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Effects of Cromakalim on the Release of Mediators in Hypersensitivity of Guinea pig

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ABSTRACT

Potassium (K^+) channels are present in airway smooth muscle cells, and their activation results in hyperpolarization and relaxation. Because these effects may have therapeutic relevance to hypersensitivity and asthma, we examined the effect of a potassium channel activator, cromakalim (BRL 34915, CK) on the release of mediators from superfused tracheal and parenchymal strips after passive sensitization with IgG_1 antibody. Both tissues were superfused with CK (2×10^{-6} M) for 30 min and challenged with CK and antigen (Ox-HSA). Using monodispersed, partially purified, highly purified guinea pig lung mast cells, we also examined the effect of CK on mediator release from these cells after passive sensitization with IgG_1 antibody (α-OA). Guinea pig lung mast cells were purified using enzyme digestion method, count current elutriation, and discontinuous Percoll density gradient. After CK pretreatment, passively sensitized mast cells were challenged with varying concentration of antigen (OA, immunological stimuli) or with varying concentration of calcium ionophore (CaI, non-immunological stimuli). Histamine (Hist) release was determined by spectrophotofluorometry, and leukotrienes (LT) by radioimmunoassy. CK pretreatment decreased Hist by 35% and LT release by 40 % in the antigen-induced tracheal tissue after IgG_1 sensitization but did not decrease the contractile response. In the antigen-induced parenchymal tissue CK decreased Hist release by 25% but poorly decreased LT. Both immunologic and non-immunologic stimuli caused a dose-dependent release of Hist and LT from monodispersed, partially purified and highly purified lung mast cells.

Verification of LT release was obtained by the use of 5-lipoxygenase inhibitor, A64077 (Zileuton). CK decreased Hist and LT release by 20% respectively in the OA-induced guinea pig lung mast cells after IgG_1 sensitization. The inhibitory effects of CK on the Hist and LT release in the Ox-HSA-induced airway smooth muscle tissues or in the OA-induced and CaI-induced mast cells after IgG_1 sensitization were completely blocked by TEA and GBC.

These studies show that guinea pig lung mast cells seem to be an important contributor to LT release, and that CK (which has been known as an airway smooth muscle relaxant) can in part act to inhibit mediator release in the antigen-induced airway smooth muscle, and that CK may also act to inhibit mediator release in the OA-induced and CaI-induced highly purified mast cells. These results suggest that Hist and LT release evoked by mast cell activation might in part be associated with K^+ channel activity.

Key Words: Cromakalim, IgG_1 antibady, Histamine, Leukotrienes, Hypersensitivity

Abbreviations were used in this paper: CK, cromakalim; Ox-HSA, oxazolone human serum albumin; α-Ox-HSA, anti-Ox-HSA; α-OA, anti-ovalbumin; OA, ovalbumin; PCA, passive cutaneous anaphylaxis; Hist, histamine; LT, leukotrienes; TEA, tetraethylammonium; GBC, glibenclamide; CaI, calcium ionophore; Ag, antigen.

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INTRODUCTION

Potassium (K^+) channels are present on a variety of cell types. Several studies have provied evidence of their existence on airway smooth muscle (Cook 1988; Kannan *et al.*, 1983; Davis *et al.*, 1982; Marthan *et al.*, 1989).

Potassium (K^+) channel opener, cromakalim (BRL 34915, CK) recently undergoing clinical evaluation as a novel antihypertensive agent, has been reported to promote the relaxation of a various kinds of smooth muscle as a consequence of potassium (K^+) channel activation (Allen *et al.*, 1986; Hamilton *et al.*, 1986; Weir and Weston, 1986; Quast and Cook, 1988; Hollingsworth *et al.*, 1987; Bray *et al.*, 1987; Hamilton and Weston, 1989). This conclusion is based on the observation that CK enhances K^+ efflux and thereby induces membrane hyperpolarization toward the K^+ equilibrium potential.

Some investigators have reported that CK has potential for relieving bronchoconstriction caused by asthma mediators, and that CK inhibits the experimental asthma caused by the IgE antibody and antigen system in guinea pigs (Arch *et al.*, 1988; Nagai *et al.*, 1991).

The pharmacology of CK has recently been reviewed (Hamilton and Weston, 1989; Cook, 1990). Asthma is among the potential therapeutic targets for CK (Willisams *et al.*, 1990). Small and Foster (1988) suggested that the hyperresponsiveness of airway in asthma might represent altered gating characteristic of membrane K^+ channels. The generation of action potentials in human airway muscle requires reduction of K^+ channel activity (Marthan *et al.*, 1989). An increased discharge of spontaneous action potential has been reported to occur during asthmatic episodes (Akasaka *et al.*, 1975). In that event, K^+ channel openers such as CK could restore responsiveness to normal and are worthy of further investigation.

Mast cells have specific receptors towards IgE and IgG_1 antibodies. Either multivalent antigen or divalent anti-IgE antibody cross-linkages with IgE on the cell membrane evokes the release of Hist from the cell (Ishizaka, 1972). Attempts to determine how mast cell secretion may be regulated included the identification of several receptors and Na^+-, K^+ and Ca^{2+}-specific ionic channels (Eleno *et al.*, 1988). It is well known that Ca^{2+} plays an important role as a second messenger during cell activation (Rasmusen and Goodman, 1977).

