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# Histamine induced elevation of cyclic AMP phosphodiesterase activity in human monocytes

C. A. Holden [1], S. C. Chan [2], S. Norris [2] and J. M. Hanifin [2]

[1] Department of Dermatology, St. Helier Hospital, Wrythe Lane, Carshalton, Surrey, England and [2] Department of Dermatology, Oregon Health Sciences University, 3181 S.W. Jackson Park Road, Portland, Oregon 97201

## Abstract

We have previously reported histamine desensitization of human blood mononuclear leukocytes resulting in reduced cAMP responses to $\beta$-adrenergic agonists, histamine and prostaglandin $E_1$. This heterologous desensitization occurred at low, micromolar histamine concentrations and was accompanied by elevation of cAMP-phosphodiesterase (PDE) activity in these cells. We have now investigated the activity of PDE in the lymphocyte and monocyte fractions of mononuclear leukocytes to determine the site of histamine effect.

PDE activity per cell was higher in monocytes ($0.075 \pm 0.070$ units) than lymphocytes ($0.026 \pm 0.08$) units). Monocytes responded to $10^{-6}$ *M* histamine stimulation with a much greater increase in PDE activity ($0.354 \pm 0.1$ units) than did lymphocytes ($0.047 \pm 0.015$ units). Histamine receptor studies, using thiazolylethylamine and chlorpheniramine as $H_1$-agonist and antagonist respectively and dimaprit and cimetidine as $H_2$-agonists and antagonists respectively, indicated that the histamine stimulation of PDE activity is mediated predominantly through $H_1$ histamine receptor in the monocytes and the $H_2$ receptor in the lymphocytes. Previously histamine had been thought to increase cyclic AMP by acting on $H_2$ receptors to activate adenylate cyclase. Our studies show that stimulation of $H_1$ or $H_2$ receptors by low histamine concentration can cause the opposite effect i.e. increased catabolism and a net reduction in cAMP levels. The localization of this effect predominantly to monocytes indicates a potentially important mechanism for histamine action on immune regulation.

## Introduction

Various mechanisms have been proposed for the desensitization or refractoriness which follows exposure of cells to agonists. Included among these are loss of binding sites due to internalization [1, 2] or alteration of the guanine nucleotide regulatory protein [3].

However, all cells contain a number of different cyclic 3′, 5′-nucleotide phosphodiesterases that catalyze cyclic nucleotide hydrolysis in various tissues [4]. One of these, a cAMP specific phosphodiesterase (PDE) appears to be present in several forms in peripheral blood mononuclear leukocytes [5, 6].

We have previously shown that human blood mononuclear leukocytes (MNL) may be cross-desensitized by exposure to low concentrations of histamine, isoproterenol or prostaglandin $E_1$, such that subsequent stimulation by any of the agents yields reduced quantities of cAMP accumulation [7]. This decrease in cAMP responsiveness was mirrored by an increase in PDE activity. In addition, kinetic experiments on par-

tially purified MNL subsets suggested a greater effect on monocyte PDE as compared to lymphocytes PDE.

In this paper we have investigated the effect of histamine at low concentrations on the PDE activity of human mononuclear phagocytes and lymphocytes purified by different methods. In addition, we have studied the effects of histamine agonists and antagonists in order to further elucidate the mechanism of the PDE activity increase.

## Materials and methods

### *Materials*

Hypaque-Ficoll (HF) and Percoll were purchased from Pharmacia Fine Chemicals, Piscataway, New Jersey; Gey's Balanced Salt Solution (GBSS), PSF (penicillin, Streptomycin and Fungizone) Mixture and RPMI-1640 from GIBCO, Grand Island, New York; Histamine dihydrochloride, cyclic AMP, cobra venom, latex beads and chlorpheniramine were purchased from Sigma Chemical Co., St. Louis, Missouri; $^3$H-cAMP, New England, Nuclear, Boston, Massachusetts; Dowex AG1X2 resin (200–400 mesh), BioRad, Richmond, California; Ready-Solv EP, Beckman Inst. Corp., San Jose, California; Thiazolylethylamine, Dimaprit, Cimetidine were gifts from Smith Kline and French Laboratories, Philadelphia, Pennsylvania.

