EFFECTS OF ANTI-ASTHMATIC DRUGS ON AIRWAY RESISTANCE AND PLASMA LEVEL OF CYCLIC AMP IN GUINEA PIG

Yumiko IWAYAMA, Chang Zer CHUNG and Issei TAKAYANAGI*

Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, Miyama Funabashi, Chiba 274, Japan

Accepted December 21, 1981

Abstract—Effects of anti-asthmatic drugs on airway resistance and plasma level of cyclic AMP were investigated in guinea pigs sensitized and non-sensitized with egg-albumin. Histamine increased airway resistance in the both groups of guinea pigs, and guinea pigs sensitized with egg-albumin were more sensitive to histamine. Anti-asthmatic drugs inhibited dose-dependently the increase of airway resistance caused by histamine. Sensitization with egg-albumin decreased the potencies of the β-adrenoceptor stimulants, salbutamol and isoprenaline, but not that of aminophylline. The plasma level of cyclic AMP was increased by salbutamol and isoprenaline, but not by aminophylline. The increased plasma level of cyclic AMP by β-adrenoceptor stimulants was not attenuated by sensitization with egg-albumin.

Beta-adrenoceptor stimulants and aminophylline are widely used as antiasthmatic drugs, which are thought to act via an increase of cyclic AMP level. It has been reported that isolated airway smooth muscle was relaxed by β-adrenoceptor stimulants and aminophylline and that the effect of these drugs is associated with the increase of intracellular cyclic AMP (1, 2).

The partial β-adrenoceptor blockade in asthma proposed by Szentivanyi (3) has been sustained by many investigators (4–6) who observed that the elevations of cyclic AMP levels in response to the β-stimulants in asthmatic patients or sensitized animals were significantly less than in controls. Moreover the decreased sensitivity to the β-stimulants in sensitized animals was observed in the mechanical response of tracheal muscle (6, 7). In the present study, we tested the correlation between the decrease of airway resistance and the increase in plasma level of cyclic AMP in the guinea pigs sensitized and non-sensitized with egg-albumin after administration of anti-asthmatic drugs.

MATERIALS AND METHODS

Sensitization: Guinea pigs of either sex were sensitized by antigen administered s.c. three times daily in a dose of 3 ml/kg. A 10% egg-albumin in saline was used as the sensitizing antigen (7). The guinea pigs were used after 3 weeks.

Mechanical response: To measure the bronchoconstriction the animals were anesthetized with urethane (0.7 ml of 25% solution/100 g s.c.), tracheomized, and ventilated by a Shinano constant volume respirator, model SM-480–7 (frequency of 7).
70/min, total volume of 10 ml). The bronchoconstriction was recorded by a Bronchospasm transducer 7020, Ugo-Basile. Drugs which were dissolved in physiological saline were injected through a polyethylene catheter into the jugular vein. The drugs were administered at intervals of 30 min.

**Determination of cyclic AMP concentration:** To estimate cyclic AMP concentration in blood plasma, 0.5 ml of blood was drawn from the carotid artery through the polyethylene catheter at a timed interval (0, 5, 15 and 30 min) after drug or antigen injection. Blood plasma samples were collected immediately in tubes containing enough ethylenediamine tetraacetic acid (EDTA) to give a final concentration of 5 mM (pH 7.4). The tubes were mixed as soon as possible and centrifuged at 4°C and the plasma cyclic AMP concentration was determined by radioimmunoassay using a Yamasa cyclic AMP assay kit. The animals were ventilated in the same conditions as for measuring the bronchoconstriction during blood drawing.

**RESULTS**

1. **The effect of histamine on airway resistance:** The increase in airway resistance developed rapidly and dose-dependently with an intravenous injection of histamine. The responses were soon over, and tachyphylaxis was not observed when histamine was administered at intervals of 30 min. The sensitivity of animals to histamine was increased by sensitization with egg-albumin. Figure 1 shows the percent increase of the bronchoconstriction caused by histamine against the basal respiratory overflow. The increased respiratory overflow caused by histamine in sensitized animals was significantly greater than that in non-sensitized (normal) animals.

