Effect of Three Novel K⁺ Channel Openers, Cromakalim, Pinacidil and Nicorandil on Allergic Reaction and Experimental Asthma

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Received November 10, 1990 Accepted February 21, 1991

ABSTRACT—The anti-allergic and anti-asthmatic activities of three potassium (K⁺) channel openers, cromakalim, pinacidil, and nicorandil, were investigated. 1) Forty-eight-hour homologous passive cutaneous anaphylaxis (PCA) in mice was not affected by cromakalim, pinacidil, or nicorandil. Ketotifen significantly inhibited the reaction. 2) Antigen-induced histamine release from sensitized guinea pig lung tissue was not affected by cromakalim, pinacidil or nicorandil (except for 10⁻⁴ M nicorandil). Salbutamol inhibited the release of histamine. 3) Histamine, serotonin and LTC₄-induced vasculitis in rat back skin was not affected by any of these three K⁺ channel openers. 4) Antigen-induced constriction of isolated sensitized guinea pig tracheal muscle was relaxed by each of the K⁺ channel openers. 5) Constrictions of isolated guinea pig tracheal muscle caused by high potassium, histamine, LTC₄, or U-46619 were clearly relaxed by each of the three K⁺ channel openers. 6) Increases of airway resistance caused by histamine, LTD₄, or U-46619 in guinea pigs in vivo were inhibited by administration of each of the three K⁺ channel openers. 7) Experimental asthma caused by the IgE antibody and antigen system in guinea pigs was inhibited by each of the three K⁺ channel openers.

Recently, there have been some reports that potassium (K⁺) channel openers are able to promote the relaxation of a various kinds of smooth muscle (1–10). The potential of such compounds to act as new anti-hypertensive and anti-asthmatic drugs via a novel mechanism is arousing great interest. Some investigators have reported that K⁺ channel activators had potential for relieving bronchoconstriction caused by asthma mediators (11–15). Allergic chemical substances, including histamine, leukotriene C₄ (LTC₄), platelet activating factor (PAF) and serotonin, were commonly used to produce bronchoconstriction because bronchial asthma is mainly caused by an allergic mechanism. Nevertheless, there is no report examining the effect of K⁺ channel openers on the allergic reaction. In the present study, we examined the effects of three K⁺ channel openers, cromakalim, pinacidil, and nicorandil, on allergic reactions in mice, rats, and guinea pigs and on experimental asthma in guinea pigs.

MATERIALS AND METHODS

Mouse ear passive cutaneous anaphylaxis

Mouse ear passive cutaneous anaphylaxis was induced by the method of Inagaki et al. (16). Briefly, 10 μl of 50-fold diluted mouse serum containing anti-dinitrophenylated ascaris (DNP-As) IgE antibody was injected into
both ears of ddY mice. Forty-eight hours after sensitization, the mice were challenged by intravenous injection of 0.25 mg dinitrophenylated bovine serum albumin (DNP-BSA) dissolved in 0.25 ml of Evans blue saline solution. Thirty minutes after the challenge, mice were sacrificed by cervical dislocation and both ears were removed to measure the amount of extravasated dye. The amount of dye was measured according to the previously described method (16). Briefly, a pair of ears was dissolved, overnight, in 0.7 ml of 1 N sodium hydroxide solution at 37°C, and 9.3 ml of a mixture of 0.6 N H₃PO₄ solution and acetone (5:13) was then added. After vigorous shaking, the precipitate was removed by filtration, and the amount of dye was measured colorimetrically at 620 nm.

Histamine, serotonin and LTC₄-induced skin reaction in rats

Reactions were carried out as described previously (17). Briefly, 0.1 ml each of histamine (2 × 10⁻² g/ml), serotonin (2 × 10⁻⁶ g/ml), and LTC₄ (5 × 10⁻⁶ g/ml) solution was injected into 3 of the 4 shaved back skin sites. Saline (0.1 ml) was injected intradermally at the remaining site. Immediately after injection, 0.25 ml of 0.5% Evans blue saline solution was injected into the tail vein. Thirty minutes later, rats were sacrificed, and the bluing skin sites were cut out in order to simultaneously measure the amount of extravasated dye (as described for PCA).

Histamine release from guinea pig lung tissue

Lungs from guinea pigs passively sensitized with anti-benzyl penicilloyl bovine γ-globulin (BPO-BGG) IgE antibody (0.5 ml antisera/ head) were chopped on a McIlwain tissue chopper and then suspended in 10 times their volume of Tyrode’s solution. Histamine release was induced by incubation with antigen (benzyl penicilloyl bovine serum albumin: BPO-BSA, 10⁻⁶ g/ml) at 37°C for 30 min. The amount of histamine in the incubation medium was measured according to the fluorescence method of May et al. (18).

