
ORIGINALS

INFLUENCE OF AMINOGLYCOSIDE ANTIBIOTICS, STREPTOMYCIN AND KANAMYCIN ON HISTAMINE SECRETION IN MAST CELLS

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Abstract Effects of kanamycin and streptomycin on histamine release from rat mast cells were examined in response of the cells to concanavalin A (Con A) plus phosphatidylserine (PS), phytohemagglutinin (PHA) plus PS or a mixture of low-molecular-weight polymers of P-methoxy-N-methylphenethylamine (compound 48/80). In the response to each of the above stimuli, kanamycin (20 mM) or streptomycin (20 mM) caused a decrease in the histamine release elicited in the presence of extracellular Ca²⁺ (1 mM), although streptomycin showed the much higher inhibitory potency than kanamycin. Similarly, streptomycin was much more effective in suppressing compound 48/80-triggered histamine release in the absence of external Ca²⁺. Histamine release in the absence of external Ca²⁺ in the response to the lectin plus PS diminished with increasing concentration of kanamycin, and in this respect streptomycin was much less effective. In the response to the lectin plus PS, external Ca²⁺ possessed potency to antagonize kanamycin (10 mM)- or streptomycin (10 mM)-caused inhibition of the histamine release, although more markedly the kanamycin-caused one. Streptomycin and kanamycin inhibit histamine release from mast cells challenged with IgE-directed secretagogue or compound 48/80, and the responsible mechanisms seem to implicate the Ca²⁺-antagonistic action on the stimulus-provoked influx of Ca²⁺ and impairment of the cellular events linked to exocytosis.

Key words: Aminoglycoside, histamine release, mast cells, rat.

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INTRODUCTION

Aminoglycoside antibiotics in common possess a variety of pharmacological actions including blockade of neuromuscular and autonomic ganglionic transmission (Sokoll and Gergis, 1981; Leaders et al., 1960; Wright and Collier, 1977), depression of cardiac contraction (Cohen et al., 1970; Adams and Goodman, 1975; Adams, 1975) and relaxation of smooth muscles (Adams and Goodman, 1975; Adams et al., 1974; Corrado et al., 1975). As the mechanism responsible for the above events has been proposed to be ascribable to Ca^{2+}-antagonistic action of the aminoglycosides at cell surface. In the blockade of synaptic transmission, a presynaptic effect of the antibiotics to reduce Ca^{2+}-dependent acetylcholine release is mentioned as an important causal factor, although reduction of postsynaptic sensitivity to the neurotransmitter has been also reported (Sokoll and Gergis, 1981; Sanders Jr. and Sanders, 1979). Histamine secretion by exocytosis in mast cells provides a convenient experimental system to analyze Ca^{2+}-dependent stimulus-secretion coupling (Cochrane and Douglas, 1974). The secretory response is inhibited by Mg^{2+} (Wenzel et al., 1985; Foreman et al., 1973), La^{3+} (Wenzel et al., 1985; Foreman and Mongar, 1973) or verapamil (Lunardi and Vugman, 1982), which are potent Ca^{2+}-antagonists in cardiac and smooth muscles (Sangborn and Langer, 1970; Haehusler, 1972; Fleckenstein, 1971). It has been reported that neomycin, gentamicin and streptomycin inhibit mast cell histamine release induced by Ca^{2+} and ionophore A23187 in a manner that is competitive with Ca^{2+} (Wenzel et al., 1985). However, the secretory response mediated by the ionophore is discriminated from histamine secretion primed by IgE-directed secretagogues such as antigen and concanavalin A or by P-methoxy-N-methylphenethylamine (compound 48 / 80) in that a series of cellular events at the surface membrane, linked to induction of Ca^{2+}-influx, are basically circumvented (Cochrane and Douglas, 1974; Foreman et al., 1973). In contrast, extensive interactions have been described between aminoglycoside antibiotics and membrane phospholipids (Sanders Jr. and Sanders, 1979; Lodhi et al., 1980; Lullman and Vollmer, 1982; Schacht, 1976; Lodhi et al., 1976). The present experiments describe actions of kanamycin and streptomycin on histamine release from rat mast cells challenged with lectin (concanavalin A, phytohemagglutinin) or compound 48 / 80.

MATERIALS AND METHODS

Mast Cells: The peritoneal mast cells were purified from male Wistar rats by bovine serum albumin (BSA) density gradient centrifugation according to the method of Sullivan et al. (1975) with minor modifications, to 90-95% homogeneity. The medium used for suspending cells contained 10 mM 4-(2-hydroxyethyl)-piperazineethanesulphonic acid, 140 mM NaCl, 2.7 mM KCl, 1 mM CaCl_{2} (unless otherwise specified), 0.1% glucose and 0.1% BSA, pH 7.0 (herein referred to as HBS).

