Schneider, 1986). Moreover, it is found that the activation of nicotinic receptors stimulated CA secretion by increasing Ca^{++} entry through receptor-linked and/or voltage-dependent Ca^{++} channels in both perfused rat adrenal glands (Wakade & Wakade, 1983) and isolated bovine adrenal chromaffin cells (Kilpatric et al, 1981; 1982; Knight & 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 & Burgoyne, 1985; Kao & Schneider, 1985; 1986; Misbahuddin et al, 1985). However, recently, Lim & 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 is 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 findings, the present results that glucocorticoids suppresses CA secretory responses induced hy membrane-depolarization and cholinergic stimulation as well as by Ca^{++}-channel activator strongly suggest that this inhibitory effect of glucocorticoids 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 is considered to be regulated by the increased intracellular Ca^{++} (Knight & Kesteven, 1983; Kao & Schneider, 1986).

Also, in the present study, pretreatment with dexamethasone or hydrocortisone did inhibit CA secretory effect evoked by cyclopiazonic acid, which is known to be a highly selective inhibitor of Ca^{++}-ATPase in skeletal muscle sarcoplarmic reticulum (Geoger & 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). Therefore, it is speculated that inhibitory effects of glucocorticoids on CA release evoked by cholinergic stimulation and/or membrane-depolarization may be associated with intracellular Ca^{++} mobilization. It has been shown that Ca^{++}-uptake into intracellular storage sites susceptible to caffeine (Iino, 1989) is almost completely abolished by treatment with cyclopiazonic acid during the preceding Ca^{++} load (Suzuki et al, 1992). This is consistent with the findings observed 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 and reduces Ca^{++}-ATPase activity in sarcoplasmic/endoplasmic reticulum, resulting in decreases in the subsequent Ca^{++} release from those storage sites and thereby reduction of Ca^{++}-dependent K^+-current.

Based upon these findings, the present experimental results suggest strongly that the inhibitory effect of glucocorticoids on the evoked CA release may be mediated by inhibiting influx of extracellular calcium as well as the release of intracellular calcium from the intracelluar storage sites in the rat adrenomedullary chromaffin cells.

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