However, the role of K^+ channel in Hist release from mast cells is still unclear, excepting Hist release by 6-tridecylresorcylic acid (TRA) from mast cells might be associated with K^+ channel activity (Takei *et al.*, 1993).

As it is already reported that effects of CK on hyperpolarization and relaxation may have therapeutic relevence to asthma and hypersensitivity, the present study aims to determine the effect of a K^+ channel opener, CK, on the release of mediators from isolated superfused tracheal and parenchymal strips after passive sensitization with IgG_1 antibody. Using purified mast cells we also examined the effect of CK on mediator release from these cells after passive sensitization with α-OA antibody.

METHOD

Animals

Hartley albino female guinea pigs, weighing about 250~350 g were used. Animals were maintained in the Research Animal Care of Yonsei University.

Immunologic techniques

Preparation of hapten-protein conjugates, immunization procedures for development of guinea pig IgG_1 antibody, antibody separation techniques with affinity-column chromatography, and quantitation of serum antibody titiers by PCA have been comprehensively described in previous articles (Ro *et al.*, 1992).

Sensitization of pulmonary tissue and mediator release and contraction

Guinea pigs were passively sensitized for specific antigen (Ox-HSA) challenge. Passively sensitized animals received intravenous injections of Ab-rich serum 1 day before being killed for study. Passive sensitization was achieved by administration of 0.5 ml/kg of serum rich in IgG_1 Ab directed against Ox-HSA (α-Ox IgG_1; PCA titer,

2560). These "standard" doses of the anti-hapten IgG serums were selected on the basis of preliminary experiments in which they were found to sensitize the guinea pig trachea for similar magnitudes of contractions induced by a maximally effective concentration (0.1 mg/ml of Ox-HSA). In all cases, the doses of sera administered were optimal for contraction and mediator release. Doses of serum higher than these levels did not elicit further contraction or mediator release (Graziano *et al.*, 1984; Undem *et al.*, 1985a).

Tracheas were trimmed of excess tissue and cut in a spiral fashion (Constantine, 1965). A parenchyma (3×3×5 mm) was obtained from the right lower lobe of the same animals. Both tissue strips were suspended in air-filled, water-jacketed tissue chambers and superfused (Fishleder and Buckner, 1984) with a Krebs bicarbonate solution of the following composition (millimolar): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 0.5; NaH_2PO_4, 1; $NaHCO_3$, 25; and glucose, 11. The solution was gassed with a mixture of oxygen (95%) and carbon dioxide (5%) and pumped from a heated (37.5℃) reservior through tygon tubing to a water-jacketed, coiled, glass tube (heat exchanger) with a Gilson Miniplus II peristaltic pump (Gilson Medical Electronics, Middleton, Wis). From the heat exchanger, the physiologic salt solution superfused the tissues at a ratè of 1.5 ml/min. The tracheal spirals and parenchymal strips were maintained at initial tensions of 5 g and 1 g, respectively, for 90 min equilibration period before Ag challenge. Changes in tension were recorded via force transducers (FT-03) on Grass model 5D or 79B polygraphs (Grass Instruments, Quincy, Mass). Histologic examination of selected trachea demonstrated that the epithelium was 95% intact with these procedures.

After equilibration, all tissues were superfused with buffer or CK (2×10^{-6} M) for 30 min and challenged with Ox-HSA or with CK and Ox-HSA dissolved in the superfusion solution. Superfusate samples were collected at the base of the tissue chamber, and each collection tube contained an appropriate amount of gelatin to make 0.1% of the collection period. Superfusate samples were collected for a 2 min period before (spontaneous release) and continuously from the beginning of challenge with Ox-HSA. Hist and LT were not found in spontaneous release samples. During Ag challenge, consecutive time intervals of superfusate collection were for 5 min, with additional 5 min collection intervals as needed. The maximum duration for Ag challenge and superfusate collection was 30 min. After collection, the superfusate samples were placed in an ice bath and stored at 4℃ until samples were analyzed for Hist and LT (see below). In experiments using TEA (8 mM) and GBC (1 μM), both tissues prepared as described above were first superfused for 20 min before CK pretreatment.

At the end of Ag challenge, trachea and parenchyma were superfused with carbachol, 10^{-3} M and barium chloride, 10^{-2} M, respectively, to elicit the maximum contractile response from each tissue. Responses to Ox-HSA were expressed as a percentage of the maximum contractile response in each tissue. Ox-HSA did not cause contraction of tissue taken from unsensitized animals.

At the end of each experiment, the trachea and parenchyma were weighed and placed in 3 ml of 0.4 N perchloric acid, and the tubes were placed in a boiling water bath for 15 min before homogenization. After centrifugation, the supernatant was analyzed for Hist content. There were no statistical differences in total Hist content between tissues in any of the experimental groups and, for 60 tissues, averaged 13.5±0.4 and 27.3±0.9 μg/gm of wet weight, respectively.

