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Influence of Cytisine on Catecholamine Release in Isolated Perfused Rat Adrenal Glands

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The aim of the present study was to determine the characteristics of cytisine on the secretion of catecholamines (CA) in isolated perfused rat adrenal glands, and to clarify its mechanism of action. The release of CA evoked by the continuous infusion of cytisine (1.5×10^{-5} M) was time-dependently reduced from 15 min following the initiation of cytisine infusion. Furthermore, upon the repeated injection of cytisine (5×10^{-5} M), at 30 min intervals into an adrenal vein, the secretion of CA was rapidly decreased following the second injection. Tachyphylaxis to the release of CA was observed by the repeated administration of cytisine. The cytisine-induced secretion of CA was markedly inhibited by pretreatment with chlorisondamine, nicardipine, TMB-8, and the perfusion of Ca^{2+}-free Krebs solution, while it was not affected by pirenzepine or diphenhydramine. Moreover, the secretion of CA evoked by ACh was time-dependently inhibited by the prior perfusion of cytisine (5×10^{-6} M). Taken together, these experimental data suggest that cytisine causes secretion of catecholamines from the perfused rat adrenal glands in a calcium-dependent fashion through the activation of neuronal nicotinic ACh receptors located in adrenomedullary chromaffin cells. It also seems that the cytisine-evoked release of catecholamine is not relevant to the activation of cholinergic M_1-muscarinic or histaminergic receptors.

Key words: Cytisine, Adrenal Gland, Catecholamine Secretion, Neuronal nicotinic Ach receptors

INTRODUCTION

Cytisine, an alkaloid found in the seeds of *Laburnum anagyroides,* is an agonist at autonomic ganglia; in binding competition studies it has the highest affinity for brain nicotinic cholinergic binding sites of all drugs examined (Romano and Goldstein, 1980; Schwartz *et al.*, 1982; Marks *et al.*, 1986; Martino-Barrows and Kellar, 1987). A comparison of binding in several brain regions has shown that [^{3}H] cytisine binding is higher in the thalamus, striatum and cortex than in the hippocampus, cerebellum or hypothalamus (Pabreza *et al.*, 1990). Cytisine is a potent ganglionic stimulant and is as potent as nicotine at stimulating dopamine release from synaptosomal preparations (Rapier *et al.*, 1990).

However, previously published observations suggest that cytisine is qualitatively different from nicotine with regard to its apparent efficacy for brain nicotinic receptors. Cytisine is only 20-30% as effective as nicotine at stimulating rubidium efflux from synaptosomal preparations (Marks *et al.*, 1993), and Luetje and Patrick (1991) reported that cytisine is a much less potent agonist than nicotine for $\alpha4\beta2$ receptors expressed in *Xenopus oocytes*. They also showed the co-application of cytisine to $\alpha3\beta2$ receptors reduced the response to ACh. More recently, Singh and Prior (1998) has found that at a low stimulation frequency, and with a $[Ca^{2+}]_o$ of 1.8 mM, the nerve evoked endplate currents from isolated rat skeletal muscles were reduced by cytisine by about 20%, and that neuronal type nicotinic ACh receptors exist on rat motor nerve terminals. Recently, it has been found that cytisine is a nicotinic acetylcholine receptor agonist that was somewhat less efficacious than nicotine in bovine adrenal chromaffin cells (Wenger *et al.*, 1997). However, Gallardo and his colleagues (1998) found that cytisine had no agonist activity on [^{3}H] norepinephrine from cultured from rat fetal locus coeruleus. Thus, there is likely not only controversy in the mode of cytisines likely

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action, but also little evidence on the cytosine-induced release of CA from the adrenal medulla. This study was undertaken to determine whether cytisine affects the release of CA in the isolated perfused rat adrenal gland model, and to establish its mechanism of action.

METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180 to 300 grams, were intraperitoneally anesthetized with thiopental sodium (40 mg/kg). The adrenal glands were isolated by previously described methods (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area exposed by the placement of a three-hook retractor. The stomach, intestine and a portion of the liver were not removed, but pushed to the right side and covered by saline-soaked gauge pads, with the urine in bladder being removed to obtain enough working space for the tying blood vessels and cannulations.

