3. Histamine and Immunology

Are the Anti-Allergic Actions of Theophylline due to Antagonism at the Adenosine Receptor

by Bertil B. Fredholm and Anita Sydbom
Department of Pharmacology, Karolinska Institutet, S-104 01 Stockholm, Sweden

Abstract
Adenosine potentiated anaphylactic histamine release from isolated rat mast cells in a dose-dependent manner between $10^{-4}$ and $10^{-2} M$. Adenosine was found to be present during a normal incubation of mast cells, but the concentration was low ($2 \times 10^{-8} M$). In rat plasma the concentration was $1.5 \times 10^{-7} M$. The effect of $10^{-4} M$ adenosine was dose-dependently inhibited by theophylline. 50% inhibition was found at $3 \times 10^{-5} M$ theophylline. Cyclic nucleotide phosphodiesterase inhibition required much higher concentrations ($IC_{50} \sim 10^{-3} M$). It is suggested that some of the anti-allergic actions of theophylline (clinical concentration range: $10^{-5}$-10$^{-4}$ M) does not involve cyclic nucleotides but may be due to inhibition of the effects of endogenous adenosine.

Introduction
There is good evidence that several cell types are endowed with cell membrane receptor for adenosine (see [1-3]). In isolated fat cells for example adenosine acting on these receptors inhibits cyclic AMP accumulation and lipolysis [3]. In blood platelets, on the other hand, adenosine activates adenylyl cyclase causing increased cyclic AMP levels and inhibition of platelet aggregation [1, 2]. In these and several other instances the effects are exerted already at submicromolar concentrations of the purine nucleoside.

Theophylline is known to exert a number of biological effects at plasma concentrations well below $10^{-4} M$ [4]. In this concentration range inhibition of adenosine effects is a prominent feature [2, 3] while inhibition of cyclic nucleotide phosphodiesterase [e.g. 5-8] is minimal. The possibility therefore exists that theophylline is clinically active, at least partly, by virtue of actions on adenosine receptors [1-3, 7, 8].

In the present communication we will present some data from isolated rat mast cells relevant to this hypothesis, and discuss these as well as data obtained from other cells and tissues involved in allergic manifestations.

Materials and methods
In these studies a high-IgE-producing strain of Hooded-Lister rats (supplied by Dr T. Karlsson, Biomed. Centre, Uppsala, Sweden) were used. The characteristics of these rats as well as the preparation of mast cells from them has been described elsewhere [9, 10]. The rats were immunized by injecting 2 x 0.5 ml pertussis vaccine (lot. nr. 120 274 from Statens Seruminstitut, Copenhagen, Denmark), containing 20 μg/ml egg albumin (Difco). Incubations were performed in phosphate buffered saline, pH 6.7, containing 0.5 mg/ml human serum albumin (Kabi). Histamine in supernatants and lysed cell residues was determined fluorometrically [11].

In some experiments the mast cell adenine nucleotide stores were labelled by preincubating for 30 min with 2-10 μCi/ml [3H]-adenine (27 Ci/mmol, New England Nuclear). Adenosine and inosine was measured either in rat plasma or in the medium obtained after incubation of mast cells by high performance liquid chromatography (Waters model 600 solvent delivery, μ-Bondapak C18-column, Model 540 absorbance monitor) using 50 mM (NH4)H2PO4 pH 5.5 containing 15% methanol as the mobile phase.

Cyclic AMP and cyclic GMP was measured in mast cells [10], following deproteinization with 10% trichloroacetic acid, its removal by four times repeated acid ethyl ether extraction, by radioimmunoassay using reagents from New England Nuclear. The samples and standards were acetylated as described by Harper and Brooker [12] to increase the assay sensitivity.

Results
As shown in Figure 1 adenosine caused a dose-dependent potentiation of histamine release induced by antigen. On the other hand, spontaneous histamine release was not significantly potentiated. The potentiation amounted to 10% already at $10^{-7} M$ adenosine and was maximal (20-25% increase) at $10^{-5}-10^{-6} M$. Inosine, the deaminated metabolite of adenosine [1, 2], was somewhat less effective, but caused a 13% increase in antigen-induced histamine release at $10^{-5} M$ concentration. Also shown in this figure are the results of determinations of adenosine and inosine in incubates of mast cells and in plasma from immunized Hooded-Lister rats. It is seen that the plasma level of adenosine was approximately 0.15 μM, and that of inosine somewhat lower. In the mast cell incubates the adenosine level was some ten times lower than in plasma. Studies on [3H]-adenine prelabelled mast cells showed that the release process is associated with an increased release of [3H]-adenosine and related compounds by the mast cells.