Guinea pig lung mast cell preparations

Guinea pig lung mast cells were isolated and purified using techniques smilar to the method previously reported (Undem *et al.*, 1985b). Briefly described here, lungs obtained from 8 to 10 unsensitized guinea pigs were each perfused with 50 ml of the modified Tyrode buffer consisting of (millimolar): NaCl, 137; NaH_2PO_4, 0.36; KCl, 2.6; $CaCl_2$, 1; $MgCl_2$, 0.5; $NaHCO_3$, 119; glucose, 5.5; gelatin, 1 g/L, pH 7.4. After removing large airways and blood vessels, the lungs were minced with a McIlwain tissue chopper. Pooled tissue was treated three times with 125 U/g tissue and 5 U/g tissue of collagenase and elastase, respectively. Times (min) of each consecutive exposure of lung fragments to the enzymes were 20, 20 and 30, respectively. Freed cells were separated from residual tissue by filtration through mesh and Nytex mesh (100 μm). The resulting cell population was washed and designed "monodispersed".

For further mast cell purification, cell suspensions (total of 4.0×10^8) obtained after the enzyme digestion were layered over gradients consisting of 10 ml of Percoll (density, 1.045 g/ml), and centrifuges at 1400 rpm for 20 min. Pelleted cells (containing mast cells) were resuspended in modified Tyrode buffer and fractionated by count current elutriation (Undem *et al.*, 1986). Cell fractionation was accomplished by decreasing rotor speed. At each rotor speed, 90 ml of cell suspension were collected and designated by number. Fraction 6 (collected at 2590 rpm) consistently contained the largest percentage of mast cells and designed "partially purified mast cell" ($3.0\sim4.0\times10^7$ cells). Fraction 6 was used alone for further purification utilizing a discontinuous Percoll density gradient (consisting of densities 1.06, 1.07, 1.08, 1.09, and 1.10 g/ml). This gradient was centrifuged at 1400 rpm for 20 min (3.5×10^7 cells/gradient). The cell band obtained between the 1.09 and 1.10 g/ml densities contained the highest purity and number ($10-20\times10^7$) of mast cells. This gradient band was removed, washed with modified Tyrode buffer and designated the "highly purity" mast cell preparation. Mast cell counts were obtained using alcian blue staining and cell viability was determined using trypan blue exclusion (Undem *et al.*, 1985b). Cell viability was consistently greater than 98%. The Purity range of Monodispersed, partially purified, and highly purified mast cells was 2~3%, 40~50%, and 90~97%, respectively.

Mediator release from mast cell

Guinea pig lung mast cells obtained after enzyme digestion (monodispersed), after fractionation by elutriation (partially purified), and after the discontinuous Percoll density gradient centrifugation (highly purified) were passively sensitized with α-OA serum (1 ml/10^6 cells) in a shaking water bath (60 min at 37℃). After this incubation period, the cells were wahed twice, resuspended in modified Tyrode buffer containing $CaCl_2$ and $MgCl_2$ and treated (15 min, 37℃) with varying concentrations of OA (0.01, 0.1, 1.0 mg/ml) or (0.01, 0.1 mg/ml). Polystyrene tubes were used for all cell incubations, and unless stated otherwise, each tube contained 2.5×10^5 mast cells suspended in 1 ml of Tyrode buffer. The mediator release reaction was terminated by placing the tubes in an ice bath. Supernatants obtained after centrifugation were taken for determination of Hist and LT. In experiments utilizing A64077 and CK, cells prepared as described above were first incubated for 60 min at 37℃ with 10 μl of 10^{-5} M A64077, 10 μl of 2×10^{-6} M CK, or vehicle prior to incubation with OA or CaI for 15 min at 37℃. In experiments using GBC (10 μM), mast cells were first incubated for 20 min before CK pretreatment.

Histamine assay

Hist was analyzed by the automated fluorometric method (with dialyzer) described by Siraganian (1974). The sensitivity of the assay is approximately 5 ng/ml of Hist. The amount of Hist in each superfusate sample was expressed as a percentage of the total tissue Hist content remaining after the proceeding collection period. Total Hist content of each cell suspension was determined after treating cell pellets with 0.4% Trition X, expressed as a percentage of the total Hist content and corrected for spontaneous release, which ranged from 2 to 4% as determined in paired sample not challenged with OA.

LT Radioimmunoassay (RIA)

The LT content of each superfusate sample or cell supernatants was determined by RIA as described previously (Aharony *et al.*, 1983). The LT antibody (#332) was diluted in buffered saline (5 mM MES, HEPES adjusted to pH 7.4 with 1 N NaOH) containing 0.1% gelatin. Each assay tube contained 100 μl of supernatant, antibody (50 μl of a 1 : 1000 dilution), and 50 μl of ^{3}H-LTD_4 (2500 to 3000 cpm) in buffered saline. Incubations were for 2 h at 4℃ and the reaction was terminated by addition of 0.5 ml dextran coated charcoal (200 mg charcoal and 20 mg dextran mixed with 100 ml buffered saline). After 5 min incubation the mixture was centrifuged at 3000 rpm at 4℃ and 0.4 ml of the supernatant was added to Aquasol (NEN Research Products) for counting by liquid scintillation spectrometry (Packard, Model 3225). Standard curves were constructed in the presence of Ag using LTD_4. The detection limit of the assay is 0.045 pmol LTD_4. LT release is expressed as pmol/10^6 cells.

