Relaxation of Airway Smooth Muscle Induced by Potassium in the Presence of Ca-Antagonists

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Accepted April 20, 1989

Abstract—We examined K+-induced relaxation instead of contraction in the presence of Ca-antagonists by measuring isometric tension in the tracheal smooth muscle isolated from guinea pigs. Cumulative administration of KCl (10–90 mM) induced a concentration-dependent contraction. When the muscle was pretreated with low concentrations of Ca-antagonists, cumulative administration of KCl caused a mild contraction, followed by a moderate relaxation. In the muscle pretreated with high concentrations of Ca-antagonists, KCl revealed a concentration-related relaxation without contraction. The potency ratios of Ca-antagonists to reverse the KCl-induced contraction to relaxation were nifedipine : verapamil : diltiazem = 94:4:1. This order of potency was quite similar to that of Ca-antagonists to relax the muscle precontracted with KCl (30 mM). Magnitudes of KCl (30 mM)-induced relaxation in the presence of Ca-antagonists were similar to those caused by Ca-antagonists in the KCl (30 mM)-precontracted muscles. Thus, K+-induced relaxation in the airway smooth muscle in the presence of Ca-antagonists may be due to the voltage-dependent increase in binding of Ca-antagonists to calcium channels.

It has been generally accepted that the airway smooth muscle displays a concentration-related contraction by the administration of KCl (10–90 mM) in terms of a depolarization mechanism. On the other hand, it has been shown that Ca-antagonists cause a relaxation of the airway smooth muscle precontracted with KCl due to the blocking effect of extracellular Ca++ entering through voltage-dependent Ca++ channels (1–3). Among the various kinds of Ca-antagonists, the effects of nifedipine and verapamil on airway smooth muscle have been well-documented. In in vitro studies, verapamil has been shown to reverse the contraction of tracheal smooth muscle in guinea pigs (4, 5) and in dogs (6), while nifedipine was even more effective than verapamil in guinea pigs (7). The preventive effect of nifedipine on human bronchoconstriction has been also reported in vitro (8, 9) and in vivo (10, 11). However, there is no available literature concerning the relative potencies for various kinds of Ca-antagonists, including diltiazem and nicardipine, on the airway tone. Since Ca-antagonists with different chemical structures may act on the smooth muscle through different subcellular mechanisms, we initially compared the potencies of nifedipine, nicardipine, verapamil and diltiazem to relax the isolated tracheal smooth muscle precontracted with KCl (30 mM). We further studied the effect of KCl on these preparations pretreated with diltiazem. Interestingly, KCl-induced relaxation instead of contraction was found in the preparation pretreated with a high concentration of diltiazem. To examine whether such a relaxation could be seen with other Ca-antagonists, we investigated the concentration-response effects for KCl in the presence of the Ca-antagonists, i.e., diltiazem, verapamil and nifedipine, at various concentrations. To ex-
plore the mechanisms of this phenomenon, we compared the relative potencies of Ca-antagonists to relax the airway smooth muscle precontracted with KCl (30 mM) and those to reverse KCl-induced contraction to relaxation, and also compared the extents of relaxations of Ca-antagonists after KCl-derived contraction and those induced by KCl after the Ca-antagonists pretreatments. Furthermore, we investigated the effects of EDRF (epithelial-derived relaxing factor) (12, 13) on the K+-induced relaxation as a possible mechanism to elucidate this phenomenon.

Materials and Methods

Isolated tracheal smooth muscle: Male guinea pigs weighing 250–750 g were sacrificed under enflurane anesthesia vaporized in a glass jar, and the tracheas were removed from the larynx to the carina. Two tracheal ring strips were prepared by the previously reported technique (14), and four preparations were obtained from each animal. Each preparation was mounted in an organ bath filled with 20 ml of Krebs-Ringer type solution maintained at 37°C and aerated with 5% CO2 and 95% oxygen. The solution contained the following chemicals: 120.7 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, 15.5 mM NaHCO3, 1.2 mM NaH2PO4 and 11.5 mM glucose.

