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Influence of Histaminergic Receptor Activation on Catecholamine Secretion in The Perfused Rat Adrenal Gland**

*Dong-Yoon Lim and Sang-Hyun Rho

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

ABSIRACT

The present study was conducted to examine the characteristics of histamine on catecholamine secretion in the isolated perfused rat adrenal gland and to clarify the mechanism of its secretory action. Histamine (37.5 to 150 ug) injected into an adrenal vein evoked a dose-dependent significant secretory response of catecholamines (CA) from the rat adrenal gland. However, upon the repeated injection of histamine (150 ug) at 120 min intervals, CA secretion was rapidly decreased after third injection of histamine. Tachyphylaxis to releasing effects of CA evoked by histamine was observed by the repeated administration.

The histamine-induced CA secretion was markedly inhibited by the pretreatment with chlorisondamine, diphenhydramine, ranitidine, Ca^{++}-free Krebs solution, nicardipine and TMB-8 while was not affected by pirenzepine. Moreover, the CA secretion evoked by ACh was considerably reduced by the prior perfusion of histamine (6.8×10^{-5} M) for 30 min.

These experimental data suggest that histamine causes secretion of CA in a calcium dependent manner from the perfused rat adrenal gland and that its secretory effect is mediated through activation of both H_1- and H_2-histaminergic receptors located in adrenal medulla, which may be associated with stimulation of cholinergic nicotinic receptors.

Key Words: Histaminergic receptors, Catecholamine secretion, Adrenal gland

It has been known that histamine acts as a neurotransmitter in the central nervous system where it, like other adrenergic nueurotransmitters, is unevenly distributed (Prell and Green, 1986; Schwartz *et al.,* 1986). Histamine induces many strong peripheral effects, such as an increase in permeability of microvessels, contraction of smooth muscles and secretion of gastric acid. Histamine is also found to evoke many central effects, such as regulation of hormonal secretions and modulation of the sleep-wake cycle (Prell and Green, 1986). Sympathoadrenal activity is significantly increased by stress stimuli, and the plasma concentrations of catecholamines (CA) reflect the degree of this activity (Kvetnansky *et al.,* 1978). In addition, sympathetic activity increases after central administration of histamine, and that effect is attenuated by ganglionic blockade (Hoffman and Schmid, 1978; Klein and Gertner, 1983; Trendelenburg, 1957).

Smith and Robinson (1970) have shown that histamine given into the perfused cat adrenal gland causes the dwindling response of CA secretion due to depletion of the "readily secretable" store of CA in the face of failure of chromaffin cell to carry on adequate synthesis under these condi-

*To whom correspondence should be addressed.

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tions. Szczygielski (1932) and Yoshizake (1973) found that CA secretion could be induced in the rat and the cat by injecting or infusing histamine directly into the adrenal gland in situ or in the isolated perfused gland. This CA secretory effect of histamine is shown to be dependent on extracellular calcium in cats (Poisner and Douglas, 1966), and is mediated by H_1-histaminergic receptors in cats and rats (Emmelin and Muren, 1949; Trendelenburg 1954; Staszewska-Barczak and Vane, 1965; Yoshizake, 1973; Robinson, 1982). Histamine is also found to evoke CA secretion from the adrenal gland of the dog (Schaepdryver, 1959; Narita, 1971) and the bovine adrenal medulla (Schneider, 1969; Shima *et al.*, 1979), the bovine adrenal medullary chromaffin cells (Noble *et al.*, 1988; Goh and Kurosawa, 1991). More recently, Schellenberg and his coworkers (1991) have reported that histamine infusion to man releases catecholamine. This secretory effect of histamine is known to be through H_1-histaminergic receptor activation, but not through H_2-receptor. However, Knigge and his celleagues (1990) found that neuronal histamine is an important mediator of the restraint stress-induced release by an action on H_1-and H_2-receptors with the central nervous system. Furthermore, histamine has been shown to inhibit sympathetic neurotransmission, in cat sympathetic ganglia (Brezenoff and Gertner, 1972), gracilis muscle, hind paw (Powell, 1979), dog heart (Lokhandwala, 1978), and pial aterioles and veins in situ (Gross *et al.*, 1983). Histamine impairs the effects of sympathetic nerve stimulation. Interference with sympathetic neuronal function is apparently mediated through H_2-receptor. McGrath and Shepherd (1976) reported that histamine-induced inhibition of norepinephrine release in vitro was reversed by a H_2-receptor antagonist and mimicked by stimulation of specific H_2-receptors.

As mentioned so far, it is clear that there are many different reports on CA secretion of histamine according to the experimental preparations, investigators and the kinds of animals. Therefore, the present study was designed to examine the effect of histamine on CA secretion in the isolated perfused rat adrenal gland and to determine the difference of H_1-and H_2-receptors activation and the role of calcium on its secretory response.

MATERIALS AND METHODS

Experimental animals

Mature male Sprague-Dawley rats, weighing 180-300 grams, were anesthetized with ether. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by placing three hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauze pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations.

A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37 ± 1℃.

Perfusion of adrenal gland

The adrenal glands were perfused by means of a ISCOP pump (WIZ Co.) at a rate of 0.4 ml/min. The perfusion was carried out with Krebs-bicarbonated 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; gulcose, 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 ± 0.5. The solution contained disodium EDTA (10 μg/ml) and ascorbic acid (100 ug/ml) to prevent oxidation of catecholamine.

Drug administration

Single injection of histamine (37.5~150 ug) or Ach (50 ug) in a volume of 0.05 ml were made into perfusion stream via a three way stopcock. In the preliminary experiments it was found that upon administration of the above drugs, secretory responses to ACh and histamine returned to preinjection level in about 4 min.

Generally, the adrenal glands were perfused with normal Krebs solution for about one hour before the experiment is initiated. The adrenal perfusate was collected in chilled tubes.

Collection of perfusate

As a rule, prior to each stimulation with histamine and ACh samples were collected (4 min) to determine the spontaneous secretion of CA ("background sample"). Immediately after the collection of the "background sample", collection of the perfusate was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Each perfustae was collected for 4 min. The CA amounts secreted in the "background sample" have been subtrated from those secreted the "stimulated sample" to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effects of various receptor antagonists on the spontaneous and histamine -or ACh-evoked secretion, the adrenal gland was perfused with Krebs solution containing the agent for 20~30 min, than the perfusate was collected for a specific time period ("background sample"), and then the medium was changed to the one containing the test agent and the perfusate were collected for the same period as that for the "background sample".