Drugs

The following substances were used: histamine

free base, oxazolone, human serum albumin, MES, HEPES, ovalbumin (fraction V), complement Freund's Adjuvant, incomplement Freund's Adjuvant, Guinea pig albumin, collagenase (type I), elastase (type I, porcine pancreatic), calcium ionophore (A23187), alcian blue, trypane blue, glibenclamide, tetraethylammonium (Sigma Chemical Co., St. Louis, MO); CK (Smithkline Beecham Pharmaceuticals, West Sussex, UK); carbamyl choline chloride (Aldrich Chemical, Inc., Milwaukee, Wis); barium chloride (JT Baker chemical Co., Phillipsburg, NY); gelatin (Difco Laboratories, Ditrotit, MI); Percoll (Pharmacia Fine Chemicals AB, Uppsala, Sweden); Triton X (Research Products International, EIK Grove, IL); A64077 (Zileuton), leukotriene antibody for RIA (Stuart Pharmaceuticals, Division of ICI Americas, Inc., Wilmington, Del): ^{3}H-LTD$_4$ (specific activity, 39 Ci/mmol, New England nuclear, Boston, Mass). α-Ox-HSA, α-OA (prepared by our laboratory). A64077 was dissolved in DMSO, CK was dissolved in 70% ethanol and diluted in buffer for use.

Statistics

Statistical analysis was performed by Student's t-test.

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RESULTS

Effect of CK on IgG$_1$-mediated pulmonary responses

In order to confirm whether CK itself has the mediator releasing effect on hypersensitivity reaction, unsensitized pulmonary tissues were challenged by Ox-HSA, 0.1 mg/ml, after varying concentration of CK pretreatment. The results show that CK did not have effect on the mediators release (not shown data). The optimal concentration of CK in the reduction of mediator release in the sensitized tissues was 2×10^{-6} M. Therefore, all experiments were performed with 2×10^{-6} M of CK. Our previous findings (Ro *et al.*, 1991), exposure

Table 1. Response of the CK (2×10^{-6} M) of superfused guinea pig trachea and parenchyma to Ox-HSA 10^{-1} mg/ml after passive sensitization with IgG$_1$ antibody@

Tissues	N	Maximum contraction(%)	Total-Histamine#(%)	Leukotrienes† (pmole/g tissue)
Tracheal strip				
Control	7	83±2.4	13.8±0.87	726±62
CK	7	78±4.7	8.8±0.67**	426±25*
TEA+CK	4	81±6.2	11.8±1.90	855±98
GBC+CK	4	79±5.6	15.0±2.00	814±106
Parenchymal strip				
Control	7	68±5.1	7.6±0.50	514±81
CK	7	61±6.0	5.7±0.29*	391±52
TEA+CK	4	55±7.2	7.8±0.66	605±75
GBC+CK	4	61±5.8	8.5±0.56	630±56

@Animals were passively sensitized by i.v. injection of IgG$_1$ antibody (α-Ox-HSA) 1 days before each experiment. Tracheal and parenchymal tissues were isolated and challenged with Ox-HSA.

Values represent total histamine found in all superfusate after Ox-HSA challenge expressed as a persentage of the total tissue histamine content.

† Values represent total leukotrienes found in superfusate of g tissue after Ox-HSA challenge.

*A value that is statistically different ($p<0.05$) from the value obtained after CK preteratment.

**$P<0.01$

to Ag (Ox-HSA, 0.1 mg/ml), resulted in tracheal and parenchymal contraction and mediator (Hist and LT) release after guinea pigs were sensitized with IgG_1 antibody. Therefore, we examined the effects of CK on the mediator release in the airway smooth muscle by exposure of Ox-HSA after passively IgG_1 sensitization.

CK pretreatment decreased Hist release by 35% and LT release by 40% in the Ag (Ox-HSA)-induced tracheal tissues after passively sensitization with IgG_1 antibody, but did not decrease the contractile responses (Fig. 1, Table 1). In the parenchyma CK decreased Hist release by 25% but poorly decreased LT (Fig. 2, Table 1).

