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Regulation of Catecholamine Secretion and Synthesis by Histamine in Primary Cultured Bovine Chromaffin Cells

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Ⅰ. INTRODUCTION

Histamine has been shown to induce the secretion of adreno-medullary catecholamines in vivo[1] which is abolished by transection of the splanchnic nerves or by ganglionic blockade.[2] More recently, it was shown that histamine has the same effect in vitro as well in the primary cultured chromaffin cells[3] and that this occurs via the H1 histaminergic receptor,[4] whose presence has been previously reported.[5] Activation of these receptors stimulates phosphotidylinositide hydrolysis,[6] which in turn can cause a variety of intracellular responses including the activation of protein kinase C(PKC), by liberating diacylglycerol(DAG) and inositol triphosphate(IP3). Since direct activation of PKC by phorbol esters has been also shown to induce catecholamine secretion[9] and the transcriptional activation of the epinephrine synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT) in chromaffin cells,[10] it is possible that the intracellular action of histamine is mediated by PKC. In the present study we tested whether this is the case by comparing the effects of histamine on the catecholamine secretion and PNMT gene expression using various pharmacological agents which influence the PKC pathway in the primary cultured bovine chromaffin cells.

Ⅱ. MATERIALS AND METHODS

Cell Culture : Primary cultures of bovine adrenal medullary cells were prepared essentially according to the published method[11] with minor modifications. Briefly, fresh adrenals were perfused with collagenase and incubated for 30min at 37℃. The medullae were dissected, minced, and further dissociated in collagenase at 37℃ for 40min. After filtering through the Spectropor membrane 105um mesh, chromaffin cells were separated on Percoll gradient at 37,000×g for 40min. Cells were plated in DMEM/F12 containing 10% fetal calf serum and two days later the cells were refed with fresh serum-free medium.

Determination of Catecholamine release : 5×10^5 cells per 15mm plate were treated with histamine and /or other agents. The bathing solution was collected at appropriate time point and the proteins removed by perchloric acid precipitation. The samples were diluted in mobile phase(0.1M sodium acetate, 0.1M citric acid, 1.5mM sodium octyl sulfate, 0.15mM NaEDTA, 1mM di-n-butylamine, in H_2O/methanol (90 : 10) and injected into HPLC. Catecholamines were separated on C18 novapak column and detected electrochemically(Waters 460 detector). All experiments were conducted using quadruplicate samples of each condition, repeated on at least two different culture preparations.

RNA Isolation : Cells were harvested after 6h and lysed in 140mM NaCl, 1.5mM $MgCl_2$, 10mM Tris-Cl, pH 8.6, 0.5% Nonidet-40 and 1mM DTT. After removing the nuclei, the supernatant was digested in 0.1M Tris-Cl pH 8.0, 2.5mM EDTA, 150mM NaCl, 1% SDS and 10ug/ml proteinase K for 15min at 50℃. After phenol/chloroform extractions, the final product was precipitated in ethanol.

Northern Blot Analysis : Total RNA isolated from 5×10^6 cells was denatured, resolved on formaldehyde gels, and transferred to Gene Screen Plus membrane. Hybridization was carried out in 50% formamide, 1% SDS, 1M NaCl, 10% dextran sulfate and the ^{32}P labelled PNMT cDNA probe[12]at the concentration of 5×10^5cpm/ml at 42℃ overnight. The blots were washed by the standard methods and autoradiographed.

Ⅲ. RESULTS

In the present study we sought to identify the intracellular mechanism with which histamine elicits its action on the chromaffin cells. As shown in Fig 1, histamine at 1uM acted as a potent secretagogue of both norepinphrine and epinephrine, causing 3.5% of total catecholamine to be released, which was 500% of the untreated control(0.7%). This degree of secretion is comparable to those by a known secretagogue reserpine[13] as well as by the potassium-induced depolarization which caused a secretion of 570% and 740% of the control, respectively. In addition, histamine induced catecholamine secretion that is comparable to that by the phorbol ester 12-O-tetradecanoyl phorbol-13-acetate(TPA). The secretion patterns for norepinephrine and epinephrine were mostly identical and thus the results of epinephrine only will be shown hereafter. During the 30min that the amount of the released catecholamines was assessed, histamine elicited a secretory respones which increased gradually, its effect being the most prominent during the initial five min(Fig 2).