Measurement of catecholamines

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayre (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981), using fluorospectrophotometer (Shimadzu Co., Japan). A volume of 0.2 ml of the perfusate was used for the reaction.

The CA content in the perfusate of stimulated glands by ACh or histamine was high enough to obtain readings several-fold greater than the reading of control samples (unstimulated). The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents. All data are presented as means with their standard errors, and the significance of differences was analyzed by Student's t-test, using the computer system as previously described (Tallarida and Murray, 1987).

Drugs and their sources

The following drugs were used: histamine chloride, acetylcholine chloride, diphenhydramine hydrochloride, nicardipine hydrochloride, 3,4,5-trimethoxy benzoic acid 8-(diethylamino) octylester (TMB-8), norepinephrine bitartrate (Sigma Chemical Co., U.S.A.), pirenzepine 2HCl (Shinpoong Pharmaceutical Manfac., Korea), chlorisondamine chloride (Ciba. Co., U.S.A.), ranitidine HCl (Glaxo. Co., England).

Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required. Concentrations of all drugs used are expressed in terms of molar base except the case of histamine or ACh in ug.

RESULTS

Histamine-evoked CA secretory responses from the isolated perfused rat adrenal gland

The resting (basal) CA secretion from the the perfused rat adrenal glands reaches a steady state after the perfusion with normal Krebs solution for 60 min before the experimental protocol is initiated. The secretory responses of the isolated perfused adrenal gland of the rat to the initial injection of a range doses of histamine (37.5~150ug) are shown in Figure 1 and Table 1. This range of doses of histamine resulted in a nearly complete dose-response relationship. Injection of 37.5 ug-histamine into the perfusion stream produced significant increase of CA over the background secretion, which was 170.7±21.9 ug for 4 min. A grandual increase in histamine administration resulted in greater amounts of CA secreted in the perfusate. After injections of 75 ug and 150 ug histamine, CA secretions were 260.5±43.8 and 448.8

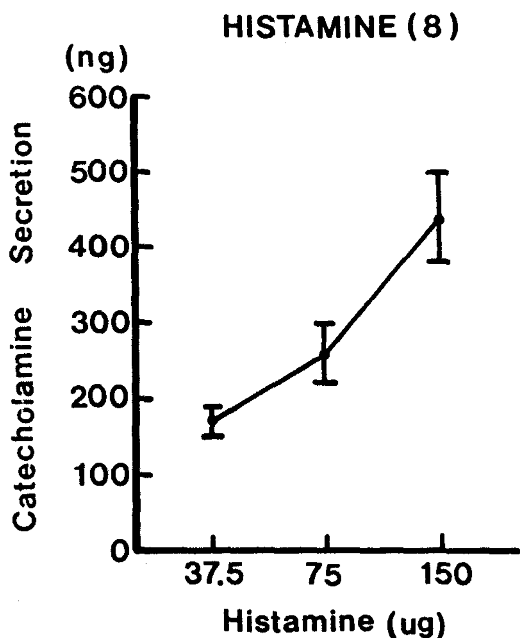

Fig. 1. Concentration-dependence of histamine-evoked secretions of catecholamines in the isolated perfused rat adrenal glands. Secretion of catecholamines (CA) was evoked by introducing injections of histamine (37.5, 75 and 150 μg) into the perfusion stream at 90~120 min intervals after perfusion with normal Krebs solution for 60 min. Following the injection of drug, the perfusate was collected for 4 min. Vertical bars and dots indicate mean with the standard error of the mean (SEM). Numeral in the parenthesis denotes number of the experimental animals. Ordinate: the amounts (ng) of catecholamines secreted from the adrenal gland for 4 min. Abscissa: doses of histamine in μg.

±68.9 ng/4 min, respectively from 8 glands. These observations are identical to those reported previously (Smith and Robinson, 1970; Suchaepdryver, 1959; Naritz, 1971; Schneider, 1969, Shima *et al.*, 1976; Kilpatrick, 1984; Noble *et al.*, 1988). When ACh (150 ug) was given into the perfusion stream via a threeway stopcock, CA secretion was 660.4± 141.2 ng for 4 min from 18 rat adrenal glands. In order to examine the tachyphylaxis to releasing effects of CA evoked by histamine, histamine at the dose of 150 ug was given into the gland three times consecutively at 120 min intervals. Figure 2

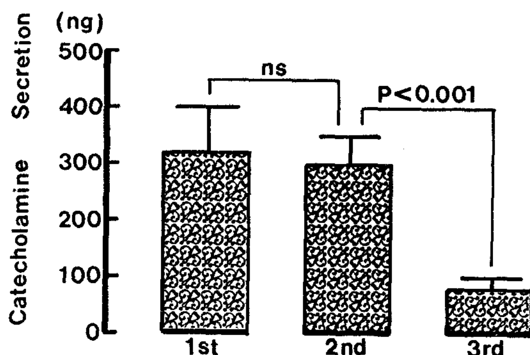

Fig. 2. The effect of the repeated injection of histamine (150 μg) on the secretory responses of catecholamines from the rat adrenal glands. Histamine (150 μg) was given repeatedly into the perfusion stream at 120 min intervals. Each column and vertical bar denote mean±SEM obtained from 8 rat adrenal glands. Other legends are the same as in Fig. 1. ns: stastistically nonsignificance

Table 1. Catecholamine secretion evoked by histamine from the isolated perfused rat adrenal gland

Type of stimulus	Dose (μg)	Time of collection (min)	Secretion of catecholamines (ng)	Number of animal
Acetylcholine	50	0~4	660.4±142.2	18
Histamine	37.5	0~4	170.7± 21.9	8
	75	0~4	260.5± 43.8	8
	150	0~4	448.8± 68.9	8

Secretions of catecholamines are expressed with mean±standard error of the mean (SEM) between pre- and post-stimulation. The adrenal glands were stimulated with the single injection of acetylcholine (50 μg) or histamine (37.5, 75 and 150 μg) in 0.05 ml into an adrenal vein. The perfusate to the drug was collected for 4 min. All data were statistically significant as compared with the corresponding basal release (P<0.001).

shows the dwindiling response when a series of three injections of 150 ug of histmaine are administered at 120 min intervels. The CA secretion of each injection of histamine was 327.5±80.3 ng (1st), 305.8±51.6 ng (2nd) and 84.7±24.9 ng (3rd), respectively from 8 adrenal glands. There was no statistical difference between 1st and 2nd secretions of CA while significant inhibition between 2nd and 3rd secretion ($p<0.001$). The present experimental results are consistent with those reported by Smith and Robinson (1970) from the perfused cat adrenal medulla.