In order to confirm whether the inhibition of the mediator release in the Ag-induced pulmonary tissues after sensitization was evoked by CK pretreatment, we examined whether CK effect was blocked by using TEA which was known as a non-specific antagonist of K^+ channels, and GBC which acted as antagonist of ATP-sensitive K^+ channels. The inhibitory effects of CK on the Hist

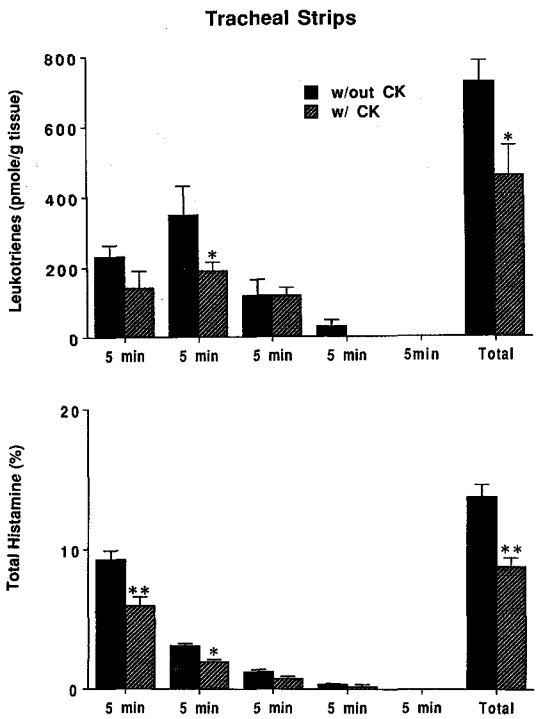

Fig. 1. Time course for Hist and LT contents in presence and absence of CK (2×10^{-6} M) after challenge with Ox-HSA, 0.1 mg/ml in superfused guinea pig tracheal strips. Animals were passively sensitized with IgG_1 antibody (α-Ox-HSA) 1 day before killed for study, and then tracheal tissues were isolated, superfused with Ox-HSA (0.1 mg/ml) after CK or vehicle pretreatment for 30 min. Solid bars represent vehicle pretreatment and the hatched bars represent CK pretreatment. Vertical lines represent the mean ±SEM of 7 experiments. The data are summarized in Table 1.

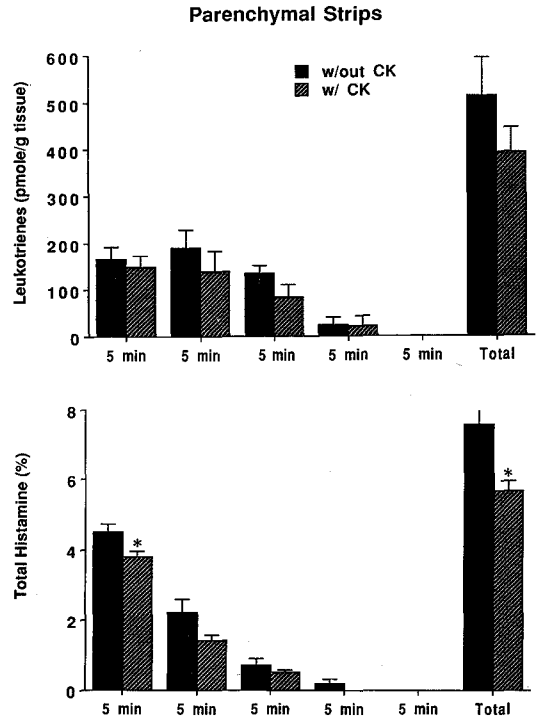

Fig. 2. Time course for Hist and LT contents in presence and absence of CK (2×10^{-6} M) after challenge with Ox-HSA, 0.1 mg/ml in superfused guinea pig lung parenchymal strips. Animals were passively sensitized with IgG_1 antibody (α-Ox-HSA) 1 day before killed for study, and then parenchymal tissues ($3\times3\times5$ mm) were isolated, superfused with Ox-HSA (0.1 mg/ml) after CK or vehicle pretreatment for 30 min. Solid bars represent vehicle pretreatment and the hatched bars represent CK pretreatment. Vertical lines represent the mean±SEM of 7 experiments. The data are summarized in Table 1.

and LT release in the tracheal and parenchymal tissues were completely blocked by TEA and GBC (Table 1).

Antigen provoked Hist and LT release from mast cells

Monodispersed, partially purified, and highly purified mast cells were passively sensitized with α-OA antibody, treated with varying concentrations of the Ag for 15 min and both Hist and LT release measured. Data obtained from these experiments are shown in Table 2. OA-induced release of LT as well as Hist was obtained in dose dependent fashion from all types of mast cells (data in Figure not shown). Peak of Hist release (approx 25~30%, both preparations; 40%, highly purifed) and LT release (10~12 pmole/10^6 cells, both preparations; 32~35 pmole/10^6 cells, highly purified) were obtained utilizing OA concentrations between 0.1 to 1.0 mg/ml. However, in the highly purified mast cells Hist and LT release were significantly increased, compared to monodispersed mast cells (Table 2). For all preparations, it was demonstrated that mediator release was the result of an immunologic reaction since it did not occur in the absence of the passive antibody sensitization step (data not shown).

As we have previously described (Ro *et al.*, 1991). Hist release was completed between 5 and 10 min after challenge. In contrast to the Hist release profile, LT release was not detectable for the first 5 min after Ag challenge. A slow release of LT was noted and it appeared to peak at approx. 60 min.

