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Hyperosmolarity Attenuates the Contraction of Rat Trachea Through the Inhibition of Phosphatidylinositol Response

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Although hyperosmolarity associated with diabetes is known to attenuate contractile response of airway smooth muscle, intracellular mechanisms involved are not fully understood. We examined the effects of hyperosmolarity on carbachol (CCh)- and aluminum fluoride (AF)-induced contractile and phosphatidylinositol (PI) responses of rat trachea. In vitro measurements of isometric tension and [3H] inositol monophosphate (IP$_1$) formed were conducted by using rat tracheal rings and slices. Hyperosmolarity solutions of 350, 450 and 600 mOsm were made with dissolving glucose in Krebs-Henseleit (K-H) solution. Hyperosmolarity attenuated dose-dependently CCh-induced contraction of rat trachea (1.86 ± 0.13 g at 300 mOsm, 1.85 ± 0.16 g at 350 mOsm, 1.37 ± 0.07 g at 450 mOsm and 0.50 ± 0.04 g at 600 mOsm, respectively), and also attenuated CCh-induced IP$_1$ accumulation (5.77 ± 0.33 Bq at 300 mOsm, 3.38 ± 0.26 Bq at 350 mOsm, 2.08 ± 0.30 Bq at 450 mOsm and 1.71 ± 0.40 Bq at 600 mOsm, respectively), and AF-induced IP$_1$ accumulation (3.93 ± 0.22 Bq at 300 mOsm, 1.63 ± 0.14 Bq at 450 mOsm and 1.02 ± 0.14 Bq at 600 mOsm, respectively). The results suggest that hyperosmolarity would inhibit G-protein-coupled phospholipase C, resulting in attenuation of CCh-induced airway smooth muscle contraction.

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Keywords: Hyperosmolarity; Contractile and phosphatidylinositol (PI) responses; Rat trachea

Materials and Methods

The studies were conducted under guidelines approved by the Animal Care Committee of Nagasaki University School of Medicine. Thirty-two male Wistar rats (Charles River, Yokohama Japan) weighing 250-350 g were used for the experiments. The rats were anesthetized with pentobarbital, 50 mg/kg intraperitoneal, and the tracheas were rapidly isolated. The contractile and phosphatidylinositol (PI) responses were measured using the tracheas isolated from the rats of same week of age.
Contractile response

The trachea was cut into 3-mm-wide ring segments with a McIlwain tissue chopper (The Mickle Laboratory Engineering, Gomshall UK). The tracheal ring was suspended between two stainless hooks and placed in a 5-mL water-jacketed organ chamber (Kishimotoika, Kyoto Japan) containing Krebs-Henseleit (K-H) solution (composition in mM: NaCl 118, KCl 4.7, CaCl₂ 1.3, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, glucose 11, Na₂-EDTA 0.05). The solution was continuously aerated with O₂: 95%/CO₂: 5% at 37°C. Isometric tensions were measured using isometric transducer (Kishimotoika, Kyoto Japan) and changes in isometric force were recorded using a MacLab system (Milford, MA). The resting tension was adjusted periodically to 1.0 g during the equilibration period. The ring was washed every 15 min and re-equilibrated to baseline tension for 60 min (Time 0).

Hyperosmolarity solutions of 350, 450 and 600 mOsm were made by adding 50, 150 and 300 mM glucose, respectively, into K-H solution. Active contraction was induced with 0.55 µM carbachol (CCh). As shown in Figure 1, the osmolarity of K-H solution was changed from 300 mOsm to 350, 450 and 600 mOsm. The ring contraction was re-induced with 0.55 µM CCh.

PLI response

Inositol 1,4,5-trisphosphate (IP₃) is rapidly degraded into inositol monophosphate (IP₁) which is recycled back to phosphatidylinositol via free inositol. Lithium inhibits the conversion of IP₃ to inositol. Thus, in the presence of Li⁺, the accumulation rate of IP₃ reflects the extent of PI response. We measured [³H] IP₃ in tracheal slices incubated with [³H] myo-inositol (Amersham, Tokyo Japan).

The trachea was cut longitudinally and chopped into 1-mm-wide slices with a McIlwain tissue chopper. The tracheal slices were preincubated for 15 min in K-H solution containing 5 mM LiCl.

Three pieces of the tracheal slice were placed in small flat-bottomed tubes containing various osmolarity of K-H solution (300, 350, 450 and 600 mOsm). An aliquot of 0.5 µCi [³H] myo-inositol was then added to each tube (final concentration 0.1 µM in 300 µL incubation volume) and the tubes were flushed with O₂: 95%/CO₂: 5%, capped, set in a shaking bath at 37°C and incubated for 30 min (Time 0). It has been demonstrated that aluminium fluoride (AF) stimulates G-protein to produce IP₃. Effects of hyperosmolarity on CCh- or AF-induced IP₃ accumulation of rat tracheal slices were determined as follows. At time 0, 0.55 µM CCh or 100 µM AF in a final concentration was added to the suspension of tracheal slices. The tubes were reaerated with 95% O₂/5% CO₂. recapped and reincubated. After additional 60-min incubation, the reaction was stopped with 940 µL chloroform: methanol (1:2 v/v). Chloroform and water were then added (300 µL each) and the phases were separated by centrifugation with 90 g for 5 min.

