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MODULATION OF MEDIATOR RELEASE FROM HUMAN BASOPHILS AND PULMONARY MAST CELLS AND MACROPHAGES BY AURANOFIN*

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Abstract—Auranofin, a new orally absorbable gold compound, inhibits IgE-(anti-IgE) and non-IgE-mediated (f-met-peptide and the Ca^{2+} ionophore A23187) histamine release from human basophils. Auranofin inhibits the release of histamine induced by phorbol myristate (TPA) and bryostatin 1 both in the presence and absence of extracellular Ca^{2+}. Increasing the Ca^{2+} concentrations in the extracellular medium does not reduce the inhibitory effect of auranofin on anti-IgE- or A23187-induced secretion. Auranofin inhibits the *de novo* synthesis of sulfidopeptide leukotriene C_4 (LTC_4) induced by anti-IgE from basophils and mast cells purified from human lung. However, in both systems auranofin has a significantly greater inhibitory effect on LTC_4 release than on histamine secretion. Finally, auranofin induces a concentration-dependent inhibition of A23187-induced leukotrine B_4 (LTB_4) release from purified human lung macrophages. These data suggest that auranofin modulates the release of preformed (histamine) and *de novo* synthesized (LTC_4 and LTB_4) chemical mediators from human inflammatory cells isolated from peripheral blood and human lung tissues.

Auranofin (2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*-triethylphosphine gold), a new orally absorbable gold compound is effective in the treatment of patients with inflammatory disorders such as rheumatoid arthritis [1, 2]. Occasional reports in the old European literature showed that chrysotherapy might be effective in the treatment of bronchial asthma [3–5]. Muranaka and co-workers showed that gold salts relieved the symptoms of asthma [6], reduced acetylcholine-induced bronchoconstriction [6, 7] and inhibited histamine release induced by anti-IgE from human basophils *in vitro* [8]. Chrysotherapy has been reported to have a corticosteroid-sparing effect in patients with severe asthma [9, 10].

The pathogenesis of allergic reactions involves the activation of IgE and non-IgE receptors on mast cells and basophils. This brings about a comlex sequence of biochemical events leading to arachidonic acid metabolism and mediator release. These include, in addition to preformed mediators such as histamine, *de novo* synthesized mediators such as Slow Reacting Substance of Anaphylaxis (SRS-A) and leukotriene B_4 (LTB_4¶) [11]. SRS-A is comprised of a series of related products of arachidonic acid metabolism: sulfidopeptide leukotrienes C_4 (LTC_4), D_4 (LTD_4), and E_4 (LTE_4). These sulfidopeptide leukotrienes display a variety of biological properties suggesting that they participate in many aspects of allergic reactions: they are potent constrictors of smooth muscle [12], enhance vascular permeability [13], and stimulate airway mucous production [14]. LTB_4 is a very potent chemotactic agent [15], which may even have important immunoregulatory functions [16]. Recent studies indicate that IgE cross-linking on human basophils and mast cells purified from human lung parenchyma results in *de novo* synthesis of immunoreactive and biologically active sulfidopeptide leukotrienes [17, 18].

Although human lung mast cells resemble human basophils in several respects, there are some differences with regard to the pharmacological control of mediator release from these inflammatory cells [19–21]. Recent evidence suggests that macrophages, either directly [22] or through the elaboration of

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¶ Abbreviations: F-met-peptide, *N*-formyl-methionyl-leucyl-phenylalanine; HSA, human serum albumin; LTB_4, leukotriene B_4; LTC_4, sulfidopeptide leukotriene C_4; LTD_4, sulfidopeptide leukotriene D_4; LTE_4, sulfidopeptide leukotriene E_4; PA, PIPES buffer (pH 7.4) containing human serum albumin; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); TPA, 12-tetradecanoyloxy-13-acetoxy-phorbol.