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Assay of Histamine Release: The mast cells suspended in HBS in a polycarbonate tube (5 × 10⁴ cells) were preincubated for 5 min at 30°C. After addition of a small volume of secretagogue to give the final volume of 1 ml, the incubation was continued for another 15 min, and terminated by placing the tube on ice. The cells were sedimented by centrifugation (300 × g, 5 min), and aliquot of the supernatant was assayed for histamine by the fluorometric method of Shore et al. (1959) with minor modifications. The histamine liberated was expressed as per cent of total cell histamine and corrected for spontaneous (non-stimulated) release. The total cell histamine was released by heating a separate cell sample at 100°C for 5 to 10 min. In experiments where effect of antibiotic on histamine release was examined, the cells were exposed to the antibiotic during the entire incubation time of 20 min.

Chemicals: Concanavalin A (Con A; Sigma Chemical Co.), phytohemagglutinin M (PHA; Difco Laboratories), P-methoxy-N-methylphenethylamine (compound 48/80; Sigma Chemical Co.), phosphatidylserine (PS; Sigma Chemical Co.), streptomycin (Boehringer Mannheim), kanamycin (P-L Biochemicals, Inc.). Other reagents were of analytical grade.

RESULTS

The dependence of histamine release from mast cells on extracellular Ca²⁺ varies with the secretagogue used. Fig. 1. shows influence of external Ca²⁺-concentration ([Ca²⁺]₀) on histamine release from the cells stimulated by Con A, PHA or compound 48/80. Con A and PHA did not elicit notable release of histamine in the presence of 1 mM Ca²⁺ without PS which is a potentiator of antigen-induced histamine secretion (Goth et al., 1971) (data not shown). When added with PS, these lectins induced histamine release to some extent even in the absence of external Ca²⁺. The histamine release was observed to be highly dependent on [Ca²⁺]₀, whereas compound 48/80-triggered histamine release showed the relatively low dependence on [Ca²⁺]₀ in consistent with the observations elsewhere (Cochrane and Douglas, 1974; Douglas and Ueda, 1973).

Kanamycin or streptomycin itself caused no valid histamine release even by raising its concentration up to 20 mM (data not shown). Fig. 2. describes effects of these antibiotics on histamine release elicited in the presence of 1 mM Ca²⁺ in the response to each of the above stimuli. Streptomycin concentration-dependently depressed histamine release in the response to Con A plus PS or PHA plus PS, markedly at the concentrations raised over 1 mM, and in this respect kanamycin possessed the less depressing potency. In the response to compound 48/80, streptomycin in concentration-range from 0.1 to 1 mM diminished histamine release to a small extent in a concentration-independent manner, and then produced a progressive decrease in the release at the concentrations over 1 mM. In contrast, kanamycin did not significantly influence the release except the somewhat variable depressing effect at the concentrations over 10 mM.
Fig. 3 presents effects of kanamycin and streptomycin on histamine release elicited in the absence of external Ca$^{2+}$ in the same responses. Streptomycin did not suppress histamine release in the response to Con A plus PS or PHA plus PS until the concentration was raised over 10 mM, whereas the histamine release gradually diminished with increasing concentration of kanamycin. Concentration dependent-effect of each antibiotic on compound 48/80-triggered histamine release under this condition was similar to that observed in the presence of external Ca$^{2+}$, but the removal of Ca$^{2+}$ appeared to result in an extenuated suppressive effect of streptomycin on the histamine release.

Fig. 1. Dependence of histamine secretion from mast cells on extracellular Ca$^{2+}$. Fresh mast cells were preincubated in HBS including varied concentrations of CaCl$_2$ for 5 min. After addition of secretagogue, the incubation was continued a further 15 min. Concentration of secretagogue: Con A, 100 μg/ml; PHA, 80 μg/ml; PS, 50 μg/ml; compound 48/80, 0.8 μg/ml. Values are the means ± S. E. (n = 2 - 4).
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Fig. 2. Concentration-dependent effect of antibiotic on histamine secretion induced in the presence of extracellular Ca\(^{2+}\). The mast cells were preincubated in HBS including various concentrations of kanamycin or streptomycin for 5 min, and incubated for another 15 min in the coexistence of secretagogue (concentrations indicated in Fig. 1-legend). Each point represents the mean ± S. E. from three to six measurements (duplicate determinations) performed with three to six different preparations.
Fig. 3. Concentration-dependent effect of antibiotic on histamine secretion induced in the absence of exogenous Ca^{2+}. The experimental conditions were the same as shown in Fig. 2, except that the incubation was carried out in the CaCl_{2}-free HBS. Each point represents the mean of duplicate determination, performed with an identical mast cell preparation.
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Fig. 4. shows influence of \([\text{Ca}^{2+}]_o\) on kanamycin- or streptomycin-caused decrease in histamine release triggered by Con A plus PS or PHA plus PS. In either secretory response, a maximal stimulative effect of \(\text{Ca}^{2+}\) on the histamine release was found at the concentrations around 1 mM (Fig. 4, control). In the response to each of these stimuli, inhibitory effect of 10 mM kanamycin on the histamine release was notably reduced by raising \([\text{Ca}^{2+}]_o\), and also the rise in \([\text{Ca}^{2+}]_o\) resulted in a decrease in the inhibitory effect of 10 mM streptomycin.