The [³H] IP₃ was separated from [³H] myo-inositol in the water phase of 750 µL by column chromatography using Dowex AG 1-X8 resin (Bio Rad, Richmond CA) in the formate form. The [³H] IP₃ formed in the tracheal slices was counted with a liquid scintillation counter and presented by becquerels (Bq). Osmolarity was measured with Osmostat (Arkray, Kyoto Japan).

Data were expressed as mean ± SEM. The results were analyzed by a one-way analysis of variance. Comparisons between groups were assessed by Scheffe’s F test. A P value < 0.05 was considered significant.

Results

Figure 1 shows a typical recording of the effects of hyperosmolarity on CCh-induced contraction of a rat trachea ring. After attenuation of CCh-induced contraction with hyperosmolarity, the CCh-induced contraction was reversed by normo-osmolar K-H solution. Hyperosmolarity attenuated dose-dependently CCh-induced contraction of rat trachea (1.86 ± 0.13 g at 300 mOsm, 1.85 ± 0.16 g at 350 mOsm, 1.37 ± 0.07 g at 450 mOsm, and 0.50 ± 0.04 g at 600 mOsm, respectively) (Figure 2), and also attenuated CCh-induced IP₃ accumulation (5.77 ± 0.33 Bq at 300 mOsm, 3.38 ± 0.26 Bq at 350 mOsm, 2.08 ± 0.30 Bq at 450 mOsm and 1.71 ± 0.40 Bq at 600 mOsm, respectively) (Figure 2), and also attenuated CCh-induced IP₃ accumulation (3.93 ± 0.16 Bq at 300 mOsm, 2.08 ± 0.30 Bq at 450 mOsm and 1.71 ± 0.40 Bq at 600 mOsm, respectively) (Figure 3- a, b). Simple correlation analysis between tension of the tracheal rings and IP₃ accumulation is shown in Figure 4. Hyperosmolarity-induced attenuation of the CCh-induced IP₃ accumulation correlated with the relaxation of rat tracheal rings (r = 0.88, P < 0.0001).
Discussion

The present results show that hyperosmolarity attenuates CCh-induced contractile and PI responses of rat trachea. The effects of hyperosmolarity on airway smooth muscle may involve one of the following mechanisms. Hyperosmolarity with high concentrations of glucose may inhibit the intracellular signal transduction pathways of the airway smooth muscle, resulting in a decrease in airway smooth muscle contraction. When muscarinic receptors in the airway smooth muscle activate phospholipase C, phosphatidylinositol-4, 5-bisphosphate (PIP$_2$) is hydrolyzed into IP$_3$ and diacylglycerol. IP$_3$ mobilizes Ca$^{2+}$ from sarcoplasmic reticulum and, at the same time, an influx of Ca$^{2+}$ occurs from the extracellular space. In the present study, hyperosmolarity attenuated CCh-induced IP$_3$ accumulation, and the attenuation of CCh-induced IP$_3$ accumulation was consistent with the attenuation of CCh-induced contraction. It has been reported that the phospholipase C coupled to G-proteins is stimulated by AF, and that AF induces IP$_3$ formation. Therefore, the present results indicate that AF stimulates the PI response, and that this response is inhibited by hyperosmolarity. Thus, hyperosmolarity with glucose would inhibit G-protein-coupled phospholipase C in the PI response, resulting in the attenuation of contractile response of rat trachea.

In contrast to the above mechanism, hyperosmolarity with high concentrations of glucose may affect the function of epithelium in the airways. Epithelium contains the factor which regulates responses of adjacent smooth muscle. Munakata et al. reported that when the trachea was precontracted with CCh, hyperosmolar stimuli (mannitol or NaCl) produced concentration-dependent relaxation, and that relaxation was not produced when the hyperosmolar stimulus was applied to the serosal surface and was markedly reduced or abolished when the epithelial surface had been physically damaged or removed. They concluded that hyperosmotic stimuli would induce
epithelial-dependent relaxation of trachea. Munakata et al.\textsuperscript{13} also examined the possibility that nitric oxide was one of the epithelium-derived relaxing factors in guinea pig airways, and concluded that although nitric oxide could relax airway smooth muscle, nitric oxide was not responsible for osmotic-induced epithelium-dependent relaxation. Hjoberg et al.\textsuperscript{14} demonstrated that the metabolites of arachidonic acid, most likely to be prostaglandin E\textsubscript{2}, were partially responsible for the relaxation induced by the increased osmolarity with NaCl. Thus, in the present study, prostaglandin E\textsubscript{2} might be involved in the attenuation by hyperosmolarity of CCh-induced airway smooth muscle contraction.

Hyperosmolarity with high concentrations of glucose may damage the airway smooth muscle, resulting in a decrease in airway smooth muscle contraction. However, as shown in Figure 1, after attenuation of CCh-induced contraction with hyperosmolarity, the CCh-induced contraction was reversed by normo-osmolar K-H solution. Thus, the attenuation by hyperosmolarity with high concentrations of glucose of CCh-induced airway smooth muscle contraction could not be attributed to damage of the airway smooth muscle.

In conclusion, hyperosmolarity with high concentrations of glucose inhibits CCh-induced contraction of rat trachea, the mechanism of which would involve the inhibition of G-protein-coupled phospholipase C resulting in attenuated PI response.

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References