In all subsequent experiments 150 ug only of histamine dose was used along with 50 ug-ACh in order to compare each other in CA secretion because tachyphylaxis to releasing effect of CA evoked by histamine was observed in the present study.

Effect of chlorisondamine on the release of CAs evoked by histamine

In order to examine the effect of chlorisondamine, a selective nicotinic receptor antagonist (Gilamn *et al.,* 1991), on histamine-evoked CA secretion, the rat adrenal gland was perfused with chlorisondamine (10^{-6} M) for 20 min before the introduetion of histamine or ACh. In the presence of chlorisondamine, the CA release evoked by 150 ug-histamine and 50 ug-ACh were greatly reduced to 124.0±27.8ng/4 min ($p<0.03$) and 144.0±28.7 ng/4 min ($p<0.01$), respectively as compared with their corresponding control responses of 868.0±199.1 ng and 584.0±155.6 ng for 4 min. Figure 3 represents the inhibitory response of chlorisondamine on CA secretion induced by histamine and ACh.

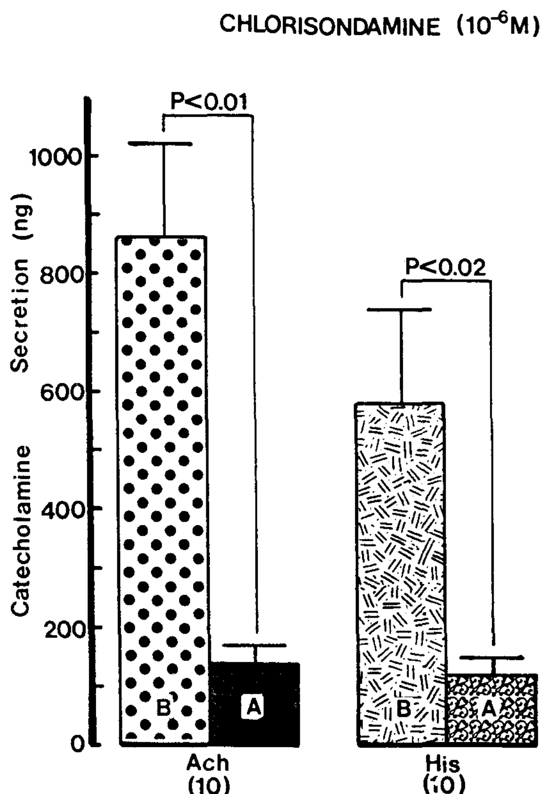

Fig. 3. The effect of chlorisondamine on histamine-evoked CA secretion. Chlorisondamine (10^{-6}M) was perfused into the adrenal gland for 20 min before introducing the drugs. Other legends are the same as in Fig. 1. "B" and "A" indicate CA secretion evoked by acetylcholine (50 μg) and histamine (150 μg) before and after treatment with a blocking agent (chlorisondamine). ACh: Acetylcholine. His: Histamine

Effect of pirenzepine on histamine-evoked CA secretion

It has been known that pirenzepine is a more selective M_1-muscarinic receptor antagoinst (Doods *et al.,* 1978; Hammer *et al.,* 1988). Thus, it would be exciting to test the influence of pirenzepine on CA secretion induced by histamine and ACh. In the present investigation, the CA release of histamine or ACh was evoked in the adrenal gland pretreated with 2×10^{-6} M -pirenzepine for 30 min. In 6 glands, histamine-evoked CA secretion in the presence of pirenzepine was 153.3±24.0 ng/4 min, which was no difference as compared with its control secretion of 176.7±20.3 ng/4 min. However, ACh-induced CA release was significiantly inhibited to 237.1±32.8 ng/4 min ($p<0.02$) by comparing with the corresponding control release of 481.4±95.2 ng/4 min. Figure 4 shows the effect of pirenzepine on CA secretion evoked by histamine and ACh.

Effect of diphenhydramine on histamine-evoked CA secretion

Diphenhydramine (10^{-5} M), a selective H_1-his-

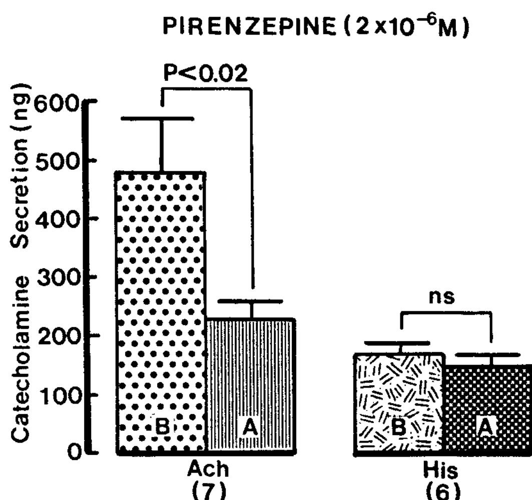

Fig. 4. The effect of pirenzepine on CA secretion evoked by histamine. Pirenzepine (2×10^{-6}M) was present 30 min before perfusion of histamine or ACh. Other legendes are the same as in Fig. 2 and 3.