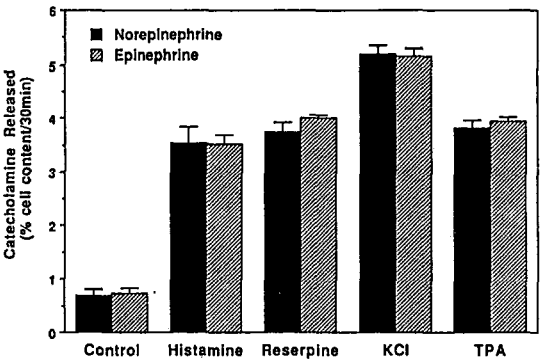

Fig 1. Effects of treatment with histamine(1uM), reserpine(100nM), KCl(59mM) and TPA(100nM) on catecholamine secretion after 30min at room temperature.

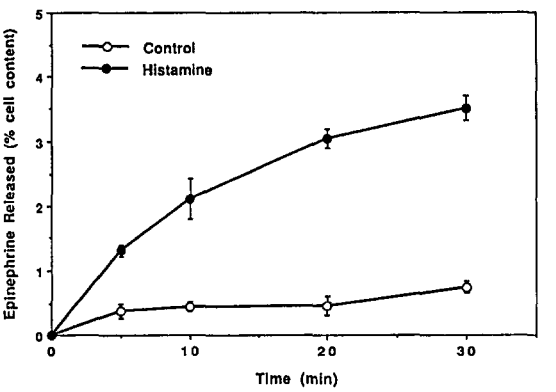

Fig 2. Time course of epinephrine secretion stimulated by 1uM histamine.

The intracellular mechanism of action of this effect was determined using a various pharmalolgical agents. As shown in Fig 3, the cotreatment with the calcium chelator EGTA caused a decrease in catecholamine release down to 22% compared to the treatment with histamine alone(100%), supporting the previous reports that the extracellar calcium is neccessary for catecholamine secretion[15, 16, 17, 18] including that stimulated by histamine.[3, 5] Whether any of the two second messengers produced by the H1 receptor activation, DAG and IP3, are involved was tested next. While IP3's primary role is to release the calcium from the intracellular stores,[18, 19, 20] blockade of this calcium release by dantrolene(50uM) inhibited

the histamine-mediated catecholamine release by only 12%. This indicates that the intracellular calcium release is unlikely to play a major role in mediating this response. On the other hand, the results of the cotreatment with histamine and TPA was not significantly higher than histamine alone, and the PKC inhibitor staurosporine(1uM) decreased the release dramatically by 86%, suggesting that the two agents may act through the common signalling pathway and that PKC activation mediates the secretion by histamine.

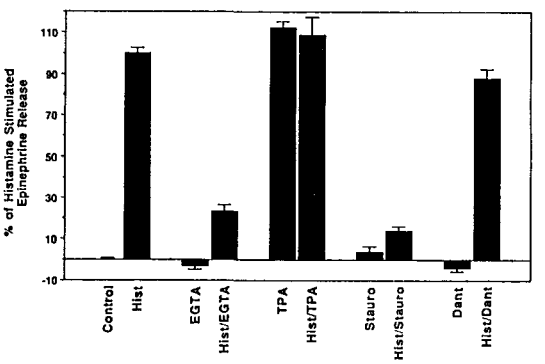

Fig 3. Effects of various agents on the histamine-induced epinephrine secretion 30min after the treatments with histamine(Hist) 1uM ; EGTA 1mM ; TPA 100nM ; staurosporine(Stauro) 1uM and dantrolene(Dant) 50uM.

To confirm that PKC is involved in the histamine responses in the chromaffin cells, its effects on the epinephrine synthesizing enzyme PNMT was examined. Fig 4 shows the results of Northern blot analysis after treating the cells for 6h with histamine and/or various agents that influence the signalling pathways. While the untreated control had very low PNMT message, histamine dramatically increased its level. The cotreatment with dantrolene(50uM) did not significantly change this effect, again showing that the internal calcium release is not important. Veratridine, a depolarizing agent which secondarily causes calcium influx, increased the PNMT mRNA to the similar degree as histamine. Cotreatment with TPA did not result in an additive effect, and the PKC inhibitor sphingosine(37. 5uM) reversed the histamine-induced effect. Thus, the data on PNMT gene regulation show similar pattern of responses as the secretion, further providing the evidence that the histamine effect on catecholamine cells is mediated by PKC. This also show that the stimulation of catecholamine secretion and the regulation of expression of its synthesizing enzyme PNMT by histamine may be mediated by the common signalling pathway.