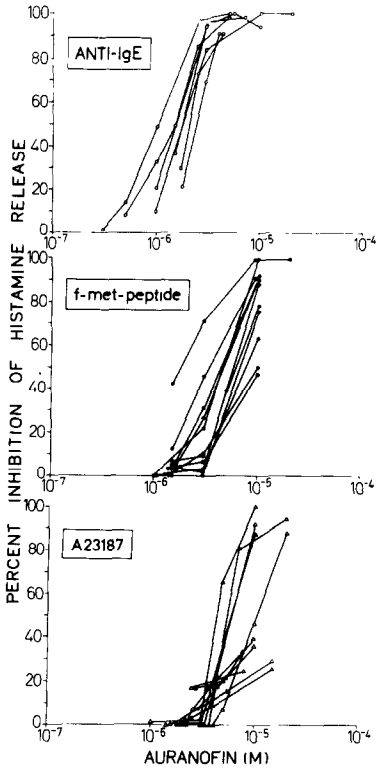

Fig. 1. Inhibition by various auranofin concentrations of histamine release from human basophils induced by anti-IgE (○), f-met-peptide (●), or the Ca^{2+} ionophore A23187 (△). Cells were preincubated for 3 min with auranofin before addition of the stimuli. Each curve represents the mean percentage inhibition of histamine release from duplicate determination on cells from different donors. The control percentages of histamine release were 51.6 ± 6.2 (anti-IgE), 50.0 ± 5.9 (f-met-peptide), and 55.3 ± 6.3 (A23187).

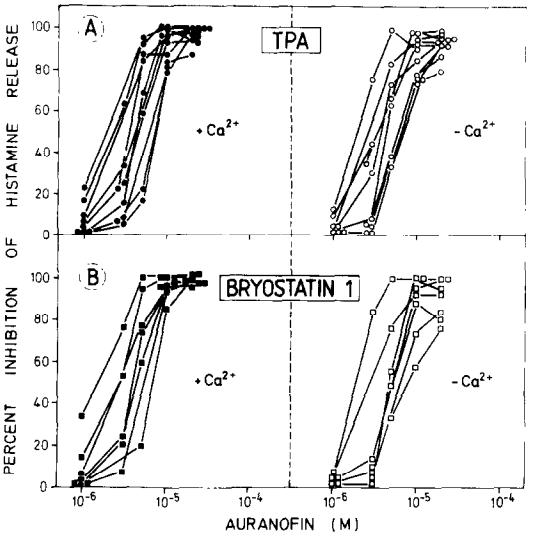

Fig. 2. Inhibition by various auranofin concentrations of histamine release from human basophils induced by TPA (N = 10) (A) or bryostatin 1 (N = 7) (B) in the presence of extracellular Ca^{2+} (1 mM) or in the absence of added Ca^{2+}. Cells were preincubated for 3 min with auranofin before addition of the stimuli. TPA (50 ng/ml) or bryostatin 1 (50 ng/ml) were then added and cells incubated for 45 min at 37°. Each curve represents the mean percentage inhibition of histamine release from duplicate determinations on cells from different donors. The control percentages of histamine release were 79.8 ± 6.0% (TPA, $+Ca^{2+}$), 47.6% ± 5.6% (TPA, $-Ca^{2+}$), 84.4 ± 3.5% (bryostatin 1, $+Ca^{2+}$), and 39.9 ± 6.2% (bryostatin 1, $-Ca^{2+}$).

"IgE-dependent factors" [23], participate in the release of chemical mediators of allergic reactions.

The present study was designed to assess the effect of pharmacological concentrations of auranofin on the release of histamine and leukotrienes from basophils and from mast cells and macrophages purified from human lung tissues. In pharmacological concentrations, auranofin appeared to be a potent inhibitor of the release of histamine and leukotrienes from human basophils and pulmonary mast cells and macrophages.