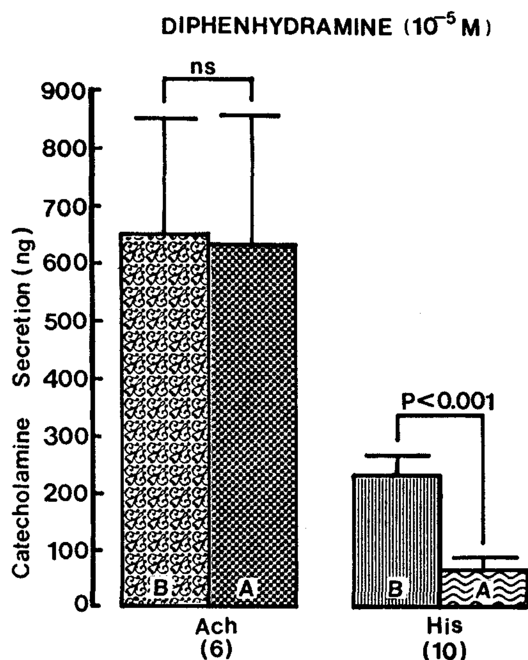

Fig. 5. The effect of diphenhydramine on histamine-induced CA secretory response. Diphenhydramine (10^{-5}M) was administered prior to injection of histamine or ACh. Other legends are the same as in Fig. 2 and 3.

taminergic receptor antagonist was preloaded into the rat adrenal gland for 20 min before the introducing histamine or ACh. In presence of diphenhydramine, histamine-induced CA secretion was clearly depressed to 66.0 ± 21.9 ng/4 min ($p<0.001$) of the corresponding control response (232.0 ± 32.6 ng/4 min) from 10 rat adrenal glands as shown in Figure 5. However, A concentration of diphenhydramine (10^{-5}M) that virtually abolished histamine (150 ug)-induced CA secretion had no effect on ACh-evoked CA secretory response from 6 glands (Fig. 5). ACh-induced CA release prior to administration of diphenhydramine was 650.0 ± 194.4 ng/4 min while that after diphenhydramine was 630.0 ± 224.8 ng/4 min.

Effect of ranitidine on histamine-evoked CA secretion

Since ranitidine is a selective and more potent

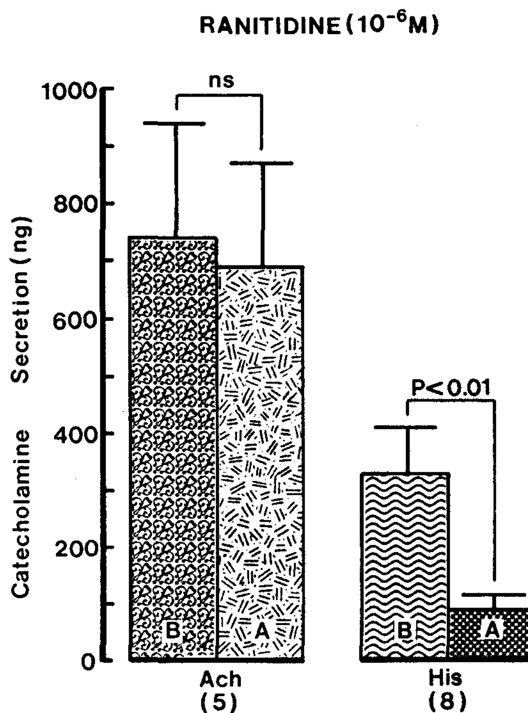

Fig. 6. The effect of ranitidine on CA serection evoked by histamine. Ranitidine (10^{-6}M) was perfused into the adrenal gland before introducing histamine or ACh. Other legends are the same as in Fig. 2 and 3.

H_2-histaminergic receptor antagonist more than a specific H_2-antagonist, cimetidine (Gilman *et al.*, 1991), it was decided to examine the effect of ranitidine on histamine-or ACh-evoked CA secretions. Ranitidine (10^{-6} M) was pretreated into the perfused rat adrenal gland for 20 min before the introduction of histamine. Under the existence of ranitidine effect, histamine-induced CA secretory response was markedly reduced to 95.0±29.4 ng/4 min ($p<0.01$) as shown in Figure 6. However, ACh-induced CA release was not affected by the pretreatemt with ranitidine (Fig. 6).

Effect of perfusion with calcium-free Krebs on histamine-evoked CA secretion

Since the secretory effect of histamine from the cat adrenal medulla (Poisner and Douglas, 1966), the bovine adrenal medullary chromaffin cells (Noble *et al.*, 1988) and the physiological release of CA and dopamine-beta-hydroxylase from the perfused cat adrenal gland is dependent on the extracellular calcium concentration (Dixon, Garcia and Kirpekar, 1975), it is of particular interest to test whether the secretory effect induced by histamine is also related to extracellular calcium ions. Thus, the adrenal gland was preloaded with calcium-free Krebs solution for 30 min before the injections of histamine and ACh.

In the absence of extracellular calcium, histamine (150 ug)-induced CA secretion was significantly attenuated to 224.0 ± 61.9 ng/4 min ($p<0.01$) as compared with the corresponding control secretion of 500.0±72.2ng/4 min from 10 rat adrenal glands. ACh-induced secretory response of CA following the pretreatment with calcium-free Krebs solution was also markedly inhibited to 501.0±72.2 ng/4 min ($p<0.01$) as compared with its control release of 770.0±116.1 ng/4 min from 10 adrenal glands. Figure 7 shows the inhibitory effect of perfusion of Ca^{++}-free Krebs on histamine-and ACh-induced CA secretion.

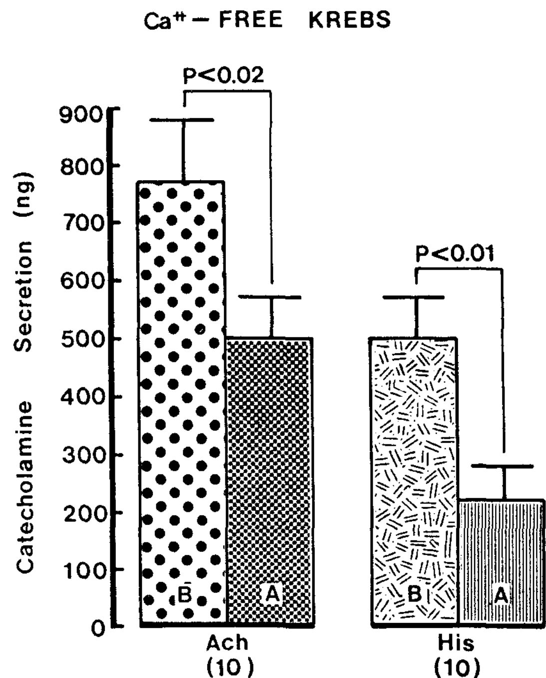

Fig. 7. The effect of perfusion of calcium-free Krebs solution on secretory response of CA evoked by histamine. Calcium-free Krebs solution was infused for 30 min prior to introduction of histamine or ACh. Other legends are as in Fig. 2 and 3.