Measurement of tension: The isometric tension of each sample was continuously measured with a strain-gauge transducer (Minebea Co., Ltd., Japan) and recorded on a pen oscillograph (San-ei Instrument Co., Ltd., Japan). The resting tension of each sample was set approximately 1.5 g prior to the drug addition. When a drug was administered, sufficient time (5–20 min) was always allowed for the steady state to be reached at each concentration. Spontaneous tension change within 30 min was less than 3% of the maximal relaxation given by isoproterenol (10^-6 M).

Epithelium impaired preparation: The tracheal epithelium was mechanically removed by rubbing with a cotton bud and it was histologically confirmed by a microscope that the epithelium was damaged in these preparations.

Drug administration: KCl was administered on the tracheal smooth muscle in its resting state or the muscle pretreated with diltiazem (3×10^-6–10^-4 M), verapamil (10^-8–3×10^-5 M) or nifedipine (3×10^-8–10^-6 M). The effects of Ca-antagonists were also tested in the presence of 30 mM KCl. All drug responses were compared with the maximal relaxation achieved by /-isoproterenol (10^-6 M) at the end of each experiment, and every response was represented as a relative percentage, taking the relaxation induced by isoproterenol as -100%. Values of pD2 were calculated as the negative logarithm of EC50 in each drug response.

Statistical analysis: Differences were compared by the Student’s t-test for two groups or analysis variance for more than two groups; P<0.05 was considered to be significant.

Results

When smooth muscles were precontracted with 30 mM KCl, Ca-antagonists caused a concentration-dependent relaxation (Fig. 1). The pD2-values for relaxant effects of Ca-antagonists are presented in Table 1. The rank order of the potencies was as follows: nifedipine : nicardipine : verapamil : diltiazem= 45:12:3:1. Differences in pD2-values among the agents were statistically significant (Table 1).

Cumulative administration of KCl caused a concentration-related contraction as shown in the control curve (Fig. 2-1). When the muscle was pretreated with Ca-antagonists, however, responses to KCl differed depending on the concentrations of KCl as well as those of Ca-antagonists used for pretreatments. When the muscle was pretreated with 10^-5 M diltiazem, KCl (10–90 mM)-induced contractions were considerably less than those of the control responses, followed by weak relaxations at high concentrations of KCl (60–90 mM) (Fig. 2-2). When the muscle was pretreated with 3×10^-5 M diltiazem, the KCl-induced contraction disappeared, and only a concentration-related relaxation was observed with 10 to 90 mM KCl (Fig. 2-3). In the presence of a high concentration of diltiazem (10^-4 M), cumulative administration of KCl caused a more pronounced relaxation (Fig. 2-4). The concentration-response curves for KCl in the absence and presence of
Fig. 1. Effects of Ca-antagonists on tracheal smooth muscle precontracted with KCl (30 mM). The vertical axis shows % of maximal relaxation obtained with $10^{-6}$ M isoproterenol. The horizontal axis represents concentration of each Ca-antagonist administered cumulatively.

1 Control

- KCl $10^{-2}$
- $3 \times 10^{-2}$
- $6 \times 10^{-2}$
- $9 \times 10^{-2}$
- Wash
- Isp $10^{-6}$M

2 with Dl $10^{-5}$M

- Dl $10^{-5}$
- KCl $10^{-2}$
- $3 \times 10^{-2}$
- $6 \times 10^{-2}$
- $9 \times 10^{-2}$
- Isp

3 with Dl $3 \times 10^{-5}$M

- Dl $3 \times 10^{-5}$
- KCl $10^{-2}$
- $3 \times 10^{-2}$
- $6 \times 10^{-2}$
- $9 \times 10^{-2}$
- W
- Isp