Influence of CK on the antigen provoked Hist and LT release from mast cells

The effect of CK on the mediator release in the Ag-induced pulmonary mast cells after passive sensitization has been examined. Hist and Lt release (both approx. 20%) by CK pretreatment only decreased by highly purified mast cells after passive sensitization (Table 2). It has also been examined whether the inhibition of the mediator re-

Table 2. Hist and LT release from passively sensitized (anti-OA antibody) guinea pig lung mast cells evoked by varying concentrations of OA in the presence and absence of CK (2×10^{-6} M)@

Cells	Ab (ml/10^6 cells)	Ag (mg/ml)	Total Hist (%)		LT (pmole/10^6 cells)	
			Control	CK	Control	CK
monodispersed cells	α-OA	OA 0.01	10.1±1.8	9.0±0.82	1.68±0.67	1.27±0.55
		0.1	27.5±1.6	24.9±1.62	10.09±1.01	8.75±0.99
		1.0	26.7±2.1	25.0±1.01	10.05±1.83	8.54±1.45
partially purified cells	α-OA	OA 0.01	13.6±0.74	13.0±0.62	1.92±0.75	1.16±0.79
		0.1	29.5±2.30	27.0±1.90	12.13±1.30	10.88±2.14
		1.0	29.3±1.50	29.0±1.30	11.91±1.47	9.21±1.48
highly purified cells	α-OA	OA 0.01	18.5±2.1$^+$	11.9±1.3*	20.19±4.13$^+$	13.04±1.11*
		0.1	32.5±2.5	22.7±1.8*	35.01±6.73$^+$	25.26±1.87*
		1.0	44.2±3.2$^+$	30.9±2.6	32.47±8.45$^+$	23.35±1.31*

@Guinea pig lung mast cells were isolated and purified by digestion, count current elutriation, and discontinuous Percoll density gradient method. Mast cell (0.25×10^6 cells) were passively sensitized by α-OA antibody and challenged by varying concentrations of OA.

$^+$A value that is statistically different ($p<0.05$) from the value obtained with the highly purified mast cell in the absence of CK pretretment.

*A value that is statistically different ($p<0.05$) from the value obtained with the highly purified mast cell in the presence of CK pretreatment.

lease in the Ag-induced pulmonary mast cells after sensitization was evoked by CK pretreatment. The inhibitory effects of CK on the Hist and Lt release in the OA-induced pulmonary mast cells after passive sensitization were completely blocked by GBC (data not shown).

Inhibitory effect of CK and A64077 on the mediator release from highly purified mast cells

In order to verify LT release from isolated guinea pig lung mast cells, the 5 lipoxygenase enzyme

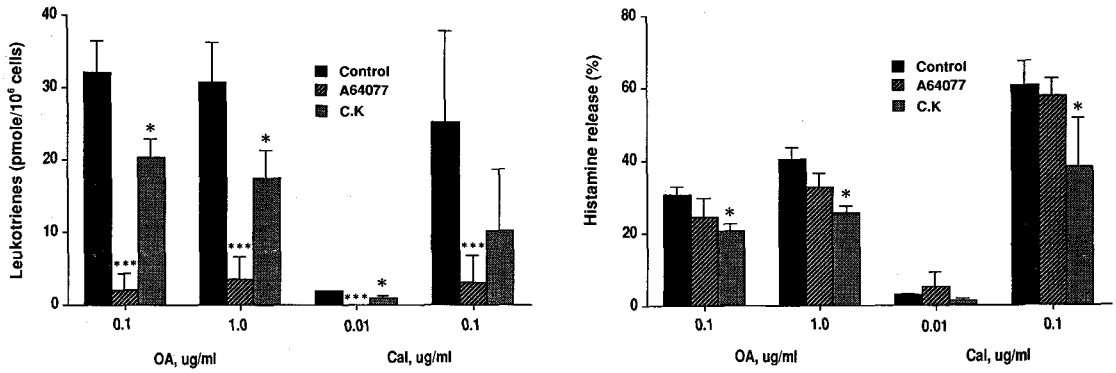

Fig. 3. Hist and LT release from passively sensitized (α-OA antibody) highly purified lung mast cells evoked by varying concentrations of OA or CaI. Guinea pig lung mast cells were isolated and purified by enzyme digestion, count current elutriation, and continuous Percoll density gradient method. Mast cell (0.25×10^6 cells) were passively snesitized by α-OA antibody and challenged by varying concentrations of OA or CaI after A64077 (10^{-5} M) or CK (2×10^{-6} M) pretreatment. Solid bars represent vehicle, the hatched bars represent A64077, and dot bars represent CK pretreatment. Vertical lines represent the mean±SEM of 7 experiments. The data are summarized in Table 3.