A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein following ligation of all the branches of the adrenal vein (if any), vena cava and aorta. Heparin (400 IU/ml) was injected into the vena cava to prevent blood coagulation prior to the ligation of the vessels and the cannulations. A small slit was made in the adrenal cortex just the opposite entrance of the adrenal vein. The perfusion of the gland was started, making sure no leakage occurred, with the perfusion fluid only being allowed to escape from the slit made in the adrenal cortex. Then the adrenal gland was carefully removed from the animal, along with ligated blood vessels and the cannula, and placed on a platform in a leucite chamber, which was continuously circulated with water heated to 37± 1°C.

Perfusion of adrenal gland

The adrenal glands were perfused by means of a peristaltic WIZ pump (ISCO Inc., St. Lincoln, NE, U.S.A.) at a rate of 0.33 ml/min. The perfusion was carried out with Krebs-bicarbonate solution, composed of the following in (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. To prevent oxidation of the CA, disodium EDTA (10 μg/ml) and ascorbic acid (100 μg/ml) were added to the solution. The solution was constantly bubbled with 95% O_2 + 5% CO_2, and the final pH of the solution maintained at 7.4~7.5.

Drug administration

The perfusion of cytisine (5×10^{-5} M) for 4 min, or a single injection of acetylcholine (5.32×10^{-3} M) in a volume of 0.05 ml, was made into the perfusion stream via a three-way stopcock. In the preliminary experiments it was found that, on the administration of the above drugs, the secretory responses to acetylcholine and cytisine returned to the preinjection level in 4 and 8 min, respectively. Prior to the initiation of the experimental protocol, the adrenal glands were perfused with normal Krebs solution for about one hour.

Collection of perfusate

As a rule, prior to stimulation with cytisine, samples were collected (4 min) to determine the spontaneous secretion of CA ("background sample"). Immediately after the collection of the "background sample", the perfusate was collected in another tube immediately as the perfusion medium containing cytisine reached the adrenal gland. Each perfusate was collected for 8 min, at 4 min intervals. To obtain the net secretion value of CA, the amount secreted in the "background sample" was subtracted from that in the "stimulated sample", and these valued are shown in all the figures.

To study the effect of a test agent on the spontaneous, and drug-evoked, secretions, the adrenal gland was perfused with Krebs solution containing the agent for 20 min. The perfusates were collected for a specified time period ("background sample"), then the medium changed to that containing the test agent, or a specific antagonist, and the perfusates collected for the same period as the "background sample". The adrenal perfusates were collected in chilled tubes.

Measurement of catecholamines

The CA content of the perfusates were measured directly by the fluorometric method of Anton and Sayre (Anton and Sayre, 1962), but without the intermediate purification alumina for the reasons described earlier (Wakade, 1981), using a fluorospectrophotometer (Kontron Co., Milan, Italy). A 0.2 ml volume of perfusates was used in reactions. The CA content in the perfusates from glands stimulated by the secretagogues in this work was high enough to obtain readings several folds greater than those of the control samples (unstimulated). The sample blank readings were also lower than those of the perfusates of the stimulated and non-stimulated samples. The CA content in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statistical analysis

The statistical significance between 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 their standard errors (S.E.M.). The statistical analyses of the experimental results were made by the computer program described

by Tallarida and Murray (1987).

Drugs and their sources

The following drugs were used: cytisine, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, nicardipine hydrochioride and 3.4.5-trimethoxy benzoic acid 8-(diethylamino) octylester (TMB-8), pirenzepine hydrochloride, ethyleneglycol tetra-acetic acid (EGTA) and diphenhydramine hydrochloride (Sigma Chemical Co., U.S.A.), and chlorisondamine chloride (Ciba Co., U.S.A.). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required. Concentrations of all drugs used are expressed in molar terms-.

RESULTS

The secretory effect of CA evoked by cytisine

When the adrenal gland was perfused with oxygenated Krebs-bicarbonate solution, for 60 min prior to the initiation of the experimental protocol, the spontaneous secretion of CA reached a steady state. The basal release of CA from the perfused rat adrenal medulla was found to be 22± 3 ng for 2 min from 8 experiments. The releasing effects to the initial perfusion of cytisine (1.5×10^{-5} M) are shown in Fig. 1. Continuous infusion of cytisine (1.5×10^{-5} M) into the perfusion stream for 60 min exerted significant responses in the secretion of CA over that of the background, as they were time-dependently reduced from 376± 52 ng (0-5 min) to 144± 12 ng (55-60 min). These observations are largely consistent with those described previously (Wenger *et al.*, 1997), although discrepancies in the efficiencies of the agonist were observed with [^{3}H] norepinephrine from cultures of fetal rat locus coeruleus cells (Gallardo *et al.*, 1998).