Effect of nicardipine on histamine-evoked CA secretion

In order to examine the effect of nicardipine, a dihydropyridine derivative and L-type Ca^{++} channel blocker (Gilman *et al.*, 1991), on histamine-evoked CA secretion, nicardipine (10^{-6} M) was preloaded into the adrenal gland for 20 min before the introduction of histamine. In the presence of nicardipine, histamine (150 ug)-induced CA release was greatly depressed to 44.0±16.1 ng/4 min ($p<0.001$) in comparison with its control response of 360.0±67.9 ng/4 min from 8 rat adrenal glands as shown in Figure 8. ACh-induced secretory effect of CA was also greatly decreased to 154.3±38.2 ng/4 min ($p<0.02$) by comparing the corresponding control secretion of 635.7±152.9 ng/4 min in 7 glands (Fig. 8).

Effect of TMB-8 on histamine-evoked CA secretion

Since it has been found that muscarinic, but not nicotinic activation causes catecholamine secretion independent of extracellular Ca^{++} in perfused adrenal glands of the cat (Nakazato *et al.*, 1988), suggesting the presence of an intracellular

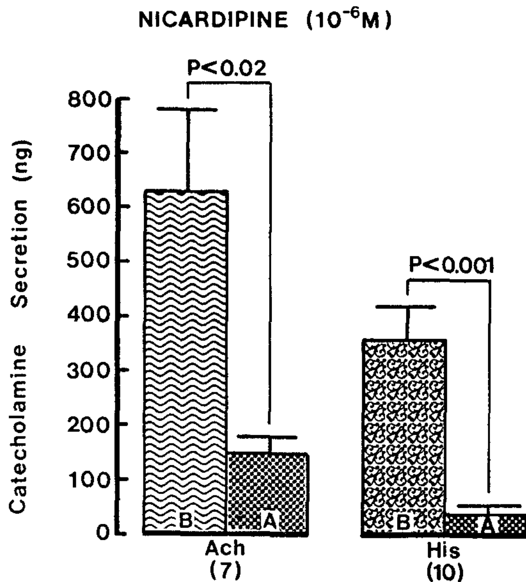

Fig. 8. The effect of nicardipine on histamine-evoked CA secretory responses. Nicardipine (10^{-6} M) was perfused for 30 min before introducing histamine or ACh. Other legends are the same as in Fig. 2 and 3.

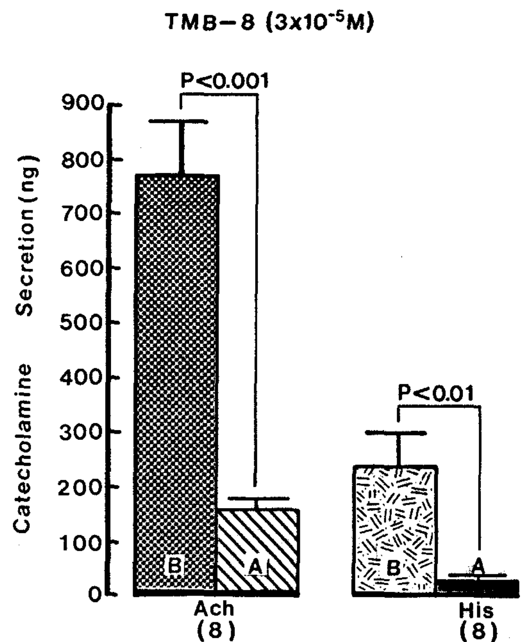

Fig. 9. The effect to TMB-8 on CA secretion induced by histamine. TMB-8 (3×10^{-5} M) was given into the perfusion stream 30 min after obtaining the corresponding control responses of histamine or ACh. Other legends are as in Fig. 2 and 3.

Ca^{++} pool linked to a muscarinic receptors, an attempt was made to test the effect of TMB-8 on histamine-induced CA secretion. In 8 rat adrenal glands, histamine-evoked CA secretion after the pretreatment with TMB-8 (3×10^{-5} M) for 20 min was clearly inhibitied to 30.0 ± 11.3 ng/4 min ($p<0.01$) in comparison with the corresponding control response of 245.0 ± 66.4 ng/4 min before TMB-8 as shown in Figue 9. ACh-induced CA following pretreatment with TMB-8 (3×10^{-5} M) was also grealty reduced to 160.0 ± 20.7 ng/4 min($p<0.001$) as compared with the control secretion of 777.5 ± 98.9 ng/4 min (Fig. 9).

Effect of histamine perfusion on ACh-induced CA secretion

In the light of the fact that histamine-induced CA secretory response was markedly blocked by chlorisondamine as in Figure 3, it is of interest to test the effect of histamine perfusion on ACh-evoked CA release. The adrenal gland was per-

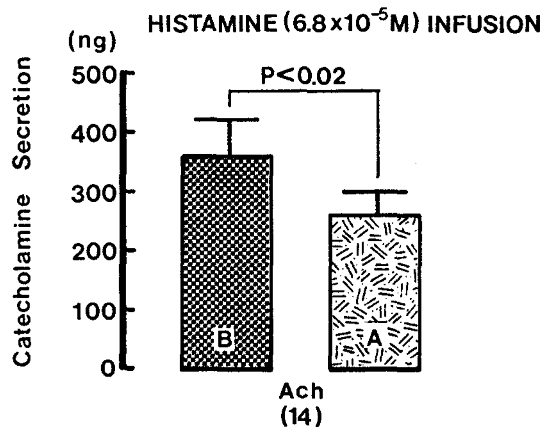

Fig. 10. The effect of histamine infusion on CA secretory responses evoked by ACh. Histamine (6.8×10^{-5} M) was perfused for 30 min after obtaining the control response of ACh-evoked CA secretory response. Other legends are the same as in Fig. 2 and 3.

fused with Krebs solution containing 6.3×10^{-5} M histamine for 30 min before ACh-injection was initiated. Under the existence of histamine effect, ACh-evoked CA secretion was significantly depressed to 265.7 ± 45.4 ng/4 min ($p<0.02$) as compared with the corresponding control secretion of 361.0 ± 65.9 ng/4 min before histamine perfusion. However, perfusion of histamine itself for 30 min did not cause CA secretion in the present experiment. Figure 10 shows the inhibitory effect of histamine perfusion on ACh-induced CA secretory response.