4 with Dl $10^{-4}$M

- Dl $10^{-4}$
- KCl $10^{-2}$
- $3 \times 10^{-2}$
- $6 \times 10^{-2}$
- $9 \times 10^{-2}$
- W
- Isp

Fig. 2. Cumulative responses of KCl in the presence or absence of diltiazem. DI (diltiazem), Isp (isoproterenol).
Table 1. Direct relaxant potencies for Ca-antagonists

| Ca-Antagonist | pD2-Values | Relative potencies |
|---------------|------------|--------------------|
| Diltiazem     | 4.93±0.09  | *                  |
| Verapamil     | 5.33±0.12  | ***                |
| Nicardipine   | 6.01±0.13  | **                 |
| Nifedipine    | 6.58±0.02  | ***                |

Each Ca-antagonist was administered after KCl (30 mM) precontraction. pD2 is the negative logarithm of EC50 in relaxation. Values represent the mean±S.E., n=5. Relative potencies are calculated from the pD2-values, taking the potency of diltiazem as 1. *P<0.05, **P<0.01, ***P<0.001.

Fig. 3. Effects of diltiazem on the concentration-response relationship for KCl. Muscle tension in the presence of each concentration of diltiazem was taken as 0%. The vertical axis shows % changes in tension expressed by the maximal relaxation obtained with 10^-6 M isoproterenol. The horizontal axis represents concentration of KCl administered cumulatively.

Increasing concentrations of diltiazem (3×10^-6–10^-4 M) are shown in Fig. 3. Similar results were obtained in the tracheal preparations pretreated with verapamil (10^-6–3×10^-5 M) and nifedipine (3×10^-8–10^-6 M) (Figs. 4 and 5, respectively). The relaxation elicited by KCl in the presence of Ca-antagonists was not influenced by the prior addition of propranolol (10^-6–10^-5 M), indicating that the involvement of β-adrenoceptors stimulated by norepinephrine released by KCl-depolarization was negligible. The concentration of Ca-antagonists that can reverse the KCl-induced contraction to relaxation was estimated from crossings at the abscissae in the individual concentration-relaxation curve for KCl in the presence of increasing concentrations of Ca-antagonists (Figs. 6, 7 and 8 for diltiazem, verapamil and nifedipine, respectively). The mean concentrations of Ca-antagonists to reverse the KCl-induced responses from contraction to relaxation at each KCl concentra-
Fig. 4. Effects of verapamil on the concentration-response relationship for KCl. Muscle tension in the presence of each concentration of verapamil was taken as 0%. The vertical axis shows % changes in tension expressed by the maximal relaxation obtained with $10^{-6}$ M isoproterenol. The horizontal axis represents concentration of KCl administered cumulatively.

Fig. 5. Effects of nifedipine on the concentration-response relationship for KCl. Muscle tension in the presence of each concentration of nifedipine was taken as 0%. The vertical axis shows % changes in tension expressed by the maximal relaxation obtained with $10^{-6}$ M isoproterenol. The horizontal axis represents concentration of KCl administered cumulatively.
Fig. 6. Concentrations of diltiazem that can reverse KCl-induced contraction to relaxation. The dashed circle indicates the reversal concentrations at which each KCl-response curve crosses the baseline. Control represents the responses to KCl (10, 30 and 60 mM) without the diltiazem-pretreatment. The vertical axis shows % changes in tension expressed by the maximal relaxation obtained with $10^{-6}$ M isoproterenol. The horizontal axis represents the concentration of pretreated diltiazem.