MATERIALS AND METHODS

Reagents. The following were purchased: RPMI 1640 with 25 mM Hepes buffer and 5.0 mM glutamine (M.A. Bioproducts, Walkersville, MD); PIPES, chymopapain, elastase type I (Sigma Chemical Co., St Louis, MO); collagenase (Worthington, Freehold, NJ); *N*-formyl-methionyl-leucyl-phenylalanine (f-met-peptide), the Ca^{2+} ionophore A23187, deoxyribonuclease and pronase (Calbiochem-Behring Co., La Jolla, CA); TPA (P-L Biochemicals Inc., Milwaukee, WI); Percoll and Dextran 70 (Pharmacia Fine Chemicals, Uppsala, Sweden); human serum albumin (HSA) (Merck Research Labs, Elkhart, NJ); [^{3}H]LTC_4 (35.7 Ci/mmol) and [^{3}H]LTB_4 (28.6 Ci/mmol) (New England Nuclear, Boston, MA). Rabbit anti human IgE-Fc_ε antibody was a generous gift from Drs Teruko and Kimishige Ishizaka, The Johns Hopkins University School of Medicine, Baltimore, MD. Synthetic LTB_4, LTC_4, LTD_4, and LTE_4 and the rabbit anti-LTC_4 antiserum were kind gifts from Dr J. Rokach (Merck Frosst, Canada, Montreal). Auranofin was kindly provided by Dr A. Astaldi (Smith Kline & French Co., Milan, Italy). Bryostatin 1 was isolated and purified as previously described [24].

Buffers. PIPES buffer (PA) consisted of 110 mM NaCl, 5 mM KCl, 25 mM piperazine-*N*,*N*′-bis (2-ethane-sulfonic acid) (PIPES), pH 7.4, and 30 mg/l HSA. This mixture is referred to as PA; PACM contains 1 mm $CaCl_2$ and 1.0 mM $MgCl_2$ in addition. Tyrode's buffer contains (g/l): NaCl, 8.0; KCl, 0.2; NaH_2PO_4, 0.05; $CaCl_2 \times 2H_2O$, 0.26; $MgCl_2 \times 6\,H_2O$, 0.25; and glucose, 1.0; pH was titrated to 7.4 with sodium bicarbonate.

Preparation of peripheral blood leukocytes containing basophils. After informed consent was obtained, blood was drawn into a final concentration of 0.008 M EDTA and 1.1% dextran 70, and allowed to sediment for 90 min at 22°. The leukocyte-rich upper layer was drawn off, pelleted (200 *g*, +4°C, 8 min), and washed twice in PA as previously

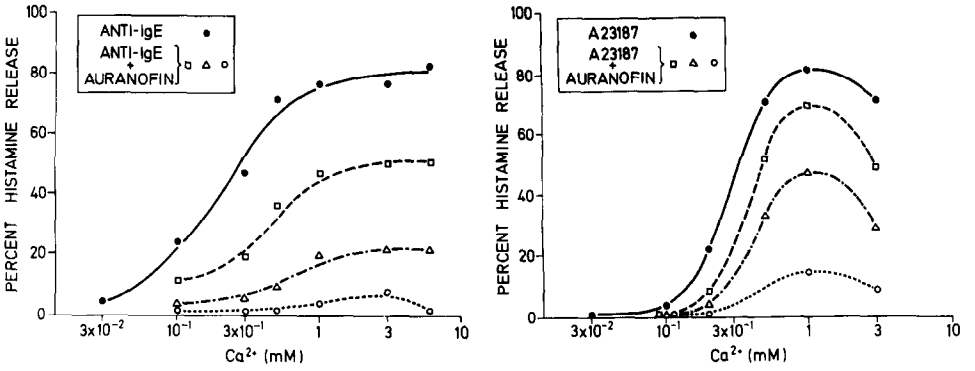

Fig. 3. (A) Effects of various concentrations of Ca^{2+} alone and in combination with auranofin (□, 1.5×10^{-6} M; △, 3×10^{-6} M; ○, 6×10^{-6} M) on anti-IgE-induced histamine release from human basophils. Cells were preincubated for 3 min with auranofin and Ca^{2+}. Anti-IgE (1 μg/ml) was then added and the cells incubated for 35 min at 37°. (B) Effects of various concentrations of Ca^{2+} alone and in combination with auranofin (□, 5×10^{-6} M; △, 10^{-5} M; ○, 2×10^{-5} M) on A23187-induced histamine release from human basophils. Cells were preincubated for 3 min with auranofin and Ca^{2+}. A23187 (2×10^{-1} μg/ml) was then added and the cells incubated for 20 min at 37°.

described [25]. These preparations, generally containing about 0.5% basophils, are referred to in this paper as "basophils". Other contaminating cells were lymphocytes, polymorphonuclear leukocytes, eosinophils, monocytes, and platelets.