Contractile studies of guinea pig tracheal muscle

Guinea pigs were stunned and exsanguinated. The trachea was excised, trimmed of excess tissue, and cut longitudinally in the cartilage tissue area. The open trachea was cut into 16 to 20 segments. Four segments, tied together to form a chain, were placed in an organ bath containing Tyrode’s solution. Changes in the tone of the preparation, with 0.5 g initial resting tension, were recorded isotonically (MEC, ME-4013, World Medical Co., Ltd.). Drugs to be examined were added cumulatively after contraction reached a plateau. Antigen-induced contraction was achieved by the addition of BPO-BSA (5 × 10⁻⁶ g/ml) to tracheal muscle obtained from guinea pigs that had been passively sensitized with anti-BPO-BGG IgE antibody, as mentioned above. Other contractions were caused by the addition of KCl (30 mM), histamine (10⁻⁶ g/ml), LTD₄ (10⁻⁷ g/ml), and U-46619 (10⁻⁶ g/ml). Relaxing potency was calculated as a percentage of the relaxation induced by isoprotenerol (10⁻⁸ g/ml).

Bronchoconstriction in vivo

Bronchoconstriction in vivo was measured by the technique of Konzett and Rössler (19). Guinea pigs were anesthetized with an intraperitoneal injection of 1.5 g/kg urethane. Tracheas were cannulated, and jugular catheters for the administration of drugs and chemical mediators were placed into the cannulas. The tracheal cannula was connected to a constant volume respirator (New England Inst., MA, U.S.A.) and the animal was artificially ventilated at a constant volume of 5 ml, at a frequency of 70 cycles/min. Changes in inflation pressure at constant airflow were measured using a pressure transducer (UGO Basel, Milano, Italy) connected to a side-arm of the tracheal cannula; changes were expressed as a percentage of the maximum increase in inflation achieved by ligating the trachea at the end of the experiment.
Experimental asthma

Experimental asthma was carried out according to the previously described method (20). In brief, guinea pigs were anesthetized with an intraperitoneal injection of 37.5 mg/kg pentobarbitone sodium. Tracheas were cannulated, and polyethylene catheters were placed in the right external jugular veins for drug and antigen administration. The tracheal cannula was then connected to a transducer coupled with a multi-purpose monitoring apparatus (Nihon Kohden, Ind., Co., RM-150 type and RM-25 type) so that respiratory rate and volume could be recorded simultaneously. At the same time, the ratio between expiration and inspiration time (expiration/inspiration ratio) was automatically calculated by computer (PC-9800, NEC, Tokyo, Japan) from the respiratory curve pattern.

Statistics

Statistical analysis was performed by Student’s t-test.

RESULTS

Homologous PCA in mice

The effects of cromakalim, pinacidil, nicorandil, and ketotifen on 48-hr homologous PCA in mice were investigated. All drugs were administered intravenously 10 min prior to antigen challenge. Ketotifen, at a dose of 300 µg/kg, inhibited dye leakage, but the other drugs showed no such inhibition (Fig. 1).

Vasculitis caused by histamine, serotonin, and LTC₄

None of the three K⁺ channel openers had any effect on histamine-, serotonin-, and LTC₄-induced vasculitis in rat back skin. Ketotifen, at a dose of 300 µg/kg, inhibited all reactions (Fig. 2).

Histamine release

Cromakalim and pinacidil had no effect on the antigen-induced release of histamine from

![Fig. 1. Effects of cromakalim, pinacidil, nicorandil, and ketotifen (Keto) on 48-hour homologous PCA in mice. Drugs were administered 10 min prior to challenge. Each group includes 8 animals. **: Statistically significant difference from the control (Cont) at P < 0.01.](image-url)
guinea pig lung tissues. Nicolandil, at a dose of $10^{-4}$ M, and salbutamol (Salbu) at a dose of $10^{-5}$ M, inhibited the release of histamine (Fig. 3).

Relaxation of contracted tracheal muscle

Figure 4 indicates the effect of the three K$^+$ channel openers on the relaxation of isolated sensitized guinea pig trachea previously contracted by antigen. Three drugs had concentration dependent relaxing activity. Cromakalim and pinacidil were more potent than nicorandil. Figure 5 indicates the relaxant activity of the three K$^+$ channel openers on KCl-, histamine-, LTD$_4$-, and U-46619-induced contraction of guinea pig tracheal muscle. In all cases, the order of relaxant potency was: cromakalim, pinacidil, and nicorandil.