In order to examine the tachyphylaxis to the cytisine-evoked releasing effects, cytisine at a concentration of 5×10^{-5} M was introduced into the perfusion stream for 4 min, on four consecutive occasions, at 30 min-intervals. In 6 rat adrenal glands, there was no statistically significant difference in the secretion of CA between the 1st and 2nd periods, while the significant inhibitions between those of the 1st and 3rd~4th periods were 48~61% of the 1st administration (Fig. 2). Therefore, in all subsequent experiments, cytisine (5×10^{-5} M) was never administered more than twice.

Effect of pirenezepine, chlorisondamine and diphenhydramine on cytisine-evoked CA secretion

In order to observe the effect of cytisine on muscarinic receptors, it would be interesting to examine the effect of pirenzepine on the release of CA evoked by cytisine. In this work, the CA output induced by cytisine was not affected in the rat adrenal gland preloaded with 2×10^{-6} M pirenzepine for 20 min. With 8 rat adrenal glands, 5×10^{-5} M cytisine-evoked CA releasing responses following the pretreatment with pirenzepine were not affected compared with their control secretions of 522± 82 ng (0-4 min) and 150± 28 ng (4-8 min), as shown in Fig. 3. There was no statistically significant difference between the cytisine-evoked CA secretory responses prior to and following pretreatment with pirenzepine.

In order to clarify the effect of chlorisondamine, a selective neuronal nicotinic receptor antagonist, on the cytisine-induced release of CA, the rat adrenal gland was preloaded with 10^{-6} M chlorisondamine for 20 min prior to the introduction of the cytisine. In the presence of chlorisondamine, the CA outputs evoked by perfusion with cytisine

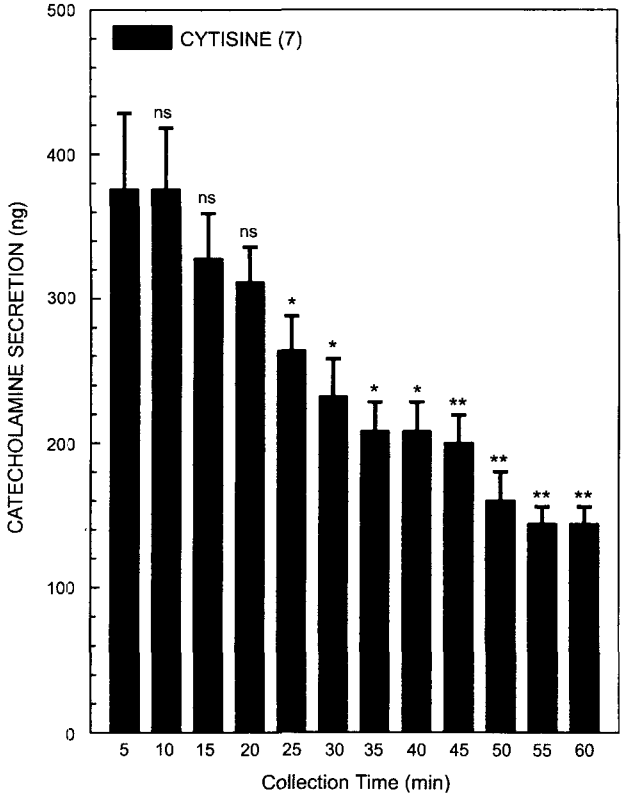

Fig. 1. Time-course effects of continuous infusion of cytisine on the secretion of catecholamines (CA) from perfused rat adrenal glands. Cytisine (1.5×10^{-5} M) was infused continuously for 60 min into an adrenal vein. Cytisine infusion was carried out after perfusion for one hour with normal Krebs-bicarbonate solution prior to the initiation of the experimental protocols. The data are expressed as the mean ± S.E. from 7 rat adrenal glands. The statistical significance was compared between the 1st group (0-5 min) and each periodic group. Abscissa: collection time (min). Ordinate: secretion of CA in ng for 5 min. The vertical columns and bars denote the means and the standard errors of the corresponding means, respectively. Number in the upper bracket indicates the number of animals used in the experiments. *: P<0.05, **P <0.01. ns: not statistically significant.