Preparation of dispersed lung mast cells. These cells were prepared as described elsewhere [18, 26]. In brief, peripheral rims of grossly normal human lung tissue were obtained from thoracotomy specimens from patients undergoing surgery for lung cancer. Tissue was dissected free of tumor, pleura, large airways and blood vessels, minced and washed with Tyrode's buffer. Fragments were retained over Nytex cloth (Tetko, Elmsford, NY, 150 micron pore size), washed at 22° and dispersed in their cellular elements by incubation with the proteases pronase, chymopapain, collagenase and elastase. These preparations (1–10% mast cells) were purified further by countercurrent centrifugation elutriation followed by centrifugation over discontinuous Percoll gradients [27]. The mast cell preparations used in the present experiments contained >95% viable cells and consisted of approximately 20–65% mast cells. These preparations are referred to in this paper as "lung mast cells".

Preparation of lung macrophages. The techniques have been described in detail [28]. In brief, lung macrophages were purified from the cell pellet obtained from the dissection of human lung tissues used for the preparation of the lung mast cells. Fragments of normal human lung tissue were retained over Nytex cloth (150 micron pore size). The filtrate which contained cell suspensions enriched in macrophages was centifuged twice and sedimented (1 *g*, 60 min, +4°). These preparations were >90% viable as determined by erythosin B dye exclusion, and were 70% macrophages as assessed morphologically or by staining for nonspecific esterase. The macrophages were further purified over discontinuous Percoll gradients [28]. For this, macrophage preparations were resuspended in Percoll (sp. g. 1.130), over which Percoll of densities 1.077 and 1.043 were layered. A gradient was then made by centrifugation, 400 *g*, 10 min, 22°. The resulting preparations generally contained 90% macrophages and were >90% viable.

Histamine release assays. In experiments with mixed leukocytes (containing basophils) 0.4 ml of the cell suspension ($2–4 \times 10^4$ basophils/tube) were placed in 12 × 75 mm polyethylene tubes (Sarstadt Inc., Princeton, NJ) and warmed to 37°; 0.2 ml of each prewarmed stimulus for release was added, and incubation was continued at 37°. At the end of the incubation, the reaction was stopped by centrifugation (1000 *g*, 22°, 2 min), and the cell-free supernatants were stored for subsequent assay of histamine [29] and LTC_4 content. Total histamine content was assessed by lysis by brief incubation of cells and supernatant with $HClO_4$ (final concentration 2%) before centrifugation. All values are based on means of duplicate or triplicate determinations. Replicates differed from each other in histamine content by less than 10%.

The numbers of lung mast cells were chosen so that each tube contained 20–40 ng histamine, the most useful range for the automated fluorometric assay [29]. Thus, the tubes contained approximately $2–4 \times 10^4$ basophils or $1–2 \times 10^4$ mast cells. Cells were preincubated for 3 min with auranofin before addition of the stimuli because it is practically the optimal time of preincubation. For calculation of histamine release as a percentage of total cellular histamine, "spontaneous" release of histamine from leukocytes (basophils) or lung cells (mast cells), which ranged from 0–10% of the total cellular histamine, was subtracted from both numerator and denominator.

High performance liquid chromatography (HPLC) and radioimmunoassay of LTC_4. HPLC was performed using a Beckman model 420 liquid chromatograph. A Waters C_{18} column was used for the separation of arachidonic acid metabolites [18]. HPLC was performed using mixtures of water/acetonitrile/methanol/acetic acid 60/33.6/5.4/1%

adjusted to pH 5.6 with 10% NH_4OH. Flow rate was 1 ml/min. The detector was set to read at a wavelength of 280 nm, with a full scale absorbance setting of 0.005 units. All HPLC runs were done first with leukotriene standards for retention time standardization, the column runs without sample for clearing, followed by a sample run. In some experiments [^{3}H]LTC_4 and [^{3}H]LTB_4 were used for time standardization in HPLC runs; fractions were collected at 1-min intervals and concentrated to dryness with a solvent concentrator. In this case 4.5 ml of a toluene-based scintillation cocktail was added and radioactivity quantitated by scintillation spectrometry using a Beckman LS 9000.