Table 2. The concentration of Ca-antagonists that can reverse KCl-induced contraction to relaxation and their relative potencies

| Ca-Antagonists | Reversal concentration ($\pm 95\%$ confidence limits) | Relative potencies |
|----------------|------------------------------------------------------|--------------------|
| Diltiazem      | $1.5 \times 10^{-5}$ M ($1.3 \times 10^{-5}$-$1.8 \times 10^{-5}$ M) | 1                  |
| Verapamil      | $3.8 \times 10^{-6}$ M ($2.9 \times 10^{-6}$-$5.1 \times 10^{-6}$ M) | 4                  |
| Nifedipine     | $1.6 \times 10^{-7}$ M ($1.2 \times 10^{-7}$-$2.2 \times 10^{-7}$ M) | 94                 |

Reversal concentration is the mean value of that obtained from KCl 10, 30 and 60 mM-induced responses. Relative potencies are calculated from each reversal concentration, taking the potency of diltiazem as 1. n=5.

The extents of relaxation (%) induced by 30 mM KCl in the presence of high concentrations of Ca-antagonists, which were normalized by the isoproterenol-induced maximal relaxation (-100), are presented in Table 3. The extents of relaxation induced by the same concentrations of Ca-antagonists in the
Fig. 7. Concentrations of verapamil that can reverse KCl-induced contraction to relaxation. The dashed circle indicates the reversal concentrations at which each KCl-response curve crosses the baseline. Control represents the responses to KCl (10, 30 and 60 mM) without the verapamil-pretreatment. The vertical axis shows % changes in tension expressed by the maximal relaxation obtained with 10^-6 M isoproterenol. The horizontal axis represents the concentration of pretreated verapamil.

Table 3. Comparison of amplitudes (% relaxation) for KCl plus Ca-antagonists under converse administrative orders

| Ca-Antagonists | KCl (30 mM) + Ca-Antagonist | Ca-Antagonist + KCl (30 mM) | Significance |
|----------------|-----------------------------|-----------------------------|--------------|
| Diltiazem (10^-4 M) | -70±4% | -64±3% | NS |
| Verapamil (3×10^-6 M) | -63±4% | -63±7% | NS |
| Nifedipine (10^-6 M) | -60±5% | -55±6% | NS |

Values represent % of the maximal relaxation obtained with isoproterenol (10^-6 M). mean±S.E., n=5. NS: not significant.

Discussion

Ca-antagonists relaxed the tracheal smooth muscle precontracted with 30 mM KCl in a concentration-dependent manner (Fig. 1). The results indicate that Ca-antagonists inhibit the voltage-dependent Ca-channels in the airway smooth muscle as generally postulated in other types of muscles (1, 2). Among various kinds of Ca-antagonists, inhibitory effects of nifedipine on bronchomotor tone were extensively studied both in vivo and in...
Fig. 8. Concentrations of nifedipine that can reverse KCl-induced contraction to relaxation. The dashed circle indicates the reversal concentrations at which each KCl-response curve crosses the baseline. Control represents the responses to KCl (10, 30 and 60 mM) without the nifedipine-pretreatment. The vertical axis shows % changes in tension expressed by the maximal relaxation obtained with 10^-6 M isoproterenol. The horizontal axis represents the concentration of pretreated nifedipine.

vitro (7-11, 15, 16). In our experiment, nifedipine was the most potent airway relaxant (Table 1). The relative potencies of Ca-antagonists for relaxant effects on the airway smooth muscle were nifedipine : verapamil : diltiazem=45:3:1. This order of potency is quite similar to that of cardiovascular inhibitory effects, especially in the negative inotropism reported previously (2, 17-19).