DISCUSSION

Secretagogues directed to IgE molecules on mast cell surface, including Con A, transiently increase \(\text{Ca}^{2+}\)-permeability of the surface membrane and lead to influx of \(\text{Ca}^{2+}\) required for trigger of histamine secretion (Foreman et al., 1977), and compound 48/80 also induces the transitory increase in \(\text{Ca}^{2+}\)-permeability (Cochrane et al., 1982). These agents also cause enhancement in metabolism of various phospholipids that has been proposed to be associated with the induction of increase in \(\text{Ca}^{2+}\)-permeability (Ishizaka et al., 1980; Hirata et al., 1979; Kennerly et al., 1979; Cockcroft and Gomperts, 1979). On the other hand, aminoglycosides possess extensive activities to interfere with binding of \(\text{Ca}^{2+}\) to various membranes such as artificial phospholipid monolayers, red cell ghost, isolated cardiac sarcolemma and cerebral tissue homogenates, and with phospholipid metabolism in renal and ear tissues (Sanders Jr. and Sanders, 1979; Lodhi et al., 1980; Lullman and Vollmer, 1982; Schacht, 1976; Lodhi et al., 1976). Therefore, a possibility can be raised that aminoglycosides influence the induction of change in \(\text{Ca}^{2+}\)-permeability or the resulting influx of \(\text{Ca}^{2+}\) via interaction with the cell membrane phospholipids.

Several findings in this study are in support of interference of kanamycin or streptomycin with \(\text{Ca}^{2+}\)-influx provoked by Con A plus PS, PHA plus PS or compound 48/80, e. g. : in response to the lectin plus PS, suppressive effect of streptomycin in concentrations up to 10 mM on the histamine release is entirely dependent on the presence of extracellular \(\text{Ca}^{2+}\) (Figs. 2 and 3); in response to 48/80, the absence of external \(\text{Ca}^{2+}\) seems to discount streptomycin-produced decrease in the histamine release (Figs. 2 and 3); in response to the lectin plus PS, external \(\text{Ca}^{2+}\) markedly reverses kanamycin (10 mM)-caused inhibitory effect on the histamine release, and also shows a potency to antagonize the streptomycin (10 mM)-caused release one (Fig. 4). It seems to be possible that strongly cationic aminoglycosides penetrate through cell membrane ineffectively and thereby would interact with the membrane in a biphasic manner, i. e., reversible binding to negatively charged phosphate groups of membrane phospholipids, at lower concentrations, and irreversible binding to cell membrane imposing conformational change of membrane at higher concentrations (Sanders Jr. and Sanders, 1979), as observed in interaction of neomycin with nerve ending membranes or with synthetic lipid monolayer (Lodhi et al., 1976). The poor antagonistic action of \(\text{Ca}^{2+}\) on streptomycin-caused inhibitory
Fig. 4. Effect of extracellular Ca\(^{2+}\) on antibiotic-produced inhibition of histamine release. The mast cells were preincubated with antibiotic (10 mM) in the CaCl\(_2\)-free HBS for 5 min. Subsequently, CaCl\(_2\) in various concentrations and secretagogue were added, and the incubation was continued a further 15 min. The concentrations of secretagogue were the same as those given in Fig. 1 (legend). Each point represents the mean of duplicate determination, performed with an identical mast cell preparation.
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effect on histamine release induced by the lectin plus PS, in comparison with the case of kanamycin, (Fig. 4) may implicate such irreversible and noncompetitive interaction with mast cell surface. Kanamycin and streptomycin also show activities to influence histamine secretion that is independent of external Ca$^{2+}$. Kanamycin is active in suppressing histamine release without Ca$^{2+}$ in the response to the lectin plus PS (Fig. 3). This effect may be related to a decrease in the availability of exogenous PS essential to the histamine release, since kanamycin is more effective in replacing Ca$^{2+}$ from PS monolayer than streptomycin (Lullman and Vollmer, 1982), suggesting that, when Ca$^{2+}$ is absent, PS prefers to interact with kanamycin rather than streptomycin. In contrast, streptomycin exerts marked suppressive effect on compound 48/80-triggered histamine release without external Ca$^{2+}$, at the concentrations over 1 mM (Fig. 3). Since compound 48/80, which induces marked histamine release in the absence of external Ca$^{2+}$, enhances phosphatidylinositol turnover in mast cells comparably to IgE-directed secretagogues, a possible involvement of the phospholipid turnover in endogenous release of Ca$^{2+}$ ions to trigger exocytosis has been pointed out (Cockcroft and Gomperts, 1979). Impediment in the phospholipid turnover may tentatively account for such effect of streptomycin, and in that case may be simultaneously connected with inhibition of the increase in Ca$^{2+}$-permeability.

In conclusion, kanamycin and streptomycin inhibit histamine release from mast cells activated by IgE-directed secretagogue and compound 48/80 via the mechanisms including the Ca$^{2+}$-antagonistic action on the stimulus-induced influx of Ca$^{2+}$, and also possess potency to interfere with the cellular events involved in histamine secretion. In addition, detailed studies on interaction between aminoglycosides and membrane phospholipids of mast cells may be useful for elucidation of Ca$^{2+}$-movements accompanied by the stimulus-secretion coupling.

REFERENCES

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