2. **The effects of bronchodilators on a histamine induced bronchospasm:** Isoprenaline, salbutamol and aminophylline attenuated bronchoconstriction caused by histamine dose-dependently when histamine (10 μg/kg, i.v.) was used as the bronchoconstrictor. Bronchodilators were administered 3 min before the injection of histamine 10 μg/kg. Figure 2 a, b shows the effects of bronchodilators on a histamine-induced bronchospasm in the sensitized (closed symbols) and the non-sensitized (open symbols) animals. The ordinate shows the response to histamine in the presence of bronchodilators. The response to histamine in the absence of bronchodilators is indicated as 100%. The inhibitory effect of the β-stimulants isoprenaline and salbutamol in the sensitized animals was weaker than that in the non-sensitized animals, but the effect of aminophylline was not changed by sensitization.
3. Changes of the cyclic AMP concentrations in blood plasma after administration of bronchodilators: The administration of antigen (egg-albumin 25 mg/kg, i.v.) to the sensitized guinea pigs increased airway resistance. The increase in airway resistance lasted for 10 min or more. No increase in airway resistance was observed by administration of egg-albumin in non-sensitized animals. These results indicate that the increase in airway resistance caused by egg-albumin is due to the antigen antibody reaction.

Figure 3 shows the changes of the plasma level of cyclic AMP caused by egg-albumin. Egg-albumin (25 mg/kg, i.v.) increased the plasma level of cyclic AMP in the guinea pigs sensitized with egg-albumin, but did not in the non-sensitized guinea pigs. Blood was drawn from the same animal at a timed interval. On the other hand, the increase in the plasma level of cyclic AMP was observed in the both guinea pigs sensitized and non-sensitized with egg-albumin when 10 μg/kg histamine was injected (Fig. 4). In normal guinea pigs, isoprenaline and salbutamol increased cyclic AMP in the plasma dose-dependently. The increase by salbutamol lasted longer than that by isoprenaline. Aminophylline did not increase cyclic AMP in the plasma (Fig. 5). Isoprenaline was
about 10 times as potent as salbutamol in increasing the plasma level of cyclic AMP. Figure 6 shows changes of the plasma level of cyclic AMP after administration of bronchodilators. The plasma level of cyclic AMP in sensitized animals was not significantly different from that in normal animals. The values estimated in the normal animals were taken from Fig. 5. These results indicate that the effects of β-adrenoceptor stimulants on the plasma level of cyclic AMP were not attenuated by sensitization.

**DISCUSSION**

It is generally accepted that an antigen-antibody reaction provides the trigger for the allergic bronchospasm. The increase in airway resistance caused by an antigen-antibody reaction was demonstrated in the present study. This increase in airway resistance was accompanied with the increase in plasma level of cyclic AMP. No increases in airway resistance and in the cyclic AMP level after administration of egg-albumin were observed in non-sensitized animals. These results indicate that an antigen-antibody reaction may release the mediator(s) of anaphylaxis which causes increase in airway resistance and plasma cyclic AMP concentration. Histamine also increases the airway resistance and plasma cyclic AMP concentration. One of mediators of anaphylaxis in guinea pigs sensitized with egg-albumin is considered to be histamine as reported previously (8).
The bronchoconstriction caused by histamine in sensitized animals was greater than that in non-sensitized animals. These results indicate the increase of histamine sensitivity after the treatment with egg-albumin. These data are consistent with the results of Antonissen et al. (9) who reported that the tracheal smooth muscle from the ovalbumin sensitized canine model of allergic asthma showed hypersensitivity and hyperreactivity to histamine when compared to that from litter mate controls in vitro.

The plasma level of cyclic AMP was increased by the β-adrenoceptor stimulants which activate adenylate cyclase but not by aminophylline which inhibits phosphodiesterase activity. On the other hand, aminophylline is said to relax the smooth muscle via the increase of intracellular cyclic AMP by inhibiting phosphodiesterase activity. Therefore, there is no good correlation between bronchodilation and the increase in the plasma level of cyclic AMP caused by anti-asthmatic drugs.

The hypothesis of partial β-adrenergic blockade in asthma has been sustained by many investigators (4-7). Our results also show that sensitization of guinea pigs with egg-albumin decreased the potencies of salbutamol and isoprenaline on inhibition of histamine-induced bronchospasm. However, the increase in the plasma level of cyclic AMP after administration of β-adrenoceptor stimulants to the sensitized animals was not attenuated as compared with normal animals. Some investigators (4-7) reported that the mechanical responses or the elevations of cyclic AMP caused by the β-stimulants were attenuated by sensitization. The present results together with the aforementioned (4-7) suggest that the plasma level of cyclic AMP appears not to exactly reflect the responses to the β-adrenoceptor stimulants.

REFERENCES