### *Leukocyte preparation*

After informed consent, blood was drawn from normal, young adult donors between 8:00 and 9:00 a.m. All subjects had avoided methyl xanthine-containing foods and beverages for the previous 14 hours, and none was receiving medication. The heparinized blood was processed in two different ways, depending upon the final method of purification of the MNL subsets.

### *Percoll separation of MNL subsets*

Heparinized blood was mixed with an equal volume of normal saline and layered on to HF and centrifuged at 400 g for 40 minutes at room temperature. The isolated MNL were washed three times in normal saline at 400 g, 300 g, and 250 g to reduce platelet numbers, and then resuspended in GBSS or phosphate buffered saline (PBS) for further studies.

Continuous Percoll gradients were preformed by mixing seven parts Percoll with six parts of a double strength PBS. Fifteen milliliter aliquots were centrifuged for 40 minutes in 17 ml polycarbonate tubes (Sorvall-Dupont #00770) at 25000 g in an angle head rotor (SS-34) of a Sorvall RC-5B centrifuge. One milliliter of the MNL ($15\times10^6$ cells) in PBS were layered onto the gradients and then spun at 1000 g in a hanging bucket rotor for 20 minutes. Two bands were produced: an upper monocyte-rich (MR) fraction and a lower lyphocyte-rich (LR) fraction, as described by Gmelig-Meyling [8]. These were aspirated from the gradients, washed with GBSS and resuspended in GBSS at $1.5–2\times10^6$ ml for characterization and PDE assay.

### *Adherence separation of MNL subsets*

Whole blood was obtained from normal donors as described above. The blood was then immediately processed in a modification of the method of Pawlowski et al. [9] designed to generate platelet-free MNL. The whole blood was diluted with an equal volume of cold, calcium and magnesium-deficient, phosphate-buffered saline (PD) and washed twice in PD by centrifugation at 150 g in a swing-out rotor. The cell pack was reconstituted to volume with PD containing 0.3 m*M* EDTA. The blood was layered onto 4 ml HF in 15 ml plastic tissue culture tubes (#3026, Falcon, Oxnard, California) and the MNL separated by centrifugation at 500 g for 30 minutes at room temperature. The MNL were washed three times in PD and then either resuspended in GBSS at $4\times10^6$/ml for histamine stimulation or at $7\times10^6$/ml for adherence in RPMI-1640 containing 15% human AB serum and PSF (Penicillin, Streptomycin, Fungizone) mixture. Three milliliters were incubated for 2 hours in 60 mm plastic dishes (Corning #25010) at 37 °C in 5% $CO_2$/95% air to allow monocyte adherence.

The supernatant was aspirated and the dishes were rinsed thrice with warm GBSS and gentle agitation to remove nonadherent cells. A cover slip, previously placed in the bottom of the dish, was removed for adherent cell characterization. The adherent cells were washed from the plastic by vigorous pipetting with PD. Complete removal

and dissociation of monocytes were monitored using an inverted, phase microscope. The adherent and nonadherent cell populations were washed and resuspended at $1-2\times10^6$/ml GBSS for PDE assay.

*Cell characterization*

Cell viability resulting from both methods of separation was monitored by trypan blue exclusion and was always greater than 95%. The MNL, MR, LR, supernatant cells and adhered cells were characterized by Giemsa and alpha napthyl acetate esterase activity (ANAE) stain [10] and by latex beads uptake.

*Histamine and histamine agonist ($H_1$ and $H_2$) activation*

Mononuclear leukocytes ($4\times10^6$/ml) isolated on HF were incubated at 37 °C in 5% $CO_2$/air for 15 minutes in 15 ml plastic conical tubes (Corning #25310) to stabilize temperature. Histamine dihydrochloride, 2-thiazolylethylamine (TEA) or Dimaprit (DIM) in GBSS was then added to half the tubes at final concentrations of $10^{-6}$ *M* and the cells were mixed and incubated, with occassional gentle inversion, for 1 hour. The optimal histamine concentrations for maximum histamine PDE activation having been determined in our previous study [7]. Subsequently, the cells were diluted with 4 parts PD, pelleted by centrifugation at 400 g, washed once more in PD, and then either purified by the Percoll or adherence methods described above, or resuspended in GBSS at a cell concentration of $1.5-2\times10^6$ cells/ml, for characterization and PDE assay. Untreated control tubes were processed in parallel but with addition of GBSS without histamine. For direct histamine, TEA or DIM stimulation of monocytes, compounds were added directly to plates containing highly purified adherent monocytes or to the non-adherent lymphocytes preparations.