DISCUSSION

The present experimental data demonstrate that histamine produces an increased secretion of CA in a calcium-dependent fashion from the isolated perfused rat adrenal glands and that this secretory effect is exerted throught activation of both H_1- and H_2-histaminergic receptors located in adrenal medulla, which may be associated with stimulation of cholinergic nicotinic receptor. It has been known that the ability of histamine to evoked adrenal secretion of CA is an important physiological mechanism during anaphylactic shock to counteract the hypotensive and bronchoconstrictor effects of histamine (Cession-Fossion and Lecomte, 1966; Lish *et al.*, 1966), and that histamine is capable of inducing release of adrenomedullary CA when given to cats or dogs intravenously (Elliot, 1912; Szczygielski, 1932; Wada *et al.*, 1940; Schaepdryver, 1959; Staszewska-Barczat and Vane, 1965; Narita, 1971), and that histamine also evokes CA release from bovine adrenal medulla (Schneider, 1969; Shima *et al.*, 1976) and from cultured bovine chromaffin cells (Livett and Marley, 1986; Goh and Kurosawa, 1991; Noble *et al.*, 1988). In the present work, the results that histamine induced a significant release of CA from the perfused rat adrenal gland are consistent with many previous reports as mentioned above. In the present rat adrenal glands, tachyphylaxis to histamine-evoked CA release was observed by the repeated perfusion of the drugs and this findings is in agreement with the evidence obtained by Smith and Robinson (1970) in the perfused cat adrenal medulla. In view of the fact that histamine-evoked CA secretion was inhibited markedly by the pretreatment with diphenhydramine, a H_1-histaminergic receptor antagonist or ranitidine, a selective and potent H_2-antagonist, it is felt that histamine evokes CA secretion by stimulation of both H_1-and H_2-receptors. In support of this idea, Knigge and his colleagues (1990) have reported that neuronal histamine is an important mediator of the restraint stress-induced release of peripheral CA by an action on H_1- and H_2-receptors with the central nervous system because central infusion of the H_1-receptor antagonist mepyramine or the H_2-receptor antagonist cimetidine prevented the release of the CA induced by histamine as well as restraint stress. Furthermore, in contrast to the present experimental results, many investigators have reported that CA secretory effect of histamine is mediated by H_1-histaminergic receptors in intact adrenal glands of cats or rats (Emmelin and Muren, 1949; Trendelenburg, 1954; Staszewska-Barczak and Vane, 1965; Yoshinzake, 1973; Robinson, 1982) and also in cultured bovine adrenal chromaffin cells (Livett and Marely, 1986; Noble *et al.*, 1988). It has been also known that H_2-receptor antagonist do not affect histamine-induced CA release. These results are not agree with the present data. However, McGrath and Shepherd (1976) reported that histamine induced inhibition of norepinephrine secretion in vitro, which was reversed by a H_2-receptor antagonist and mimiked by stimulation of specific H_2-receptors, However, some investigators have shown that histamine infusion elevated plasma CA even in humans (Smith *et al.*, 1985; Vigorito *et al.*, 1983).

In the present study, histamine-induced CA secretory effect was greatly depressed by prior treatment with chlorisondamine, an autonomic ganglionic blocking agent. This result seems that histamine-induced CA release is associated with cholinergic nicotinic receptors in adrenal medulla. This data are disagreed with those reported by Livett and Marley (1986) who showed that CA secretion induced by histamine was unaffected by hexamethonium. Even in the intact cat and the rat, splanchnicotomy and ganglionic blockade do not reduce the effect of histamine on adrenal CA release (Szczygielski, 1932; Slater and Dresel, 1952; Trendelenburg, 1954; Staszewska-Barczak and Vane, 1965; Cession-Fossion and Lecomte, 1966;

Yoshizake, 1973). However, in dogs, this secretion of adrenomedullary CA induced by histamine was completely abolished by transection of the splanchnic nerves or by ganglionic blockade, suggesting that it is mediated indirectly by a central reflex mechanism (Wada *et al.*, 1940; Staszewska-Barczat and Vane, 1965). In addition, the present experiment finding that ACh-induced CA release was significantly inhibited by prior perfusion of histamine for 30 min suggest that histamine-induced CA secretion may be assoicated with cholinergic nicotine receptors similar to that of ACh. Pretreatment with pirenzepine, a selective M_1-muscarinic receptor antagonist (Doods *et al.*, 1987; Hammer *et al.*, 1988) did not affect the CA secretion of histamine while did inhibit markedly ACh-induced secretory effect of CA. This indicates that histamine-evoked CA secretion is not associated with M_1-muscarinic receptor activation.