Table 3. Hist and LT from passively sensitized (anti-OA antibody) highly purified lung mast cells evoked by varying concentrations of OA and CaI in the presence and absence of A64077 or CK@

Ab (mg/ml)	(μg/ml)	N	Total Histamine (%)			Leukotrienes (pmole/10^6 cellsl)		
			control	A64077	CK	control	A64077	CK
α-OA	OA							
	0.1	7	30.5±2.5	24.5±5.3	20.7±1.8*	32.1±4.3	2.1±2.1***	20.4±2.3*
	1.0	7	40.2±3.2	32.5±3.9	25.4±2.1*	30.7±5.4	3.5±2.9***	17.5±3.7*
α-OA	CaI							
	0.01	7	2.8±0.5	5.2±3.7	1.2±0.4	1.9±0.1	0***	0.9±0.3*
	0.1	7	60.2±6.8	57.4±4.5	38.2±6.2*	25.1±12.7	3.0±3.6***	10.2±8.42

@Guinea pig lung mast cells were isolated and purified by digestion, count currente elutriation, and discontinous Percoll density gradient method. Mast cells (0.25×10^6 cells) were passively sensitized by anti-OA antibody and challenged by varying concentration of OA after A64077 (10^{-5} M) or CK (2×10^{-6} M) pretreatment.

*A value that is statistically different ($p<0.05$) from the value obatined with the highly purified mast cell in the presence of CK pretreatment.

***A value that is statistically different ($p<0.001$) from the value obtained with the highly purified mast cell in the presence of A64077 pretreatment.

inhibtor A64077 was utilized. In these experiments, both immunologic (OA) and non-immunologic (CaI) stimuli were utilized. Figure 3 illustrates the a dose dependent release of Hist and LT can be obtained with the CaI. In further experiments, we examined whether A64077 and CK could alter Hist release using CaI as well as OA as the secretagogue. Figure 3 (lower panel) demonstrates that A64077 had no appreciable effects on Hist release from highly purified lung mast cells using either secretagogue, but CK significantly decreased Hist release using either secretagogue. The effect of A64077 and CK on LT release from gunea pig lung mast cells after challenge with OA or CaI is shown in Figure 3 upper panel. When mast cells were challenged with OA or CaI after incubation for 1 h with A64077, no LT release was observed. But, after incubation of mast cells for 1 h with CK, LT release (20~25%) was significantly decreased. In previous experiments it was determined that the LT standard curve was not affected by the presence of A64077 or CK alone. The data in Figure 3 were described in Table 3.

DISCUSSION

Recently, much attention has been paid to airway smooth muscle relaxation and the bronchodilating activity of K^+ channel openers in vitro and in vivo (Allen *et al.*, 1986; Arch *et al.*, 1988; Nielsen-Kudsk *et al.*, 1989; Nagai *et al.*, 1991). In the unsensitized and sensitized guinea pig trachea, CK is a relaxant agent with the pharmacological profile of a K^+ channel opener (Hamilton and Weston, 1989; Cortijo, *et al.* 1992). This pharmacological property suggests the possible therapeutic potential of K^+ channel openers in the treatment of hypersensitivity and bronchial asthma (Cook, 1988; Baird, 1988).

Since many possible mechanisms, including allergic responses, are believed to be involved in the onset and development of bronchial asthma, the present study attempted to evaluate the effects of a new class of K^+ channel opener, CK, on allergic reactions. From the results of this study, it appears that CK is effective on allergic Hist and LT release in the tracheal tissues taken from passively sensitization (Fig. 1, Table 1), but, in the parenchymal strips, CK is poorly effective on allergic LT release (Fig. 2, Table 1). The latter results may suggest that certain inhibitory action in parenchymal tissue level may be partially involved in CK-induced inhibition of LT release. The data on allergic reaction by CK have not been reported yet. The inhibition of Hist and LT release elicited by CK was suppressed by TEA (8 mM) and GBC (10 μM) (Table 1). GBC is potent blocker of ATP-sensitive K^+ channels in the airway smooth muscle. This type of ion channel is suppressed by increased intracellular concentration of ATP (Nielsen-Kudsk *et al.*, 1990). The present results therefore suggest that the inhibitory effects of mediator release in the airway smooth muscle produced by CK may involve activation of a GBC sensitive K^+ channel. These data are in agreement with other recent finding that there may be open K_{ATP} present in smooth muscle cell membrane (Nielsen-Kudsk *et al.*, 1990; Black JL *et al.*, 1990).

Allen *et al* (1986) reported that the inhibition of spontaneous tone elicited by CK was suppressed by procaine (5 mM) but remained unaffected in the presence of TEA (8 mM). But, we found that the inhibtion of mediator release elicited by CK was suppressed by TEA. Therefore, the present results support that the inhibitory effects of mediator release in the airway smooth muscle evoked by CK may be involved by selective and non-selective K^+ channel activity.

It is still contraversal if LT is released from the isolated and purified guinea pig lung mast cells. We report that LT is released from guinea pig mast cells (Table 2). These results are in agreement with Doran *et al.* (1993, submitted article). Confirmation of LT was obtained by utilizing the 5-lipoxygenase inhibitor A64077 (Fig. 3, Table 3). A64077 has been shown to be a potent, and selective inhibitor of the 5-lipoxygenase enzyme (Carter *et al.*, 1991). And it has also been shown in a number of studies to be effective in reduction of LT synthesis in humans following oral administration (Bell *et al.*, 1992). A64077 has also been demonstrated to be important in the treatment of diseases such as inflammatory bowel disease, asthma and rheumatoid arthritis (Collawn *et al.*, 1992). In our present study, the Ag-induced release of LT bioactivity by lung mast cells was in-

dependent of cell purity (Table 2). These data and our current work would strongly suggest that the LT are derived from mast cells rather than other contaminating cell types.