*Histamine blocking studies*

In order to ascertain whether increased PDE activity resulted from the action of histamine on histamine receptors, we performed experiments employing cimetidine as an $H_2$-receptor blocker and chlorpheniramine as an $H_1$-receptor blocker. MNL were suspended in GBSS at $4\times10^6$ ml with varying concentrations of cimetidine or chlorpheniramine. Identical controls were similarly treated in the absence of the antagonists. After 15 minutes, histamine, TEA or DIM ($10^{-6}$ *M*) was added to half the tubes, and they were incubated for an additional hour. The cells were washed in PD and then layered onto Percoll, as described above. The MNL, MR, and LR fractions were finally suspended at $1.5-2\times10^6$ cells/ml of GBSS for PDE assay.

*Cyclic AMP phosphodiesterase assay*

The cells were freeze thawed thrice, sonicated at 50 W for 2 minutes by a Braunsonic 2000 (B. Braun, Melsungen, Germany) and assayed for PDE activity using modification of the procedure of Thompson et al. [11].

Homogenate (200 µl) was added to 200 µl of substrate containing 0.025 µ*M* cAMP, 200000 cpm $^3$H-cAMP in 40 m*M* Tris HCl buffer (pH 8.0) containing 3.75 m*M* beta-mercaptoethanol and 50 m*M* $MgCl_2$. For kinetic studies cAMP concentrations ranged from 0.02 to 250 µ*M*.

After 10 minutes incubation at 30 °C, the reaction was stopped by boiling for 45 seconds and snap freezing in ethanol/dry ice mixture. The mixtures were further incubated with excess 5′-nucleotidase (cobra venom) for ten minutes at 30 °C and then mixed with 1 ml of AG1X2 resin (200–400 mesh) for at least half an hour at 4 °C. The samples were counted in Ready-solv EP and enzyme activity was expressed as pmol cAMP hydrolyzed per minute per $10^6$ cells (equivalent to 1 unit).

Km's were estimated from Eadie-Hofstee plots by linear regression. Statistical analysis was performed using the Student T test for unpaired means except when unstimulated controls were compared to the stimulated level, then paired means were used.

## Results

*Histamine stimulation of percol separated subpopulations*

Percoll gradients separated the histamine treated or control MNL ($78\pm6$% lymphocytes, $22\pm6$%

monocytes, and <1% polypmorphonuclear leukocytes) into MR (60±5% monocytes) and LR (94±4% lymphocytes) fractions. The PDE activity of the control cells varied according to their constitution (Table 1), being highest in the MR and lowest in the LR. Both MNL and MR had significantly more PDE activity than did the LR fraction ($p < 0.05$). Prior exposure to histamine, histamine agonists or antagonists did not alter cell proportions after Percoll.

Histamine exposure caused increased PDE activity in all three cell preparations. The net increase was 0.28 pmol/min/$10^6$ in the MR, 0.073 pmol/min/$10^6$ in the MNL and 0.021 pmol/min/$10^6$ in the LR with $p$ values of $<0.005$, $<0.005$ and $<0.01$ (respectively), significantly different from their respective control levels (Table 1). The increase in PDE activity in the MR was 4 times greater than that in the MNL and 13 times that in the LR fractions.

*Histamine stimulation of adherence separated subpopulations*

The initial studies clearly indicated that the monocyte enriched preparations were the main source of basal and histamine stimulated PDE activity and that the $10^{-6}$ $M$ histamine concentrations was optimal [7]. We next studied more highly purified monocyte preparations. We first carried out adherence separation on histamine-treated and untreated MNL preparations. The MNL prepared for adherence separation of subsets had a similar composition to those used for Percoll purification (76±3% lymphocytes and 24±3% monocytes). However, separating the cells by adherence produced supopulations of different composition compared to Percoll separated MR and LR. Mononuclear phagocytes comprised 93±6.8% of adherent cells while the supernatant cells were 95±3.8% lymphocytes ($n=8$). Platelet counts were <30000/$10^6$ cells for all samples. No differences in differential cell counts were noted between control and histamine-treated cell populations. Histamine did not alter the numbers of adherent or nonadherent cells.

