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The Effect of β-adrenargic Agonists on Ca^{2+} Sensitivity in Tracheal Smooth Muscle

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Abstract: To determine whether a reduction in the sensitivity to Ca^{2+} is involved in relaxation by β-adrenergic receptor agonists, we examined correlation between force generation and intracellular Ca^{2+} concentration in the inhibitory effects of isoproterenol (ISO) on methacholine (MCh)-induced contraction. Fura-2 was loaded in tracheal smooth muscle of guinea pigs and tension and fluorescence ratio F_{340}/F_{380} as an index of intracellular Ca^{2+} concentration were recorded simultaneously. Addition of 0.3 μM ISO caused an inhibition in 1 μM MCh-induced contraction and a reduction in intracellular Ca^{2+} concentration. The reduction in intracellular Ca^{2+} concentration was, however, much smaller than that of tension. In conclusion, Ca^{2+} sensitivity in addition to Ca^{2+} influx plays an important role in β-adrenergic action in airway smooth muscle.

Key words: calcium sensitivity, beta-adrenergic agonists, smooth muscle, isoproterenol

Linear relationship of contraction and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) has been generally accepted in variable smooth muscles. Recently, dissociation between force generation and $[Ca^{2+}]_i$ have been raised in contractile agonists such as methacholine (MCh), histamine, and leukotrienes in airway smooth muscle (Somlyo and Somlyo, 2000). In these studies, the increase in contractility by these agonists did not accompany with linear elevation of $[Ca^{2+}]_i$.

β-agonists are used as bronchodilators in the treatment of asthma. However, underlying physiological mechanisms have not been clarified yet. Relaxation of airway smooth muscle caused by β-agonists has not been studied in the relevance of Ca^{2+} sensitivity.

To elucidate correlation between a reduction in contraction and $[Ca^{2+}]_i$ in the inhibitory effects of isoproterenol (ISO) on MCh-induced contraction, both tension and fluorescence ratio F_{340}/F_{380} for measurement $[Ca^{2+}]_i$ were recorded in the fura-2 loaded tracheal smooth muscles of guinea pigs simultaneously.

Materials and Methods

1. Tissue Preparation and Solution

Male guinea pigs (300–350 g) were killed by injection of overdose of anesthetics (150 mg/kg pentobarbital, i.p.) and tracheas were excised. The tracheal rings were opened by cutting longitudinally at the cartilaginous region, and the epithelium was dissected out. The normal bathing solution was composed of (in mM): NaCl 137, $KHCO_3$ 5.9, $CaCl_2$ 2.4, $MgCl_2$ 1.2, and glucose 11.8, bubbled with a mixture of 99% O_2 and 1% CO_2 (pH 7.4). The bathing solution was filled in the organ bath at a constant flow of 3 ml/min. To abolish the spontaneous tone, 2 μM indomethacin was present throughout the experiments. The temperature of the organ bath was maintained at 37 °C.

2. Isometric Tension Recording and Measurement of Fura-2 Fluorescence

The tissues were placed horizontally in an organ bath (0.6 ml volume). Muscle strips containing four cartilaginous rings, one for isometric tension recording and three for $[Ca^{2+}]_i$ measurements, were prepared. One end of a cartilaginous ring was fixed to the chamber, and the other end was connected to a force-displacement transducer to monitor isometric tension. Each ends of three cartilaginous rings were fixed to the chamber for $[Ca^{2+}]_i$ measurements. Muscle strips were treated with 10 μM acetoxymethyl ester of fura-2 (fura-2/AM) for 5 h at room temperature (22–24 °C). After the loading, the chamber was filled with the normal solution at 37 °C for 30 min to wash out the extracellular fura-2/AM before the measurements. Isometric tension and the fura-2 fluorescence of muscle strips were measured simultaneously, using a displacement transducer and a spectrofluorometer (CAF-110; Japan Spectroscopic, Tokyo, Japan). The mucosal side of the muscle strips was exposed to

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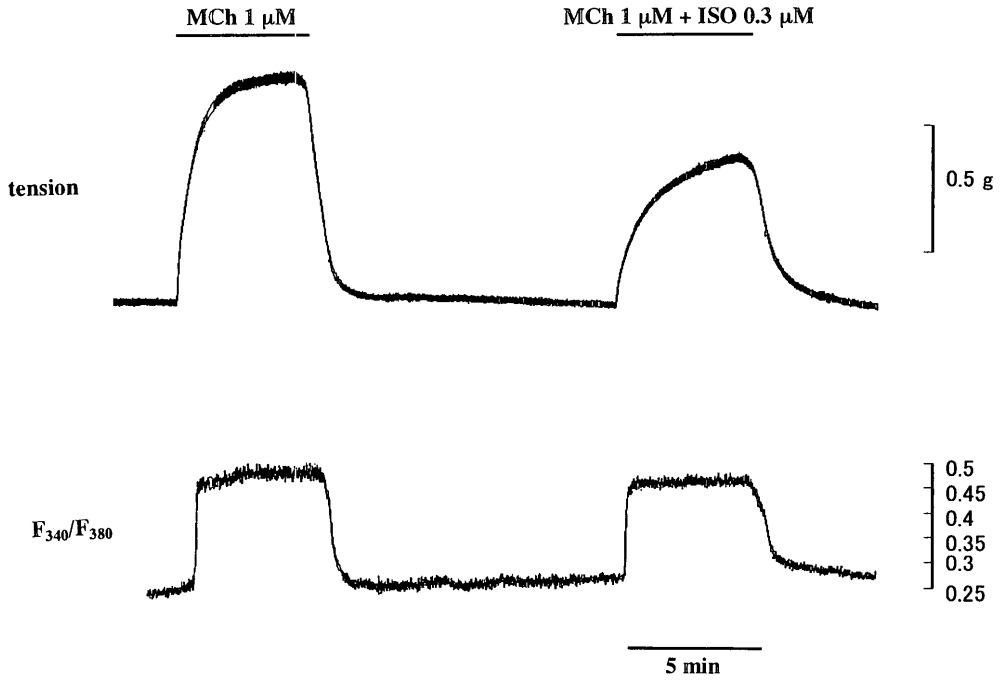