Because LT as well as Hist can be released from guinea pig mast cells, we examined that influence of CK on mediator release from isolated guinea pig mast cells. In the present study, it appears that CK is effective on allergic Hist and LT release only in highly purified mast cells using OA (immunologic stimuli) and CaI (non-immunologic stimuli) as the secretagogues, and the inhibition of mediator release evoked by CK is blocked by GBC. Nagai *et al.*, (1992) reported that none of the K^+ channel openers including CK were effective on allergic Hist release. The reason that they did not observe CK effects on the allergic Hist release seems to be that they used monodispersed mast cells instead of highly purified mast cell. In our present results, we did not observe the inhibition of mediator release by CK in the monodispersed and partially purified mast cells, too. Therefore, it could be possible that Hist release-inhibiting material and LT-producing lung cell other than the mast cells, might be in the crude lung preparations.

It is well known that certain cellular events which occur during allergic reactions are Ca^{2+}-dependent phenomena (Middleton, 1980). Some investigators have demonstrated that allergic mediator release are Ca^{2+}-dependent reactions (Richie *et al.*, 1984; Rossi *et al.*, 1982; Nagai, 1987). As Ca^{2+}-dependent cellular responses usually cross link to K^+ movement, it is possible that reactions (membrane phospholipids break down, membrane enzyme activity, Ca^{2+} entry etc.) are in part modified by K^+ channel openers. At present, however, further experiments may be necessary to clarify the biochemical responses in cellular event modified by K^+ channel opener, CK.

These results show that guinea pig lung mast cells seem to be an important contributor to LT release, and that CK can in part act to inhibit mediator release in the Ag-induced airway smooth muscle, and that CK may act to inhibit mediator release in the OA-induced and CaI-induced highly purified mast cells. these results suggest that Hist and LT release evoked by mast cell activation might in part be associated with K^+ channel activity.

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=국문초록=

Cromakalim이 해명의 과민반응 매개체 유리에 미치는 영향

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K^+통로는 기도 평활근 세포에 존재하며 이들 통로가 활성화되면 평활근의 과분극의 결과 이완작용이 나타난다. K^+통로의 이런 효과는 과민반응과 천식 치료에 응용될 수 있으므로 우리는 K^+통로 개방제인 cromakalim (BRL34915, CK)이 IgG_1 항체로 감작시킨 기도 및 폐조직으로 부터 유리되는 매개체 유리에 미치는 영향을 조사하였다. 피동적으로 감작된 두 조직은 2×10^{-6} M의 CK로 30분동안 superfusion시킨 후 CK와 항원 (Ox-HSA) 0.1 mg/ml로 자극하였다. 또한 비만세포를 이용하여 CK의 효과를 조사하였다. 해명 폐조직 비만세포는 효소에 의한 digestion method (monodispersed; 미분리 정제), count current elutriation에 의한 방법(partially purified; 부분분리정제), 그리고 discontinuous Percoll방법(highly purified; 순수분리정제)에 의해 순수 분리되었다. CK로 전처치한후, 피동적으로 감작된 비만세포는 OA와 CaI의 여러 농도에 의해 자극되었다. 유리된 Hist은 spectrophotofluorometry에 의해, LT는 면역방사법에 의해 측정되었다.

CK 전처치는 IgG_1 감작후 항원에 의해 자극된 기도 조직에서 His 유리량을 35%까지, LT유리량은 40%까지 감소시켰으나 기도 평활근 수축력에는 반응을 나타내지 못하였다. 항원 유도 폐조직에 있어서 CK전처치는 Hist유리량을 25%까지 감소시켰으나 LT 유리에는 미약한 감소를 나타내었다. 해명의 미분리정제, 부분분리정제, 그리고 순수 분리 정제된 비만세포로부터 Hist과 LT은 면역자극(OA)이나 비면역자극(CaI)에 의해 농도 의존적으로 유리되었다. 비만세포에서 유리된 LT는 5-lipoxygenase억제제인 A64077에 의해서 억제됨이 확인되었다. CK전처치는 OA 유도 및 CaI유도 해명 폐조직 비만세포에서 Hist과 LT 유리량을 20%까지 감소시켰다. IgG_1감작후 Ox-HSA유도 기도 평활근 조직이나 혹은 OA유도 및 CaI유도 비만세포에서 Hist과 LT유리에 미치는 CK의 억제효과는 TEA와 GBC에 의해 완전히 봉쇄되었다.

이상의 결과에서 폐조직 비만세포는 LT를 유리할 수 있는 세포로 간주되며, 기도 평활근 이완제로 알려져 있는 CK은 특수 항원 유도 기도 평활근조직에서 매개체 유리를 부분적으로 억제하며, CK은 또한 OA유도 및 CaI로 유도된 순수분리 정제된 비만세포에서 매개체 유리를 부분적으로 억제하는 것으로 보아 비만세포가 활성화시 야기되는 여러 생화학적 현상중에서 미약하나마 K^+통로가 관여할 것으로 사료된다.