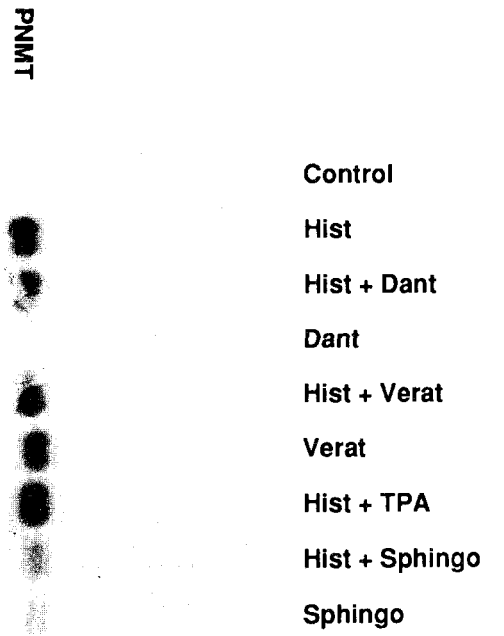

Fig. 4 Effects of various agent on the steady state mRNA level of PNMT 6h after the treatments with histamine(Hist) 1uM; dantrolene(Dant) 50uM; veratridine(Verat) 2uM; TPA 100nM; and sphingosine(Sphingo) 37.5uM

Ⅳ. DISCUSSION

Norepinephrine and epinephrine play a critical role as neurotransmitters in the central as well as peripheral nervous systems. In addition, they are released as hormones from adrenal medulla under conditions of fear or stress thereby coordinating a "fight or

flight" response that prepares one to combat an enemy or flee from danger. This physiological response obviously crucial for survival is well-controlled in vivo and the availability of these catecholamines is mainly determained by the enzymes responsible for their synthesis. The present study demonstrates that the neurotransmitter histamine induces secretion of catecholamines and increases PNMT gene expression which is primarily mediated by activation of PKC.

PKC is a calcium/phospholipid dependent kinase whose intracellular actions are diverse.[21] These responses are mediated by phosphorylation of specific target proteins which elicits changes in the intracellular biochemical reactions. In catecholamine cells, PKC activation has been shown to be involved in short term responses by activating one of the synthesizing enzymes tyrosine hydroxylase(TH)[22,23,24] as well as eliciting the secretory processes.[9] Our present results that the patterns of catecholamine secretion by activation of PKC by TPA and by histamine are indistinguishable and that the histamine responses are blocked by calcium blockers and PKC inhibitors suggest that the histamine-induced catecholamine secretion is mediated by this kinase. PKC is also involved in the long term responses by increasing the gene expression of TH and PNMT[10] which is thought to occur through the AP1 site in the upstream regulatory region of the TPA-responsive genes.[25] Our results that the pattern of PNMT gene expression after histamine treatment is similar to PKC activation and inhibited by PKC inhibitors show that the histamine action on PNMT is mediated primarily by PKC. The reason that the previous investigators could not see the induction of PNMT by histamine[26] seems to be due to the longer time point, 24h, at which the effect was looked at, while our data demonstrate that this induction occurs dramatically within 6h. Thus, histamine acts on catecholamine synthesis by governing both the short term and long term regulations, inducing the secretion of catecholamines as well as activationg the gene transcription of PNMT, supporting the stimulation-secretion-synthesis coupling.[27] Thus, it is possible that the target proteins for PKC in these cells include those involved in secretion as well as the proteins that confer TPA responsiveness at the gene transcription level such as Fos or Fos-related proteins as suggested for TH.[28]

V. ACKNOWLDEGEMENTS

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=국문초록=

일차배양 소부신수질 세포에서 histamine에 의한 catecholamine유리 및 합성 조절

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황 온 유 · 나 도 선 · 송 규 영 · 조 성 우 · 이 재 담

Histamine은 부신 수질 세포의 H_1 수용체 활성화를 통한 phosphatidylinositide 가수 분해와 함께 catecholamine유리를 촉진시킨다고 알려진 바 있다. 본 논문에서는 histamine의 세포 내 작용 기전을 밝히고자 histamine이 catecholamine 생합성 효소인 phenylethanolamine N-methytransferase(PNMT)의 유전자 발현 및 catecholamine유리에 미치는 영향을 연구하였다. Histamine에 의한 catecholamine유리는 세포 외 calcium 유입에 의존적이었으나, 세포 내 저장 calcium과는 무관하였고, reserpine 및 탈분극에 의한 유리와 비슷한 수준이었다. Protein kinase C(PKC)활성제 TPA에 의한 유리와 구별 될 수 없었으며, 그 억제제인 staurosporin에 의해 억제되었다. 또한 histamine은 PNMT mRNA를 증가시켰는데 이 현상은 TPA에 의한 증가와 동일하며, dantrolene에 의한 차이는 없었으나, PKC 억제제인 sphingosine에 의해 억제되었다. 이상의 결과는 histamine이 chromaffin세포에서 PKC활성화를 통하여 catecholamine 유리 및 PNMT 유전자 발현을 증가시킴으로 catecholamine의 유리와 합성을 연결시킴을 시사하였다.

Key words : Histamine, Protein Kinase C, Catecholamine, Phenylethanolamine N-methyltransferase