Fig. 1 A reduction in Ca^{2+} sensitivity by ISO
Upper trace: A typical record of 1 μM MCh-induced contraction in the absence and presence of 0.3 μM ISO. Lower trace: A typical record of F_{340}/F_{380} induced by 1 μM MCh and 1 μM MCh with 0.3 μM ISO.

the excitation light, and the light emitted from the strip was collected into a photomultiplier through a 500-nm filter. The intensities of fluorescence due to excitation at 340 (F_{340}) and 380 (F_{380}) nm were measured after background subtraction. The absolute amount of $[Ca^{2+}]_i$ was not calculated because the dissociation constant of fura-2 for Ca^{2+} in smooth muscle cytoplasm is known to be different from that obtained *in vitro* (Konishi et al, 1988). Therefore, the ratio of F_{340} to F_{380} (F_{340}/F_{380}) was used as a relative indicator of $[Ca^{2+}]_i$. Measurements of tension and F_{340}/F_{380} were performed after MCh-induced contraction was established. Percent inhibition for tension and F_{340}/F_{380} was expressed taking the response to the normal bathing solution as 100%.

3. Experimental Protocol

1 μM MCh was applied for 5 minutes and wash out for 15 minutes. Next, 1 μM MCh was applied to the identical tissues for 5 minutes in the presence of ISO (0.01–1 μM). ISO was applied 3 min prior to MCh.

4. Materials

ISO, MCh, and Indomethacin were obtained from Sigma Chemical (St. Louis, MO). Fura-2/AM was from Dojin Laboratories (Kumamoto, Japan). Fura-2/AM was dissolved in dimethyl sulfoxide (DMSO), and the final DMSO concentration did not exceed 0.5%. Neither drug affected the fura-2 fluorescence ratio at the concentrations used.

Results

In tracheal smooth muscle of guinea pig, perfusion of 1 μM MCh markedly evoked contraction and increase $[Ca^{2+}]_i$ above baseline. Further application of 0.3 μM ISO caused a remarkable inhibitory action on the 1 mM MCh-induced contraction (Figure 1). The inhibitory action caused by ISO was evaluated by the percentile decrease in contraction. The values were 70 ± 14% (n=6). In spite of this clear reduction of tension, ISO caused minimum change in F_{340}/F_{380} ($[Ca^{2+}]_i$) (26 ± 7%, n=6).

The larger decrease in contraction compared to F_{340}/F_{380} ($[Ca^{2+}]_i$) were also observed in all experiments ISO consisted for 0.01 to 1 μM.

Discussion

This report has demonstrated for the first time that relaxation by β-agonists is involved in a decrease in sensitivity to Ca^{2+} in airway smooth muscle. A decrease in $[Ca^{2+}]_i$ (Ca^{2+} mobilization) is generally considered to play an important role in b-adrenergic action. However, our results indicated that the inhibitory effects of ISO on MCh-induced contraction are mediated by Ca^{2+} desensitization because this agent relaxed airway smooth muscle without a substantial change in $[Ca^{2+}]_i$. In our precious study, SKF-96365, a non-selective Ca^{2+} channel blocker, produced relaxation in MCh-induced contraction, but did not accompany marked reduction in $[Ca^{2+}]_i$ (Ito et al, 2001, 2002). The mechanism underlying relaxation by β-ago-

nists is different from that by a non-selective Ca^{2+} channel blocker.

The decrease in Ca^{2+} sensitivity by β-agonists might be explained by the relevance of RhoA, a small monomeric G protein. The Ca^{2+} sensitization is defined as force generation without an increase in $[Ca^{2+}]_i$, and RhoA is recently shown to enhance in MCh-induced Ca^{2+} sensitivity (Somlyo and Somlyo, 2000). RhoA activity is inhibited by an elevation of cAMP and an actuation of PKA, which are signal transduction processes in β-agonists action (Sakai et al, 2003; Qaio et al, 2003). Therefore, b-agonists may cause relaxation at least in part mediated by a reduction in RhoA-induced Ca^{2+} sensitization.

Our results may provide the evidence that an inhibition in Ca^{2+} sensitization is useful for bronchodilator therapy against asthma attack.

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