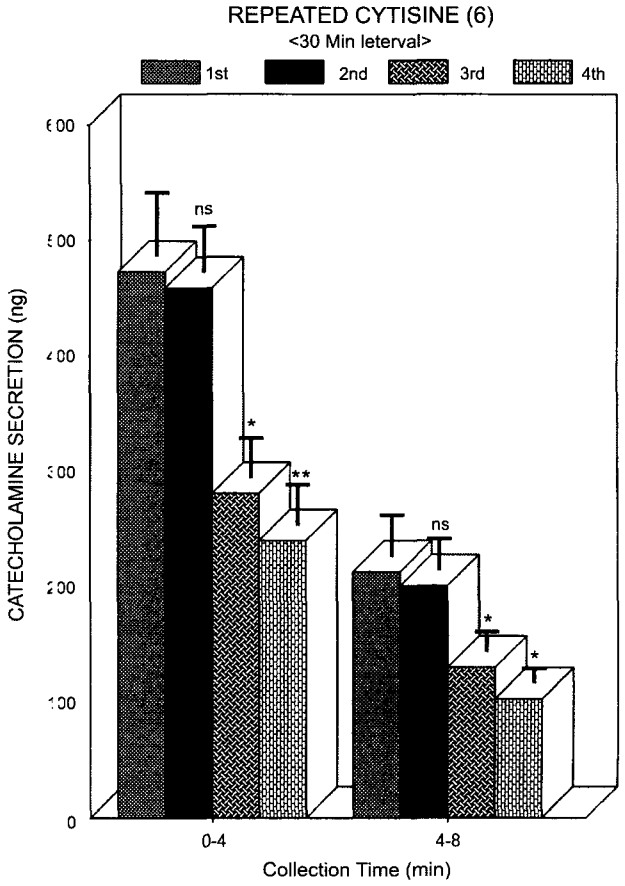

Fig. 2. The effects of repeated administration of cytisine on the secretion of CA in perfused rat adrenal glands. Cytisine (5×10^{-5} M) was perfused into an adrenal vein for 4 min at 30 min-intervals for about 60 min after the initiation of perfusion with normal Krebs solution. The perfusate was collected for 8 min at 4 min-intervals. These results were obtained from 6 rat adrenal glands. The statistical significance was compared between the 1st group and each of the other groups. *: P<0.05 **: P<0.01. ns: not statistically significant.

(5×10^{-5} M) were inhibited significantly, to 4~16% of that of the control compared with the corresponding control releases of 384± 54 ng (0-4 min) and 144± 31 ng (4-8 min) from 8 experiments, as shown in Fig. 3.

Diphenhydramine (10^{-5} M), a selective H_1-histaminergic receptor antagonist, was preloaded into the rat adrenal gland for 20 min prior to the introduction of the cytisine (5×10^{-5} M). In the presence of diphenhydramine, the cytisine-induced CA secretion remained unchanged compared to the corresponding control responses of 232± 32 ng (0-4 min) and 110± 12 ng obtained from 8 rat adrenal glands, as shown in Fig. 4.

The effect of perfusion of Ca^{2+}-free Krebs, nicardipine and TMB-8 on cytisine-evoked CA secretion

It has been found that the physiological releases of CA and dopamine-β-hydroxylase, from the perfused cat adrenal gland, are dependent on the extracellular calcium concentration (Dixon, Garcia and Kirpekar, 1975). It was of particular interest to test whether the secretory effect induced by cytisine was also. Therefore, the adrenal gland was preperfused with calcium-free Krebs solution containing 5×10^{-3} M EGTA for 20min prior to the introduction of cytisine. Under the perfusion of calcium-free Krebs solu-