When the samples were to be assayed by HPLC, the supernatants were extracted and concentrated. C_{18} Sep-Pak cartridges (Waters Associates, Milford, MA) were pre-wet with 2 ml of methanol (100%) and 5 ml of water. Supernatants (1 ml) were applied to the column and then the cartridges were washed with 5 ml of 20% methanol. Leukotrienes were eluted with 1 ml of 100% methanol. The methanol was evaporated to dryness under a nitrogen stream. The residue was dissolved in 0.1 ml of the mobile phase and finally loaded on a 20 μl sample loop for subsequent chromatography. Recoveries of leukotrienes using this technique ranged from 65 to 95%. Recovery was calculated by the use of PGB_2 (10 ng) as internal standard.

LTC_4 was measured by radioimmunoassay as described previously [20], using dextran-coated charcoal as the separation technique. The rabbit anti-LTC_4 antiserum has been characterized and its cross-reactivity for heterologous ligands described [30]. Auranofin did not interfere with the radioimmunoassay of LTC_4.

Statistical analysis. Comparison of IC_{50} values were performed after log transformation by means of paired or unpaired Student's *t*-test where appropriate; geometric means and ranges are reported.

RESULTS

Effect of auranofin on IgE- and non-IgE-mediated histamine release from human basophils

In a first series of experiments, we evaluated the effect of pharmacological concentrations of auranofin on histamine release from human basophils induced by a variety of immunological and non-immunological stimuli. The concentrations of auranofin ($1–5 \times 10^{-6}$ M) used correspond to the gold concentrations (0.6–1 μg/ml) achieved *in vivo* during auranofin treatment [31] and did not affect spontaneous LDH or histamine release (data not shown). Auranofin caused concentration-dependent inhibition of anti-IgE-induced histamine release in one series of experiments (Fig. 1). Both the Ca^{2+} ionophore A23187 and f-met-peptide cause histamine release from human basophils by mechanisms distinct from the IgE-mediated process [32, 33]. Auranofin was significantly more active against anti-IgE-induced release than f-met-peptide- or A23187-induced histamine secretion (Fig. 1). The IC_{50} averaged 1.6×10^{-6} M (range 10^{-6} to 2.4×10^{-6} M) (anti-IgE), 5.3×10^{-6} M (range 1.8×10^{-6} to 1.1×10^{-5} M) (f-met-peptide; $P < 0.001$) and 1.9×10^{-5} M (range 4.3×10^{-6} to 5×10^{-4}) (A23187; $P < 0.001$).

Effect of extracellular Ca^{2+} on the inhibition by auranofin of histamine release from basophils

We have previously shown that another metal, zinc, is a potent inhibitor of IgE-mediated release of histamine from basophils, competing with Ca^{2+} for the same binding site (i.e. Ca^{2+} channel) activated by these stimuli [32]. We therefore investigated the interaction between Ca^{2+} and auranofin in two series of experiments.

We first investigated whether auranofin modulated histamine release stimulated in the absence of extracellular Ca^{2+}, using the tumor promoter TPA, which stimulates histamine release from human basophils without the addition of extracellular Ca^{2+} [34]. We also used bryostatin 1, a macrocylic lactone isolated from a marine invertebrate (*Bugula neretina*) [24]. Bryostatin 1 and TPA activate different isoforms of protein kinase C leading to the activation of different enzymatic pathways [35]. We tested the effect of auranofin on TPA- ($N = 10$) and bryostatin 1-induced ($N = 7$) histamine release both in the presence and absence of extracellular Ca^{2+}. Figure 2 shows that auranofin (10^{-6}–2×10^{-5} M) caused concentration-dependent inhibition of histamine release from human basophils induced by both TPA and bryostatin 1 regardless of the presence of extracellular Ca^{2+}. The IC_{50} averaged 3.5×10^{-6} M (range 1.8×10^{-6} to 7×10^{-6} M) (TPA, $+Ca^{2+}$), 4.6×10^{-6} M (range 1.9×10^{-6} to 7×10^{-6} M) (TPA, $-Ca^{2+}$), 4.0×10^{-6} M (range 1.5×10^{-6} to 7.0×10^{-6} M) (bryostatin 1, $+Ca^{2+}$), 4.9×10^{-6} M (range 1.9×10^{-6} to 6.6×10^{-6} M) (bryostatin 1, $-Ca^{2+}$).