The PDE activities of the control, unstimulated MNL, adherent and non-adherent cells (Table 2) were not significantly different from those of the previous experiment. Histamine desensitization caused a net increase of 0.055 pmol/min/$10^6$ cells of PDE activity in the MNL. This increase reached significance with a $p$ value of $<0.01$. The increase in the non-adherent cells (0.025 pmol/min/$10^6$ cells) reached significance at $p<0.05$. However, the greatest increase of PDE activity was seen in the adherent cells (0.146 pmol/min/$10^6$ cells). The increase in PDE activity in the adherent cells reached statistical significance with a value of $p < 0.05$.

Previous studies had shown that $10^{-6}$ $M$ histamine increased MNL activity by 15 minutes with a maximal effect at 60 minutes [7]. Preliminary time course ecperiments carried out on adherent monocytes in this study also showed monocyte PDE to be stimulated by histamine, TEA and DIM at 15 minutes, but the increase was maximal at 60 minutes.

In addition the specific antagonists showed maximal blocking of the histamine, TEA and DIM effects with 15 minutes preincubation and no greater effect was noticed when preincubation was extended to 60 minutes. Consequently in all subsequent studies of $H_1$- and $H_2$-agonists and antagonists on PDE activity a 15 minute preincubation with the antagonists and a 60 minute subsequent incubation with agonists were employed.

The $H_1$-agonist TEA, at $10^{-6}$ $M$, significantly enhanced PDE activity in adherent cells (Table 2) but not in nonadherent cells. In contrast, the enzyme activity was significantly elevated in both leukocyte populations by the $H_2$-agonist, dimaprit ($10^{-6}$ $M$), but to a lesser extent compared with the TEA effect on monocytes.

The increase in PDE activity caused by dimaprit on the non-adherent cells, was more than equi-

**Table 1**
Cyclic AMP phosphodiesterase activity in control and histamine activated mononuclear leukocyte subpopulations purified by Percoll centrifugation (pmol cAMP/min/$10^6$ cells ±S.D., $n=6$).

| | Control | Histamine ($10^{-6}$ $M$) pretreatment |
|---|---|---|
| Mixed mononuclear leukocytes (78±6% lymphocytes) | 0.046±0.025 | 0.119±0.017 $p<0.005$ |
| Monocyte-rich (MR) (60±5% monocytes) | 0.075±0.070 | 0.354±0.100 $p<0.005$ |
| Lymphocyte-rich (LR) (94±4% lymphocytes) | 0.026±0.008 | 0.047±0.015 $p<0.01$ |

**Table 2**
Cyclic AMP phosphodiesterase activity in control, histamine and histamine agonist pre-treated mononuclear leukocyte subpopulations purified by adherence separation (*p*mol cAMP/min/$10^6$ cells ±S.D.).

| | Control | $10^{-6}$ *M* histamine | $10^{-6}$ *M* TEA* | $10^{-6}$ *M* DIM** |
|---|---|---|---|---|
| Mononuclear leukocytes (76±3% lymphocytes) | 0.037 ±0.016 ($n=5$) | 0.092±0.033 ($p<0.01$) ($n=5$) | | |
| Adherent cells (93±6.8% lymphocytes) | 0.0792±0.031 ($n=10$) | 0.225±0.12 ($p<0.05$) ($n=8$) | 0.276±0.046 ($p<0.01$) ($n=5$) | 0.141±0.027 ($p<0.05$) ($n=5$) |
| Non-adherent cells (95±3.8% lymphocytes) | 0.028±0.012 ($n=10$) | 0.053±0.0137 ($p<0.05$) ($n=8$) | 0.036±0.008 (ns) ($n=5$) | 0.076±0.027 ($p<0.01$) ($n=5$) |

$n$ = number of experiments; * TEA = thiazolylethylamine; ** Dimaprit.

**Table 3**
Cyclic AMP phosphodiesterase activity increase in mononuclear leukocyte preparations exposed to histamine immediately or after a preparative delay (*p*mol cAMP/min/$10^6$ cells ±S.D.).

| | $n$ | $\Delta$ |
|---|---|---|
| Delayed stimulation | | |
| Monocytes (94–99% pure) | 4 | 0.014±0.002 |
| MNL (75% lymphocytes) | 2 | 0.037±0.010 |
| Immediate stimulation | | |
| MNL (75% lymphocytes) | 2 | 0.128±0.008 |

$\Delta$ = Mean change from basal level expressed as *p*mol cAMP/min/$10^6$ cells ±standard deviation.
$n$ = Number of experiments.