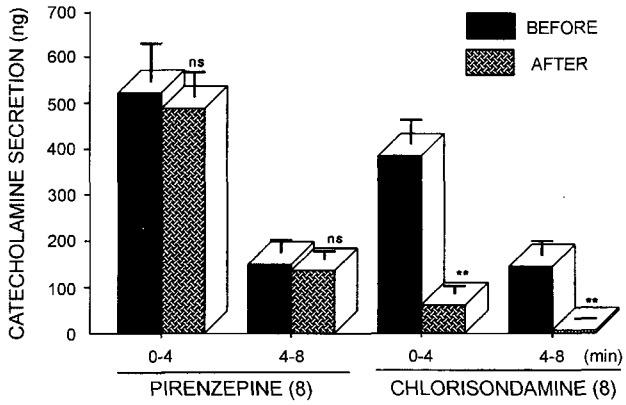

Fig. 3. The effects of pirenzepine and chlorisondamine on the secretion of CA evoked by cytisine in the perfused rat adrenal glands. Secretion of CA evoked by cytisine (5×10^{-5} M) was evoked 20 min after perfusion of the adrenal gland with Krebs solution containing pirenzepine (2×10^{-6} M) or chlorisondamine (10^{-6} M). "BEFORE" and "AFTER" indicates the amount of CA released by cytisine before and after the preloading with pirenzepine or chlorisondamine. Statistical differences were compared between the amount of CA evoked by pirenzepine or chlorisondamine "AFTER" and "BEFORE" pretreatment. A statistical difference was obtained when comparing the secretory effect of cytisine on CA after preloading with pirenzepine or chlorisondamine to their corresponding controls. Other legends are the same as in Fig. 1. **: P<0.01. ns: not statistically significant.

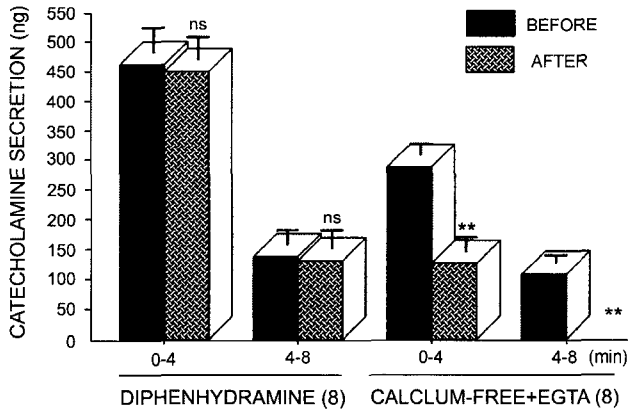

Fig. 4. The effects of diphenhydramine and Ca^{2+}-free Krebs-perfusion on the secretion of CA evoked by cytisine in the perfused rat adrenal gland. Secretion of CA evoked by cytisine (5×10^{-5} M) was induced 20 min following perfusion of adrenal gland with Krebs solution containing diphenhydramine (10^{-5} M) or Ca^{2+}-free Krebs solution containing EGTA (5×10^{-3} M). Other legends are the same as in Figs. 1 and 3. **: P< 0.01, ns: not statistically significant.

tion containing EGTA in 8 rat glands, the CA releasing responses to cytisine (5×10^{-5} M) were significantly inhibited to 44% (0-4 min, P<0.01) and 0% (4-8 min, P<0.01) of their corresponding control responses, as shown in Fig. 4.

In order to investigate the effect of nicardipine, a dihydropyridine derivative and L-type Ca^{2+} channel blocker (Hardman *et al.*, 1995), on the cytisine-evoked secretion of CA, nicardipine (10^{-6} M) was preloaded into the adrenal gland for 20 min. In the presence of nicardipine, the release of CA induced by perfusion of cytisine (5×10^{-5} M) for 4 min was greatly depressed to 30% (0-4 min, P<0.01) and 0% (4-8 min, P<0.01), in 12 rat adrenal glands compared with their corresponding control responses. Fig. 5 illustrates that nicardipine inhibits the secretory responses of CA evoked by cytisine.

It has been reported that muscarinic, but not nicotinic activation, causes the secretion of CA independently of extracellular calcium in the perfused cat adrenal glands (Nakazato *et al.*, 1988). The presence of an intracellular calcium pool is linked to the muscarinic receptors, and TMB-8, an intracellular calcium antagonist, inhibits both the nicotinic and muscarinic stimulation-induced release of CA in the rat adrenal glands (Lim *et al.*, 1992). We attempted to test the effect of TMB-8 on the cytisine-evoked secretion of CA. In 10 rat adrenal glands, secretions of CA evoked by cytisine (5×10^{-5} M) after preloading with TMB-8 (10^{-5} M) for 20 min were significantly blocked to 42% (0-4 min, P<0.01) and 12% (4-8 min, P<0.01) in comparison with their corresponding control responses, as shown in Fig. 5.