Generally, the indispendible role of calcium in the neurosecretory process has been well estabilished. Yet, according to the assumptions of Baker and Knight (1978; 1980), the relationship between the concentration of intracellular calcium and the transmitter release has not been determined in nerve terminals. As mentioned above, calcium plays the crucial role in process of depolarization neurotransmitter release coupling in many types of secretory cells (Douglas, 1968; Schulz and Stolze, 1980; Williams, 1980). In the present work, removal of extracellular Ca^{++} inhibited greatly CA secretion evoked by histamine of ACh. The secretory effect of CA by histamine is apparently dependent on extracellular calcium. However, in the present experiment, the reason for considerable response to histamine even in the absence of extracellular Ca^{++} is not clear. It may be that chromaffin cells of the rat adrenal gland contain an intracellular store of calcium which participates in the secretion of CA as shown in the bovine adrenal gland (Baker and Knight, 1978). Such a store may not be easily depleted by mere removal of extracellular calcium. Some investigators (Boxler, 1968; Ohashi *et al.*, 1974; Casteels and Raeymeakers, 1979; Malagodi and Chiou, 1974; Takahara *et al.*, 1990) reported that intracellular stores of calcium have been shown to play some role in contraction of smooth muscle produced by noradrenaline or ACh in Ca^{2+}-free medium. Moreover, in the present study, the finding that considerable response to histamine is still remained in the presence of a Ca^{++}-channel blocker nicardipine although the secretory effect of CA evoked by histamine is significantly diminished by pretreatment with Ca^{++}-entry blocker may support above the hypothesis. Both inorganic (Co^{2+} and Ni^{2+}) and organic (verapamil, nifedipine and D-600) Ca^{2+} channel blockers significantly reduce release of CA in the cultured bovine chromaffin cells (Noble *et al.*, 1988; Goh and Kurosawa, 1991). In addition, in terms of the fact that prior administration of TMB-8 inhibited clearly CA secretion evoked by histamine as well as that by ACh in the present perfused rat adrenal gland, it is felt that histamine-evoked CA release may be exerted at least partly through mobilization of calcium from the intracellular stroe located within chromaffin cells. TMB-8 [3,4,5-trimethoxybenzoate 8-(N,N-diethylamino)octylester], a benzoic acid derviative is known well to act by preventing mobilization of calcium from intracellular stores without altering Ca^{++} influx into stores (Charo *et al.*, 1976; Chiou and Malagodi, 1975; Rubin *et al.*, 1980; Smith and Idea, 1979; Wiedenkeller and Sharp, 1984). In support of the present results, Yamada and his colleagues (1988) have found that the secretory effect of CA evoked by caffeine is inhibited by TMB-8 from perfused cat adrenal glands in the absence of extracellular calcium. This similar result was also obtained from the rat adrenal gland (Lim *et al.*, 1991). Moreover, it is known that ACh and pilocarpine cause a partial increase of CA release from both guinea pig adrenal glands (Nakazato *et al.*, 1984) and perfused cat adrenal glands (Nakazato *et al.*, 1988) with Ca^{++}-free lock solution. More recently, Goh and Kurosawa (1991) have reported that stimulation with histamine in culture bovine chromaffin cells induces a continuous secretion of CA via the H_1-receptor activation, in addition to a transient and nonspecific secretion at higher doses. At least a part of the continuous CA secretion is induced by a sustained intracellular calcium rise due to the continuous Ca^{2+} influx through Ca^{2+} channels. These channels are sensitive to dihydropyridine, but do not appear to be identical to the L-type voltage-sensitive Ca^{2+} channel. From the present experimental results CA secretion evoked by histamine is exerted through stimulation of H_1-and

H_2-histaminergic receptors and is still remained considerably even after removal of Ca^{2+} from the Krebs solution, at least partly being due to the liberation of Ca^{++} from the internal stores. This is consistent with the observations that histaminergic stimulation in the cultured bovine chromaffin cells, by inducing rapid turnover of phosphoinositides, causes accumulation of inositol phosphates (Noble *et al.*, 1986; Plevin and Boarder, 1988; Standerman and Pruss, 1990), which in turn induce Ca^{2+} liberation from the internal stores (Berridge, 1987).

REFERENCES

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

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

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

Berridge MJ: *Inositol triphosphate and diacylglycerol: two interacting second messengers. Annu Rev Biochem 56: 159-193, 1987*

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

Brezenoff HE and Gertner SB: *The action of polymixin B and histamine on ganglionic transmission. Can J Physiol Pharmacol 50: 824-831, 1972*

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

Cession-Fossion A and Lecomte J: *Sur les modes de stimulation de la medullo-surrenale du rat. Archs int Pharmacodyn 160: 124-132, 1966*

Charo IF, Feinnman and Detwiler TC: *Inhibition of platelet secretion by an antagonist of intracellular calcium. Biochem Biophys Res Commun 72: 1462-1467, 1976*

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

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

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

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

Elliot TR: *The control of the suprarenal glands by the splanchnic nerves. J Physiol 44: 374-409, 1912*

Emmelin N and Muren A: *Effect of antihistamine compounds on the adrenal liberation from the suprarenals. Acta Physiol Scand 17: 345-355, 1949*

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

Goh Y and Kurosawa A: *Characterization and Ca^{2+} requirement of histamine-induced catecholamine secretion in cultured bovine chromaffin cells. J Neurochem 57: 1249-1257, 1991*

Gross PM, Harper MA, Teasdale GM: *Interaction of histamine with noradrenergic constrictor mechanisms in cat cerebral arteries and veins. Can J Physiol Pharmacol 61: 756-763, 1983*

Hammer R, Monferini E, Deconti L, Giraldo E, Schiavi GB and Landsky H: *Ditinct muscarinic receptor subtypes in the heart and in exocrine glands. In cellular and molecular basis of cholinergic function. Ed. by Dowall MJ and Hawthorne JN, New, Ellishorwood, pp 56-63, 1988*

Hoffman WE and Schmid PG: *Cardiovascular and antidiuretic effects of central histamine. Life Sci 22: 1709-1715, 1978*

Kilpatrick DL: *Ion channels and membrane potential in stimulus-secretion coupling in adrenal paraneurons. Can J Physiol Pharmacol 62: 477-483, 1984*

Klein MC and Gertner SB: *Studies on the mechanism of cardiovascular action of central injections of histamine. Neuropharmacology 22: 1109-1114, 1983*

Knigge U, Matzen S and Warberg J: *Histamine as a neuroendocrine regulator of the stress-induced release of peripheral catecholamines. Endocrinology, 126: 1430-1434, 1990*

Kvetnansky R, Sun CL, Lake CR, Thoa N, Torda T and Kopin IJ: *Effect of handling and forced immobilization on rats plasma levels of epinephrine, norepinephrine, and dopamine-beta-hydroxylase. Endocrinology 103: 1868-1874, 1978*

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

Lish PM, Robbinson SI and Peters EL: *Specificity of antihistamine drugs and involvement of the adrenergic system in histamine deaths in the guinea-pig. J Pharmacol Exp Ther 153: 538-543, 1966*

Livett BG and Marley PD: *Effects of opioid peptides and morphine on histamine-induced catecholamine secretion from cultured bovine adrenal chromaffin cells. Br J Pharmacol 89: 327-334, 1986*

Lokhandwala MF: *Inhibitor of sympathetic nervous system by histamine with H_1 and H_2 receptor agonists. J Pharmacol Exp Ther 206: 115-122, 1978*

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

McGrath MA and Shepherd JT: *Inhibition of adrenergic neurotransmission in canine vascular smooth muscle by histamine. Cir Res 39: 566-573, 1976*

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

Nakazato Y, Ohga A, Oleshansky M, Tomita U and Yamada Y: *Voltage-independent catecholamine release mediated by the activation of muscarinic receptors in guinea-pig adrenal glands. Br J Pharmacol 93: 101-109, 1988*

Narita S: *Comparative studies on the adrenal medullary and cortical response to histamine. Tohoku J Exp Med 104: 349-357, 1971*