**Table 4**
Comparison of $H_1$ and $H_2$ antagonist effects on histamine-stimulated phosphodiesterase activity in mononuclear leukocytes.

| Antagonist | $n$ | IC-50* |
|---|---|---|
| Chlorpheniramine ($H_1$) | 3 | $2.5\times10^{-5}$ *M* |
| Cimetidine ($H_2$) | 6 | $4.0\times10^{-6}$ *M* |

* IC-50 represents the concentration, derived by linear regression analysis, causing 50% inhibition of histamine stimulation.

valent to the effect of equimolar concentrations of histamine. In contrast adherent cells only showed an increase in PDE activity by dimaprit equal to 40% of the histamine effect. A far greater effect was seen on adherent cells by the $H_1$-agonist TEA which caused an increase of the same magnitude as equimolar histamine.

The histamine stimulation of MNL before adherence isolation of monocytes left open the possibility that histamine was acting indirectly (e.g. via lymphocytes) to stimulate PDE activity. In order to determine whether other leukocytes were required for histamine elevation of PDE activity in monocytes purified by adherence, the adherent cells or MNL controls were exposed to $10^{-6}$ *M* histamine added directly to culture plates. While basal PDE activity was similar to levels detected in previous experiments above, histamine caused an increase of only 0.014 pmol/min/$10^6$ monocytes (Table 3) and 0.037 pmol/min/$10^6$ MNL after being held in culture plates during the prolonged (2 hour) monocytes adherence procedure. The MNL PDE enzyme activity increase after this delayed stimulation was less than one-third that on MNL stimulation immediately after Hypaque-Ficoll separation (Table 3).

*Antagonist studies*

Results of studies with the $H_1$- and $H_2$-antagonists chorpheniramine and cimetidine are shown in Table 4 which compares $IC_{50}$ determinations for cimetidine and chlorpheniramine in MNL preparations. The $IC_{50}$ of the $H_1$-antagonists was 1000-fold higher than that of cimetidine ($p<0.001$). At micromolar concentrations, the $H_2$-effect of histamine was only partially blocked. As reported in the previous section TEA and DIM specifically enhanced PDE activity of the adherent cells and DIM raised the enzyme activity of the non-adherent cells. The TEA provoked increase in PDE activity was completely abolished by $10^{-4}$ *M* chlorpheniramine and the dimaprit induced enzyme activity was prevented by $10^{-6}$ *M* cimetidine. Again a far greater concentration of the $H_1$-antagonist was needed to block the enzyme increase.

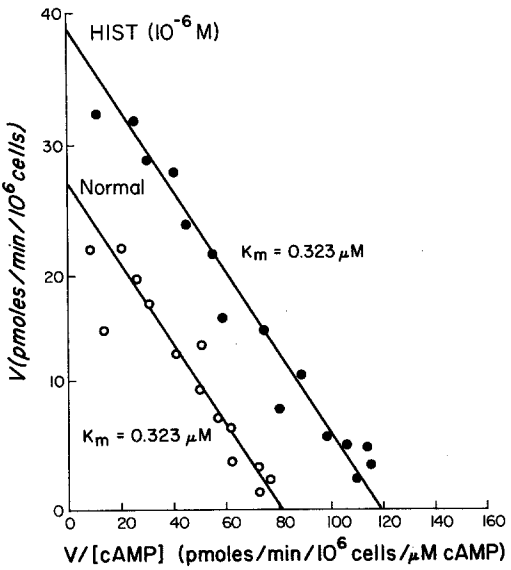


**Figure 1**
Eadie-Hofstee plots of PDE in untreated and histamine-treated normal monocyte-phosphodiesterase. Substrate concentration ranged from 0.02 to 250 $\mu M$. Km's were determined from the slopes of the linear regression lines and $V_{max}$ from the Y-intercepts.