We have found a unique phenomenon that the KCl-induced response was converted from a contraction to a relaxation in the airway smooth muscle in the presence of Ca-antagonists. The KCl-induced relaxation was dependent on the concentrations of both Ca-antagonists and KCl. This phenomenon was observed not only in the tracheal muscles pretreated with diltiazem but also with verapamil and nifedipine. In the presence of low concentrations of Ca-antagonists, the KCl-induced contraction was significantly attenuated. In the presence of medium concentrations of Ca-antagonists, KCl-induced contraction was converted to the relaxation only at high concentrations of KCl elicited a concentration-related relaxation starting from a low concentration of KCl (10 mM). Apparently, it is difficult to explain these contrary phenomena by general pharmacological considerations. However, the relative potencies of Ca-antagonists to relax the KCl-derived contraction are well consistent with those to reverse the response to KCl (Tables 1 and 2). The relative potencies of Ca-antagonists to relax airway smooth muscle are identical to those in vascular smooth muscle (17, 18) and cardiac muscle (17, 19). This implies that KCl-induced relaxation in the presence of Ca-antagonists may involve the primary calcium antagonistic effect of these compounds (18). Furthermore, the magnitude of KCl-induced relaxation in the Ca-antagonist-pretreated muscle was quite similar to that of Ca-antagonist-induced relaxation in the KCl-pre-
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contracted muscle. The results strongly suggest that the relaxant effect of the Ca-antagonist requires the depolarization of tracheal smooth muscle. It is generally believed that potassium-induced contraction is the result of a depolarization-dependent increase in Ca influx into the smooth muscle cells (20). It has also been shown that the depolarization potentiates the calcium channel blocking effect (2, 18). Thus, it is possible that KCl-induced depolarization enhances the blocking effect of Ca-antagonists, resulting in a decreased intracellular Ca²⁺ and, consequently, a relaxation. But, how can KCl relax the basal tension of airway smooth muscle in the presence of Ca-antagonists? It is considered that the voltage-dependent Ca-channel is operative to maintain the resting tone of airway smooth muscle since Ca-antagonists are able to decrease the resting tension in a concentration-dependent manner (Fig. 2). Moreover, since the EC50 of Ca-antagonists to relax the resting tone is higher than that to antagonize the 30 mM KCl-induced contraction (21), it seems possible that the depolarization-induced increase in the binding of Ca-antagonists to calcium channels results in a more effective inhibition of the channels.

Another possibility is that KCl induces both contractile and relaxant actions on airway smooth muscle and that Ca-antagonists selectively inhibit the contractile mechanism, thereby unmasking the KCl-induced relaxation. In the vascular smooth muscle, it has been reported that KCl enhances Na⁺-K⁺ ATPase activity which results in an increased electrogeneric transport of sodium and potassium and, consequently, a relaxation due to hyperpolarization (22). It has been shown that the K⁺-induced relaxation depends on the previous inhibition of Na-K ATPase. Further, K⁺-induced relaxation is rather transient and the magnitude reflects the duration of previous exposure of the smooth muscle to a potassium free environment. We did not pre-treat the preparations with potassium-free solution in the present experiment. Moreover, even a high concentration of KCl, which could overcome the hyperpolarization elicited by Na⁺-K⁺ ATPase activation, produced greater relaxation in the present experiments. In these respects, K⁺-induced relaxation associated with Ca-antagonists in the present study may not be attributable to the Na⁺-K⁺ ATPase activation mechanism.

The other explanation is the hyperosmotic stimuli that have been reported to induce epithelial-dependent relaxation in the guinea pig trachea (23). In fact, a hyperosmolar state which resulted from the cumulative administration of KCl should have existed, since an osmotic control was not carried out in the present experiment. However, this does not seem to be the case because the hyperosmolar KCl caused only a concentration-related contraction in a control experiment (Fig. 2-1), and the muscle pretreated with a high concentration of Ca-antagonist displayed a relaxation with a low concentration of KCl (10 mM), which was not considered to produce a hyperosmolar effect. In addition, since the epithelial-impaired preparation in our experiments still caused a marked KCl-induced relaxation under the same condition as an intact preparation, the effect of EDRF could be also ruled out.

In summary, in the airway smooth muscle of the guinea pig, the KCl-induced contraction was converted to a relaxation in the presence of Ca-antagonists, such as nifedipine, verapamil and diltiazem. The potassium-induced relaxation seems to be due to the enhanced blocking effects of Ca-antagonists resulted from the depolarization-induced increase in their binding to Ca-channels.

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