The effect of cytisine infusion on ACh-evoked CA release

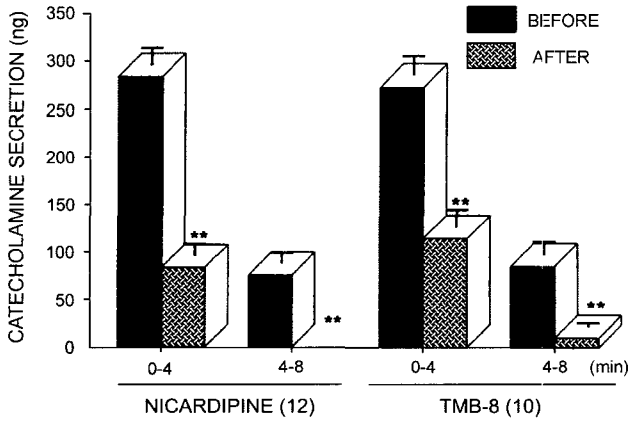

Fig. 5. The effects of nicardipine and TMB-8 on cytisine-evoked CA secretory responses in the perfused rat adrenal glands. Nicardipine (10^{-6} M) or TMB-8 (10^{-5} M) was perfused for 20 min before introducing cytisine (5×10^{-5} M). Other legends are the same as in Figs. 1 and 3. **: P<0.01.

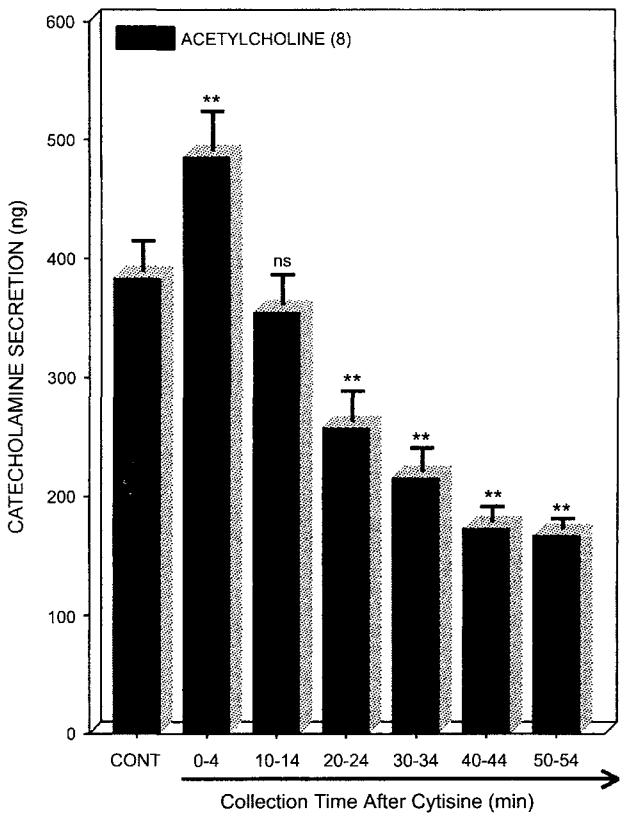

Fig. 6. The effects of cytisine-infusion on the ACh-evoked CA secretion in the perfused rat adrenal glands. ACh (5.32×10^{-3} M)-evoked CA secretory response was induced at 10 min intervals during the infusion of cytisine (5×10^{-6} M) for 60 min. "CONT" indicates the amount of Ach-evoked CA release before preloading with cytisine. Other legends are the same as in Figs. 1 and 3. **: P<0.01, ns: not statistically significant.