In a second series of four experiments, we investigated the auranofin-induced inhibition of both anti-IgE- and A23187-stimulated histamine release at various concentrations of extracellular Ca^{2+} (from 0.1 to 6 mM). Figure 3 shows a typical experiment in which raising the Ca^{2+} concentrations in the extracellular medium did not reduce the inhibitory effect of auranofin on anti-IgE- or A23187-induced histamine secretion.

Effect of auranofin on IgE-mediated release of LTC_4 from basophils

In a group of 11 experiments, the effect of auranofin on the release of histamine and LTC_4 from human peripheral blood basophils was examined. *In vitro* studies indicate that basophils challenged with anti-IgE are the major, perhaps the only, source of LTC_4 in human peripheral blood leukocytes [18]. Table 1 shows that auranofin inhibited the anti-IgE-induced release of histamine and LTC_4 in a concentration-related fashion. The IC_{50} for the release of histamine was between 9×10^{-7} and 2.4×10^{-6} M, and the IC_{50} for the release of LTC_4 was between 1.5×10^{-7} and 10^{-6} M. It is clear from these results that auranofin inhibits the LTC_4 release more effectively than it does histamine secretion induced by anti-IgE. The IC_{50} averaged 4.6×10^{-7} M (LTC_4) and 1.6×10^{-6} M (histamine) ($P < 0.001$).

The radioimmunoassay we employed to detect

Table 1. Effect of auranofin on IgE-mediated histamine and leukotriene C_4 (LTC_4) release from human basophils*

	Experiment 1		Experiment 2		Experiment 3	
Auranofin (M)	LTC_4	Histamine	LTC_4	Histamine	LTC_4	Histamine
No drug	68	48	64	52	38	51
2.5×10^{-7}	ND	ND	47	49	32	52
5×10^{-7}	52	42	35	42	28	42
1×10^{-6}	34	36	9	25	21	33
2.5×10^{-6}	2	10	3	4	0	6
5×10^{-6}	0	2	0	2	0	0

* Cells were preincubated with auranofin for 3 min before the addition of anti-IgE (2×10^{-1} μg/ml).

Values are expressed as ng of immunoreactive $LTC_4/10^6$ mast cells and percentage histamine release. Each value is the mean of duplicate determinations.

ND, not done.

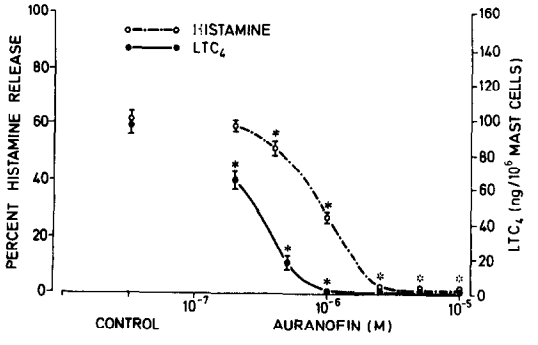

Fig. 4. Effect of various concentrations of auranofin on histamine and LTC_4 release induced by anti-IgE from mast cells purified (64%) from human lung tissues. The cells were preincubated for 3 min with auranofin; anti-IgE (3 μg/ml) was then added and cells were incubated for 35 min at 37°. Each symbol represents the mean ± SE of triplicate determinations. *$P < 0.001$ compared with control.