*Enzyme kinetic studies*

Because the major portion of the histamine-stimulated PDE activity is present in monocytes, we next examined the effect of histamine on the Km of the monocyte-PDE. The Km of PDE from histamine-treated ($10^{-6}$ *M*, for 1 hour) monocytes was virtually identical to that of the untreated cells (Km = 0.32 $\mu M$) as determined from the slope of the Eadie-Hofstee plot (Figure 1), confirming previous findings using other kinetic analysis [7]. $V_{max}$ for histamine-treated monocyte-PDE was 0.39 pmol/min/$10^6$ cells compared to 0.26 pmol/min/$10^6$ cells in untreated cells.

## Discussion

Our data demonstrate a significant increase of MNL PDE activity when cells are exposed to low concentrations of histamine. This confirms previous experiments that showed an association between cyclic AMP desensitization by histamine and increased PDE activity [7]. We have utilized a new separation technique that effectively removed plateletes from MNL and the effect is clearly leukocyte directed.

We have demonstrated that the major portion of the MNL response to histamine resides in the monocytes. Although there may be an effect of preparation on cyclic nucleotide metabolism [12] the increase in monocyte PDE activity was observed whether the cells were purified by Percoll or adherence separation. These purification methods are entirely different and thus the findings are not the result of a preparative effect. In addition the adherence method of monocyte separation gave samples of very high purity with little contamination by other mononuclear cells.
Lymphocyte-rich preparations showed a relatively smaller increase in PDE activity, considerably less than in the monocyte population. These observations agree with previous data which showed differing enzyme kinetics of lymphocyte PDE compared with monocyte PDE [7].
Our data cannot exclude an indirect effect of histamine on monocytes via another mononuclear leukocyte subgroup. Indeed when monocytes were stimulated directly after purification the increase in PDE activity was lower than when MNL were exposed. This was shown to be due only in part to the preparative delay.
In addition the data shows the MNL subgroups to be sensitive to different $H_1$- and $H_2$-agonists. The monocytes were predominantly affected by $H_1$-agonists with only a lesser effect by $H_2$-agonists. In contrast lymphocyte PDE was only increased by $H_2$-agonists. These effects seem to be mediated via the receptors because they could be blocked by the appropriate $H_1$- and $H_2$-antagonists, chlorpheniramine and cimetidine respectively.
However, the effect of the antagonists on histamine induced elevation of MNL PDE complicates the situation. The histamine induced increase of MNL PDE could be blocked by cimetidine at $10^{-8}$ *M* and by chlorpheniramine at $10^{-5}$ *M*. This either implies that one or both of these antagonists are not completely specific or that there is interaction between the receptors or cell subsets.
It is possible that exposure of lymphocytes to $H_2$-receptor agonists induces release of a soluble factor that sensitizes monocytes to the effect of $H_1$-receptor stimulation. Thus the use of low concentrations of cimetidine would prevent the large increase in PDE activity attributable to the monocytes. Certainly other authors have reported the formation of lymphocyte factors induced by histamine stimulation [13].

The kinetic data indicates that the PDE induced in monocytes by histamine exposure is similar to the normal monocyte and suggests the elevated activity was not the result of alteration of the affinity of the enzyme. In addition previous studies have shown the monocyte PDE is a relatively specific cAMP phosphodiesterase because it is uniquely sensitive to inhibition by RO-20-1724 a highly specific cAMP PDE inhibitor [14].

Although the increase in PDE activity is receptor mediated the precise intracellular mechanism is unknown. Recent evidence has suggested TEA ($H_1$) mediated protein kinase-C phosphorylation may cause covalent modification of the enzyme leading to its activation [15]. This may provide a mechanism for the regulation of $H_2$-receptor mediated increase in adenylate cyclase production of intracellular cAMP.

In this study we have demonstrated that one of the mechanisms by which histamine affects target cells is by increasing the rate of endogenous cAMP degradation mediated through either $H_1$ and $H_2$-receptors or $H_2$-receptors alone dependent on the leukocyte subpopulation. Other agents that produce an increase in PDE activity, such as isoproterenol, also appear to be blockable at the receptor level [16]. The situation with regard to the histamine receptor is clearly complicated as illustrated by the fact that there are several different $H_1$ and $H_2$-blockers perhaps representing different classes of receptors [17, 18].

However, there is no doubt that monocytes are highly sensitive to histamine induced elevation of cyclic AMP PDE activity. The altered cellular function resulting from reduced cyclic AMP levels may be of importance in the regulation of the immune control of IgE production. Particularly in pathological conditions such as atopic diseases that have a high tissue histamine content.

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