Being as the cytisine-induced release of CA was markedly inhibited by the pretreatment with chlorisondamine, as shown in Fig. 3, it was of interest to examine the influence of cytisine perfusion on the ACh-induced CA secretory responses. The adrenal gland was preloaded with cytsine (5×10^{-6} M) for 20 min prior to the introduction of ACh. In the presence of cytisine, the ACh-induced CA output was greatly enhanced to 127% (0-4 min, P<0.01) of the control response for the first 4 min, but was subsequently time-dependently inhibited to 93% (10-14 min, ns), 67% (20-24 min, P<0.01), 56% (30-34 min, P<0.01), 45% (40-44 min, P<0.01) and 44% (50-54 min, P<0.01) of the corresponding CA secretion (384± 26 ng), as shown in Fig. 6. However, in the absence of cytisine, the ACh-induced CA output was unaltered over the same period (data not shown).

DISCUSSION

These experimental data strongly suggest that cytisine causes secretion of CA from the isolated perfused rat

adrenal gland in a calcium-dependent fashion, through the activation of neuronal nicotinic ACh receptors located on the rat adrenomedullary chromaffin cells.

In support of this idea, it has been found that cytisine is a nicotinic acetylcholine receptor agonist, and that it was somewhat less efficacious than nicotine in bovine adrenal chromaffin cells (Wenger *et al.*, 1997). However, Gallardo and his colleagues (1998) suggested that cytisine has no agonist activity on [^{3}H] norepinephrine from cultured rat fetal locus coeruleus. In the present work, upon the repeated administration of cytisine (5×10^{-5} M) at 30 min intervals in the perfused rat adrenal gland, the CA secretory response rapidly decreased after the third perfusion of cytisine. Moreover, the release of CA evoked by the continuous infusion of cytisine was gradually time-dependently reduced from 15 min after the initiation of cytisine infusion. Tachyphylaxis to the releasing effects of CA evoked by cytisine was observed on repeated administration. In support of this finding, Collect and Story (1984) found that the release of CA evoked by DMPP declined abruptly between the first and second periods of exposure to DMPP in isolated rabbit adrenal glands and guinea pig atria. This reduction may be due to the agonist desensitization of the nicotinic receptors. In this study, the repeated perfusion of cytosine, four times at 30 min intervals, also produced a desensitization-like effect (tachyphylaxis) between the 1st and 3rd~4th periods. However, Lim and Hwang (1991) have found that the repetitive perfusion of DMPP in the isolated perfused rat adrenal gland did not produce any desensitization-like effect (tachyphylaxis) between the 1st and 2nd~3rd periods. From these results, the existence of different species in the secretion of CA evoked by cytosine can not be excluded.

Generally, the adrenal medulla has been employed as a model system for the study of numerous cellular functions involving not only noradrenergic nerve cells, but neurons also. One such function is neurosecretion. During the neurogenic stimulation of the adrenal medulla, ACh is released from the splanchnic nerve endings and activated cholinergic receptors on the chromaffin cell membrane (Viveros, 1975). This activation triggers a series of events, known as stimulus-secretion coupling, culminating in the exocytotic release of CA, and the components of the secretory vesicles, into the extracellular space. ACh, the physiological presynaptic transmitter at the adrenal medulla, releases CA and dopamine-β-hydroxylase in a calcium-dependent secretory process (Viveros *et al.*, 1968; Dixon *et al.*, 1975).

The cytisine-induced release of CA was due presumably to the exocytosis of CA storage vesicles, subsequent to the activation of nicotinic ACh receptors in the rat adrenomedullary chromaffin cells, since it was almost completely abolished in the presence of chlorisondamine in our work. Chlorisondamine is known to be a selective antagonist of neuronal nicotinic cholinergic receptors (Hardman *et al.*, 1995). However, in this study, the cytisine-evoked CA secretory response was not affected by pretreatment with pirenzepine, while it was greatly inhibited by pretreatment with chlorisondamine. This finding indicates that cytisine-evoked CA release is not exerted by the stimulation of muscarinic ACh receptors. In general, muscarinic receptor subtypes have been recognized in many tissues (Eglen and Whiting, 1986). Receptor binding studies have supported the classification of muscarinic receptors into M_1 and M_2 based on the selectivity profile of pirenzepine; receptors with a high affinity for pirenzepine are designated as M_1 and those with low affinity as M_2 receptors (Hammer *et al.*, 1980; Hammer and Giachetti, 1982). Doods and his colleagues (1987) have classified muscarinic receptors into M_1 (pirenzepine sensitive, neuronal), M_2 (cardiac) and M_3 (smooth muscle and glandular). In view of the above studies, our finding that cytisine-evoked CA release was not affected by pretreatment with pirenzepine strongly demonstrates that cytisine-evoked CA secretion is not mediated through the activation of M_1-muscarinic receptors in the perfused rat adrenal gland.