Fig. 2. Effects of cromakalim, pinacidil, nicorandil, and ketotifen (Keto) on vasculitis caused by histamine, serotonin, and LTC$_4$. Each drug was administered i.v. 10 min prior to the elicitation of reaction. Each group includes 8 animals. **: Statistically significant difference from the control (Cont) at $P < 0.01$.

Fig. 3. Effects of cromakalim, pinacidil, nicorandil, and salbutamol (Salbu) on antigen-induced histamine release from chopped lung tissue of guinea pigs passively sensitized with BPO-BGG IgE antibody. Each group includes 6 to 7 experiments. **: Statistically significant difference from the control (Cont) at $P < 0.01$. 
maka\textsuperscript{l}im > pinacidil > nicorandil.

**Bronchoconstriction in vivo**

Increases of airway resistance caused by histamine, LTD\textsubscript{4}, and U-46619 in guinea pigs, in vivo, were inhibited by administration of each of the three K\textsuperscript{+} channel openers. All drugs inhibited the U-46619-induced reaction rather than the histamine- and LTD\textsubscript{4}-induced responses (Fig. 6).

**Experimental asthma**

In experimental asthma, each drug, at a dose of 1 mg/kg, inhibited the decrease of tidal volume and the increase of the expiration/inspiration ratio (Fig. 7).

**DISCUSSION**

Recently, much attention has been paid to the bronchodilating activity of K\textsuperscript{+} channel openers (11-15). This pharmacological property suggests the possible therapeutic potential
of K⁺ channel openers in the treatment of bronchial asthma (21, 22). Since many possible mechanisms, including allergic responses, are believed to be involved in the onset and development of bronchial asthma, in the present study, we chose to evaluate the effects of these novel K⁺ channel openers on allergic reactions. From the results of this study, it appears that none of the K⁺ channel openers are effective on allergic histamine release and vasculitis. Moreover, they are also ineffective on allergic mediator-induced vasculitis. However, in contrast, these three agents are effective on the contractile responses caused by allergic mechanisms in guinea pig trachea. In addition, the three agents are also effective on the bronchoconstriction caused by allergic chemical mediators.

It is well-known that certain cellular events which occur during allergic reactions are Ca²⁺-dependent phenomena (23–25). Indeed, some investigators, including ourselves, have demonstrated that allergic histamine release and vasculitis are Ca²⁺-dependent reactions (26–28). As Ca²⁺ dependent cellular responses usually cross link to K⁺ movement, it is possible that reactions are modified by K⁺ channel openers. However, in the present experiments, allergic histamine release and vasculitis were not modified by K⁺ channel openers except for those due to 10⁻⁴ M nicorandil. Thus it is unlikely that the effectiveness of 10⁻⁴ M nicorandil was a result of K⁺ channel opening. According to Endoh et al. (29), nicorandil inhibits cyclic AMP phosphodiesterase activity at concentrations between 3 ×
10^{-3} and 10^{-2} M, which may result in an elevation of intracellular cyclic AMP level. These concentrations are higher than our effective concentration by one or two orders of magnitude. Nevertheless, it is possible the phosphodiesterase inhibitory action may be partially involved in nicorandil-induced inhibition of histamine release.

Regarding the bronchodilating mechanisms of K^+ channel openers, it is possible that mechanisms other than K^+ channel opening may participate in the actions of nicorandil and pinacidil. Endoh and Taira (30) and Holzman (31) reported that nicorandil stimulated cyclic GMP formation in arterial smooth muscle. Furthermore, Allen et al. (32) reported that nicorandil caused clear relaxation of tracheal tone in guinea pigs through a nitrate-dependent action, probably related to the production of cyclic GMP. These evidence suggest that an additional pharmacological action of nicorandil may participate in its bronchodilating activity.

Recently, some investigators reported that mechanisms other than K^+ channel opening are involved in the vasodilating action of pinacidil (33, 34). At present, however, it still remains unsolved what types of additional mechanisms are involved in the bronchodilating action of nicorandil and pinacidil. Further experiments will be necessary to clarify the bronchodilating mechanism of nicorandil, pinacidil and cromakalim.

In addition to the present observations, the three K^+ channel openers demonstrate an anti-asthmatic activity in guinea pigs. There is
a report indicating that cromakalim has an anti-asthmatic action in guinea pigs (11). We confirmed the efficacy of all three K⁺ channel openers in our experimental asthma. This suggests that a K⁺ channel opener is a useful anti-asthmatic drug.

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