LTC_4 detects LTD_4 and LTE_4 less well [30]. Therefore, it is possible that the apparent inhibition of LTC_4 release was due to an increased rate of conversion of LTC_4 to the less cross-reactive metabolites, LTD_4 and LTE_4. To rule out this possibility, supernatants from challenged controls and auranofin (5×10^{-6} M)-treated cells were extracted and analysed on HPLC. Fractions corresponding to the known retention times of LTC_4, LTD_4, and LTE_4 were evaluated. Auranofin did not enhance the conversion of LTC_4 to LTD_4 and to LTE_4 (data not shown). Therefore, it is unlikely that the inhibitory effect of auranofin was due to increased catabolism of LTC_4.

Effect of auranofin on anti-IgE-induced histamine and LTC_4 release from human lung mast cells

In a series of experiments we evaluated the effect of auranofin on anti-IgE-induced mediator release from mast cells purified from lung tissues. Previous studies have shown that mast cells are the major, perhaps the only, source of LTC_4 in human lung cells challenged with anti-IgE [17]. Figure 4 shows the results of a typical experiment representative of a series of 10 experiments. Auranofin caused concentration-dependent inhibition of anti-IgE-induced histamine and LTC_4 release from human lung mast cells. Again, it is clear from these results that auranofin inhibits LTC_4 release more effectively than it does histamine secretion induced by anti-IgE. In this series of experiments the IC_{50} for histamine release was 1.6×10^{-6} M (range 7.5×10^{-7} to 3×10^{-6} M) and the IC_{50} for LTC_4 release was 3.6×10^{-7} M (range 2×10^{-7} to 10^{-6} M) ($P < 0.001$).

Effect of auranofin on A23187-induced LTB_4 release from human lung macrophages

In a final series of experiments the effect of auranofin on LTB_4 release from human macrophages purified from lung tissue was examined. In this study we found that highly purified (>95%) human lung macrophages synthesized LTB_4 when challenged with the Ca^{2+} ionophore A23187. LTB_4 was identified on chromatograms of A23187-stimulated supernatants of human lung macrophages. The peak comigrated with LTB_4 on HPLC and showed the characteristic spectrum with peaks at 260, 270, and 280 nm [36]. Auranofin concentration-dependently inhibited A23187-induced release of LTB_4 from human lung macrophages (Fig. 5). In the experiment shown cells were preincubated with three concentrations of auranofin (3×10^{-6}M, 7×10^{-6} M and 10^{-5} M) before challenge with A23187. At the end of incubation, supernatants were extracted for HPLC analysis. The results of a typical experiment representative of four experiments indicated that auranofin induced concentration-dependent inhibition of A23187-induced LTB_4 release from purified human lung macrophages.

DISCUSSION

The results of the present study indicate that auranofin, an oral gold-containing medication, is a potent inhibitor of the release of preformed (histamine) and *de novo* synthesized chemical mediators (LTC_4 and LTB_4) from human basophils and lung mast cells and macrophages. The inhibitory effect *in*