Adenosine ($10^{-5} M$) caused a small, but significant ($p < 0.05$), increase in mast cell cyclic AMP content – from
Histamine and Immunology

Potentiation of anaphylactic histamine from isolated rat mast cells by adenosine (upper panel). The results are expressed as percentage increase over the corresponding controls (average release 27 ± 2%). Mean ± S.E.M. of 3–6 determinations. ▲ — adenosine; ▼ — inosine. In the lower panel is shown that the spontaneous histamine release is not significantly increased by adenosine. Below the abscissa are shown the results of determinations (n = 6) of adenosine (▲) and inosine (▼) in mast cell incubates (inc.) and in rat plasma. The vertical lines represent S.D. Note that the concentrations in plasma are sufficient to cause a highly significant potentiation of mast cell histamine release.

The effects of theophylline on isolated rat mast cells are shown in Figure 2. It may be seen that the adenosine potentiation is markedly inhibited at concentrations at which the mast cell cyclic nucleotide phosphodiesterase is unaffected. It can also be seen that at therapeutic concentrations theophylline only marginally (about 6%) inhibits anaphylactic histamine release. This inhibition may have been due to antagonism of the effect of adenosine present during incubation of mast cells. Intrapolation of the data shown in Figure 1 indicates that endogenous adenosine would increase histamine release by 5–8%. Since theophylline is a competitive antagonist of adenosine effects [1–3] the action of endogenous adenosine would be essentially completely antagonized.

Discussion

It has been reported that adenosine potentiates histamine release induced by anti-IgE, Con A, compound 48/80 and the ionophore A23187 [13]. The present results show that such potentiation is also demonstrable with antigen-induced histamine release. The effect of adenosine is not inhibited by the adenosine uptake blocker, dipyridamole [13], suggesting that the effect is due to activation of some "receptor" at the mast cell surface as has been shown in several other cell types (see [1–3]). The effect occurs at the concentration of adenosine shown here to be present in rat plasma (1–2 × 10^-7 M). In man and dog the basal levels are even higher (2–4 × 10^-7 M) [14, 15]. It has been shown that plasma levels reflect the free tissue concentrations under basal conditions [15]. Thus, even under these circumstances adenosine is present in sufficient concentrations to cause a significant increase in histamine release. Various experimental interventions (see [1–3]) increase the levels of adenosine further. It seems reasonable to assume that potentiation of histamine release by adenosine occurs physiologically.

Figure 1
Potentiation of anaphylactic histamine from isolated rat mast cells by adenosine (upper panel). The results are expressed as percentage increase over the corresponding controls (average release 27 ± 2%). Mean ± S.E.M. of 3–6 determinations. ▲ — adenosine; ▼ — inosine. In the lower panel is shown that the spontaneous histamine release is not significantly increased by adenosine. Below the abscissa are shown the results of determinations (n = 6) of adenosine (▲) and inosine (▼) in mast cell incubates (inc.) and in rat plasma. The vertical lines represent S.D. Note that the concentrations in plasma are sufficient to cause a highly significant potentiation of mast cell histamine release.

0.61 ± 0.07 (n = 18) to 0.86 ± 0.14 (n = 9) nmol/10^6 cells.

The cyclic GMP content was also somewhat increased from 0.34 ± 0.02 (n = 19) to 0.42 ± 0.05 (n = 9) (p < 0.05). However, the ratio cyclic AMP/cyclic GMP was unaffected (1.97 ± 0.26 in controls vs. 2.00 ± 0.22 in adenosine-treated mast cells).

In Figure 2, it may be seen that the adenosine potentiation is markedly inhibited at concentrations at which the mast cell cyclic nucleotide phosphodiesterase is unaffected. It can also be seen that at therapeutic concentrations theophylline only marginally (about 6%) inhibits anaphylactic histamine release. This inhibition may have been due to antagonism of the effect of adenosine present during incubation of mast cells. Intrapolation of the data shown in Figure 1 indicates that endogenous adenosine would increase histamine release by 5–8%. Since theophylline is a competitive antagonist of adenosine effects [1–3] the action of endogenous adenosine would be essentially completely antagonized.

Discussion

It has been reported that adenosine potentiates histamine release induced by anti-IgE, Con A, compound 48/80 and the ionophore A23187 [13]. The present results show that such potentiation is also demonstrable with antigen-induced histamine release. The effect of adenosine is not inhibited by the adenosine uptake blocker, dipyridamole [13], suggesting that the effect is due to activation of some "receptor" at the mast cell surface as has been shown in several other cell types (see [1–3]). The effect occurs at the concentration of adenosine shown here to be present in rat plasma (1–2 × 10^-7 M). In man and dog the basal levels are even higher (2–4 × 10^-7 M) [14, 15]. It has been shown that plasma levels reflect the free tissue concentrations under basal conditions [15]. Thus, even under these circumstances adenosine is present in sufficient concentrations to cause a significant increase in histamine release. Various experimental interventions (see [1–3]) increase the levels of adenosine further. It seems reasonable to assume that potentiation of histamine release by adenosine occurs physiologically.