In the present study, the cytisine-evoked release of CA was not influenced by the presence of diphenhydramine, demonstrating that the cytisine-evoked secretion of CA is not relevant to the activation of histaminergic receptors in the perfused rat adrenal medulla.

The indispensable role of calcium in the neurosecretory process is well established. According to the assumption of Baker and Knight (1978; 1980), the relationship between the concentration of intracellular calcium and the transmitter release has not been determined in the nerve terminals. As mentioned above, calcium plays a crucial role in the depolarization-neurotransmitter release coupling process in many types of secretory cells (Douglas, 1968; Schulz and Stolze, 1980; Williams, 1980). Furthermore, it has been found that nicotinic (but not muscarinic) stimulation also releases ACh from the chromaffin cells by a calcium-dependent mechanism (Mizobe and Livett, 1983). The activation of nicotinic receptors stimulates the secretion of CA by increasing Ca^{2+} entry through receptor-linked, and/or voltage-dependent Ca^{2+} channels, in perfused rat adrenal glands (Wakade and Wakade, 1983) and isolated bovine adrenal chromaffin cells (Kilpatrick *et al.*, 1981; 1982; Knight and Kesteven, 1983).

In the present study, removal of extracellular Ca^{2+} markedly depressed the secretion of CA evoked by cytisine. The pretreatment of nicardipine, a dihydropyridine derivative, and an L-type Ca^{2+} channel blocker, also blocked the cytisine-evoked CA release. The secretory effect of cytisine apparently seems to be dependent on extracellular calcium. However, in this experiment, the reason for the considerable response to cytisine in the Ca^{2+}-free Krebs plus

EGTA solution is unclear. In the presence of TMB-8, an inhibitor of the intracelluar calcium stores, the cytisine-evoked secretion of CA was greatly inhibited in the perfused adrenal gland. TMB-8 is also known to inhibit caffeine-induced $^{45}Ca^{2+}$ release from, but not its uptake by, a sarcoplasmic reticulum preparation of skeletal muscle (Chiou and Malagodi, 1975), and in isolated bovine adrenomedullary cells (Mibahuddin *et al.*, 1985; Sasakawa *et al.*, 1984). Moreover, it has been shown that the caffeine-evoked secretion of CA from the perfused cat adrenal gland in the absence of extracellualr calcium is also inhibited (Yamada *et al.*, 1988).

Therefore, this experimental result suggests that chromaffin cells of the rat adrenal gland contain the intracellular calcium store that participates in the secretion of CA, as shown in bovine adrenal glands (Baker and Knight, 1978). Such a store may not be easily depleted by the mere removal of extracellualr calcium. Some investigators (Boxler, 1968; Ohashi *et al.*, 1974; Casteels and Raeymaeker, 1979; Malagodi and Chiou, 1974; Takahara *et al.*, 1990) reported that intracellular stores of calcium have been shown to play some role in the contraction of smooth muscle produced by noradrenaline or ACh in Ca^{2+}-free media.

Interestingly, in this study, the reason why the secretory responses of CA evoked by ACh were time-dependently depressed in the presence of cytisine (continuous infusion) is unclear. In support of this idea, Papke and Heinemann (1994) reported cytisine to be a true partial agonist for β_2-containing ACh receptors, and as such can inhibit the response of these receptors to ACh through a competitive mechanism. Although cytisine was relatively ineffective in stimulating current, the coapplication of cytisine and ACh reduced the responses to ACh (Papke and Heinemann, 1994). Therefore, bearing these facts in mind, it is felt that cytisine has a partial agonist activity.

Taken together, these experimental data suggest that cytisine can significantly effect the secretion of CA from the perfused rat adrenal gland in a calcium-dependent fashion, through the activation of neuronal nicotinic ACh receptors located in adrenomedullary chromaffin cells. It also seems that the cytisine-evoked catecholamine release is not relevant to the activation of cholinergic muscarinic, or histaminergic receptors.

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