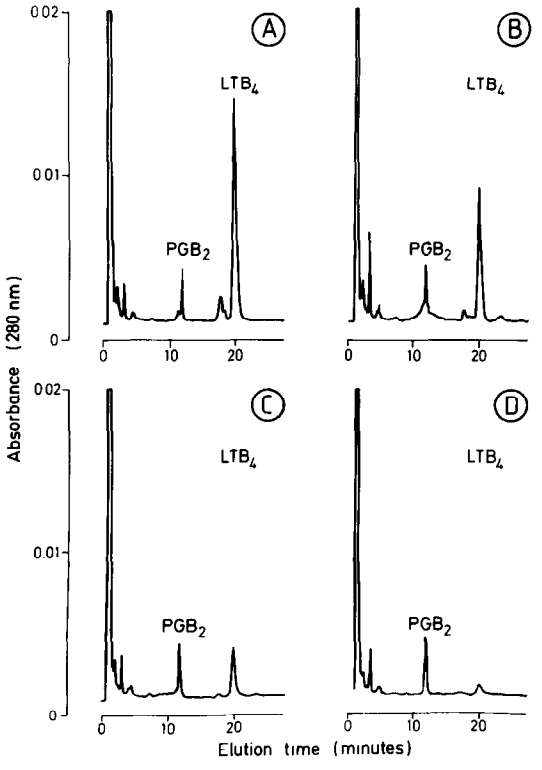

Fig. 5. Reverse phase HPLC of A23187-stimulated supernatants of purified human lung macrophages. Cells (10^6) were preincubated for 3 min with buffer (A) or with auranofin (3×10^{-6} M, B; 7×10^{-6}, C; 10^{-5} M, D) before the addition of A23187 (1 μg/ml). At the end of incubation (20 min) supernatants were extracted using a Sep-Pak cartridge and concentrated under a nitrogen stream. Samples (20 μl) were applied to a C-18, 5 μm reverse phase octadecylsilyl HPLC column and leukotrienes were detected by their UV absorbance at 280 nm. The migration of authentic LTB_4 and PGB_2 standards is indicated at their retention times.

vitro becomes detectable at auranofin concentrations corresponding to the gold concentrations achieved *in vivo* during auranofin treatment.

Because auranofin inhibits histamine release induced by several immunological and non-immunological stimuli that presumably increase intracellular Ca^{2+} by different means, it is suggested that the inhibitory effect observed in our study was not exerted through the Ca^{2+} channel. This hypothesis is supported by the observation that the inhibitory effect of auranofin on both TPA- and bryostatin 1-induced release was independent of the presence of extracellular Ca^{2+}. These results provide evidence that the inhibitory effect of auranofin occurs regardless of inhibition of Ca^{2+} uptake.

These findings are part of a broader project on the possible role of endogenous and exogenous metals in the modulation of inflammatory reactions. We have previously shown that zinc, in physiological concentrations, inhibits IgE-mediated histamine release from human basophils [32] and lung mast cells [37] and acts as a natural Ca^{2+} antagonist with a dissociation constant (K_d) of the zinc–Ca^{2+} receptor on human basophils of 10^{-5} M [32].

Auranofin was a potent inhibitor of release of both preformed and *de novo* synthesized chemical mediators from human basophils and mast cells. In both systems auranofin had greater inhibitory effect on LTC_4 release than on histamine secretion. Although the reasons for this phenomenon are still unclear, the fact that it occurred in both basophils and mast cells suggests that a metabolic step common to these cells has more effect in the modulation of LTC_4 release than in the secretion of histamine. It is of interest that other compounds that presumably do not have the same mechanism of action as auranofin, such as forskolin and isoproterenol, have a greater inhibitory effect on IgE-mediated release of LTC_4 than on histamine secretion [18, 38].

A number of early reports suggested that chrysotherapy had beneficial effects in asthma [3–5]. Furthermore, an oral form of gold, no longer available, led to clinical improvement in a majority of asthmatic patients [39, 40]. However, the use of gold compounds in this disease was apparently pursued only in Japan. In a double-blind study Muranaka *et al.* [6] showed that gold compounds relieved the symptoms of asthma and reduced the need for bronchodilators and corticosteroids. The same investigators showed that gold compounds reduced bronchial responsiveness to acetylcholine in asthmatic patients [7] and inhibited guinea-pig tracheal contractions induced by histamine [41]. More recently, it was found that auranofin also inhibited the *in vitro* response of the guinea-pig trachea to leukotriene D_4 and, more important, to specific antigen [42]. In two recent studies, the corticosteroid-sparing effect of chrysotherapy in the treatment of asthma has been confirmed [9, 10].

The mechanism(2) of action of gold in asthma is obscure. Gold compounds have no consistent effect on IgE synthesis [7]. Auranofin inhibits rat PCA and anti-IgE-induced release of histamine from rat lung [43] and human basophils [8]. We now show that auranofin is a potent inhibitor of IgE- and non-IgE-mediated secretion of chemical mediators from human basophils and leukotrienes from lung mast cells and macrophages. The IgE- and non-IgE-mediated release of histamine and leukotrienes from human basophils and pulmonary mast cells and macrophages plays a central role in the pathophysiology of human inflammatory disorders [11, 44]. Therefore, our results *in vitro* in models relevant to human allergic and inflammatory diseases [44, 45] might have a bearing on the pharmacological control of these conditions.

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