Noble EP, Bommer M, Liebisch B and Herz A: *H_1-Histaminergic activation of catecholamine release by chromaffin cells. Biochem Pharmacol 37: 221-228, 1988*

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

Plevin R and Boarder M: *Stimulation of formation of inositol phosphate in cultures of bovine adrenal chromaffin cells by angiotensin II. histamine, bradykinin, and carbachol. J Neurochem 51: 634-641, 1988*

Poisner AM and Douglas WW: *The need for calcium in adrenomedullary secretion evoked by biogenic amines, ploypeptides and muscarinic agents. Proc Soc Exp Bioi Med 123: 62-64, 1966*

Powell JR: *Effects of histamine on vascular neuroeffector transmission. J Pharmacol Exp Ther 208: 360-365, 1979*

Prell GD and Green JP: *Histamine as a neuro-regulator. Annu Rev Neurosci 9: 209-254, 1986*

Robinson RL: *Histamine-induced catecholamine secretion from the cat adrenal medulla is mediated primarily by the H_1 receptor. Fedn Proc 41: 1060-1066, 1982*

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

Schaepdryver AF De: *Physio-pharmacological effects on suprarenal secretion of adrenaline and noradrenaline in dogs. Archs int Pharmacodyn 121: 222-253, 1959*

Schellenberg RR, Ohtaka H, Paddon HB, Bramble SE and Rangno RE: *Catecholamine response to histamine infusion in man. J Allegy Clin Immunol 87: 499-504, 1991*

Schneider FH: *Secretion from the cortex-free bovine adrenal medulla. Br J Pharmac 37: 371-379, 1969*

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

Schwartz JC, Arrang JM, Garbarg M and Korner M: *Properties and roles of the three subclasses of histamine receptors in brain. J Exp Biol 124: 203-224, 1986*

Shima S, Kawashima Y, Hirai M and Kouyama H: *Studies on cyclic nucleotides in the adrenal gland. V. Adenylate cyclase in the adrenal medulla. Jap J Pharmac 26: 711-717, 1976*

Slater IH and Dresel PE: *The pressor effect of histamine after autonomic ganglionic blockade. J Pharmac Exp Ther 105: 101-107, 1952*

Smith DJ and Robinson RT: *The dwindling secretory response of the perfused adrenal medulla of the cat to repeated injections of histamine. J Pharm Exp Ther 175 (3): 641-648, 1970*

Smith R, Grossman A, Boyce MJ, Besser GM, Ress LH: *Effect to histamine infusion on circulatory methionine-enkephalin and catecholamine concentrations. Neurosci Lett 55: 289-292, 1985*

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

Stauderman KA and Pruss RM: *Different patterns of agonist-stimulated increases of ^{3}H-inositol phosphate isomers and cytosolic Ca^{2+} in bovine chromaffin cells: comparison of the effects of histamine and angiotensin II. J Neurochem 54: 946-953, 1990*

Staszewska-Barczak J and Vane JR: *The release of catecholamines from the rat adrenal medulla by histamine. Br J Pharmacol 25: 728-742, 1965*

Szczygielski J: *Die adrenalinabsondernde Wirkung des Histamins und ihre Beeinflussung durch Nikotin. Archs Exp Path Pharmak 166: 319-332, 1932*

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

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

Trendelenburg U: *The action of histamine and pilocarpine on the superior cervical ganglion and the adrenal glands of the cat. Br J Pharmacol 9: 481-487, 1954*

Trendelenburg U: *Stimulation of sympathic centers by histamine. Circ Res 5: 105-109, 1957*

Vigorito C, Russo P, Picotti GB, Chiariello M, Poto S, Marone G: *Cardiovascular effects of histamine infusion in man. J Cardiovasc pharmacol 531-537, 1983*

Wada M, Fuzii K, Sibuta M and LI. M-C: *The action of histamine upon the epinephrine secretion from the suprarenal gland in non-anaesthetized dogs. Tohoku J Exp Med 37: 433-445, 1940*

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

Wiedenkeller DE and Sharp GWG: *Unexpected potentiation of insulin release by the calcium store bloker TMB-8. Endocrinology 114: 116-119, 1984*

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

Yamada Y, Teraoka H, Nakazato Y and Ohga A: *Intracellular Ca^{2+} antagonist TMB-8 blocks catecholamine secretion evoked by caffeine and acetylcholine from perfused cat adrenal glands in the absence of extracellular Ca^{2+}. Neuroscience Lett 90: 338-342, 1988*

Yoshizake T: *Effect of histamine, bradykinin and morphine on adrenaline release from rat adrenal glands. Jap J Pharmac 23: 695-699, 1973*

=국문초록=

흰쥐 관류부신에서 Histamine 수용체 활성화가 Catecholamine 분비작용에 미치는 영향

조선대학교 의과대학 약리학교실

임 동 윤·노 상 현

흰쥐 관류부신에서 histamine의 catecholamine (CA) 분비작용의 특성과 기전을 규명코자 연구한 결과는 다음과 같다. Histamine (37.5∼150 μg)을 부신정맥내에 주사 하였을 때 현저한 용량 의존성의 CA 분비작용을 나타내었다. 그러나 histamine (150 μg)을 120분 간격으로 반복투여시 제3차 투여시부터는 CA 분비효과가 뚜렷이 감소하였다. 즉, histamine의 반복투여로 인한 반응급강현상을 관찰할 수 있었다.

Histamine의 CA 분비작용은 chlorisondamine, diphenhydramine, ranitidine, Ca^{++}-free Krebs 용액의 관류, nicardipine 및 TMB-8 등의 전처치로 유의하게 억제 되었으나 pirenzepine의 전처치에 의해서는 별다른 영향을 받지 않았다. 더우기 histamine (6.8×10^{-5} M)으로 30분간 관류시킨 후에 ACh (50 μg)의 CA 분비작용이 상당히 억제됨을 나타내었다.

이상과 같은 연구 결과로 보아 histamine은 흰쥐 적출관류 부신에서 현저한 CA 분비작용을 나타내었으며 칼슘 의존성이었다. 이러한 CA 분비작용은 H_1- 및 H_2- 양수용체의 활성화를 통해서 일어나며 또한 부신의 nicotine 수용체와도 관련성이 있는 것으로 사료된다.