

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 (IP₁) 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 IP₁ 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 IP₁ 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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Introduction

The infrequency of diabetes mellitus and bronchial asthma in the same individual is generally recognized.¹ Douglas et al.² reported that despite the lack of clinical evidence of respiratory involvement, patients with diabetic autonomic neuropathy reduced airway vagal tone. Heaton et al.³ reported the diminished bronchial reactivity to cold air in diabetic patients with autonomic neuropathy. These changes can be ascribed to either diabetic neuropathy or an increase in neuronal inhibitory muscarinic M₂ receptor function.⁴ However, other mechanism such as hyperosmolarity with high concentrations of glucose may be involved in the infrequency of diabetes mellitus and bronchial asthma in the same individual. Hyperosmolar stimuli (mannitol or NaCl) produced concentration-dependent relaxation when the trachea was precontracted with carbachol (CCh), a muscarinic receptor agonist.⁵ When muscarinic receptors in the airway smooth muscle activate phospholipase C, phosphatidylinositol-4, 5-bisphosphate is hydrolyzed into Inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol. IP₃ mobilizes Ca²⁺ from sarcoplasmic reticulum,

and at the same time, an influx of Ca²⁺ occurs from the extracellular space.⁶ Although hyperosmolarity with mannitol or NaCl attenuates CCh-induced contractile response of airway smooth muscle, intracellular mechanisms involved are not fully understood. Thus, this study was designed to examine the effects of hyperosmolarity with glucose on the contractile and PI responses of 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.

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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.

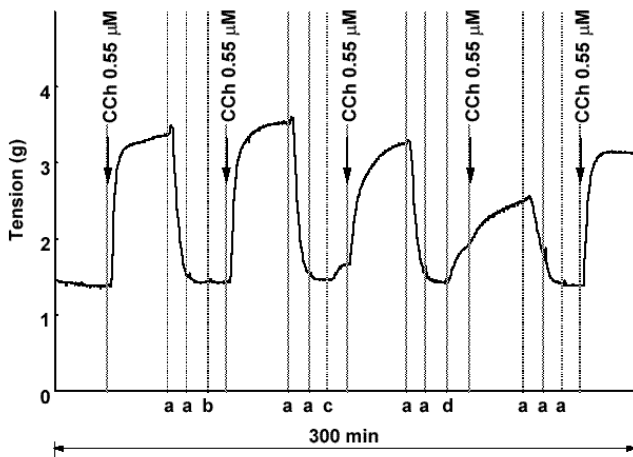


Figure 1. A typical recording of the effects of hyperosmolarity on CCh-induced contraction of a rat tracheal ring. **a.** Wash with iso-osmolar (300 mOsm), **b.** Wash with hyperosmolar (350 mOsm), **c.** Wash with hyperosmolar (450 mOsm), **d.** Wash with hyperosmolar (600 mOsm). After attenuation of CCh-induced contraction with hyperosmolarity, the CCh-induced contraction was reversed by normo-osmolar K-H solution.

PI 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 IP₁ to inositol. Thus, in the presence of Li⁺, the accumulation rate of IP₁ reflects the extent of PI response. We measured [³H] IP₁ 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.^{7,8} Effects of hyperosmolarity on CCh- or AF-induced IP₁ 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] IP₁ 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] IP₁ formed in the tracheal slices was counted with a liquid scintillation counter and presented by becquerels (Bqs). 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 IP₁ 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 IP₁ 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) (Figure 3-a, b). Simple correlation analysis between tension of the tracheal rings and IP₁ accumulation is shown in Figure 4. Hyperosmolarity-induced attenuation of the CCh-induced IP₁ accumulation correlated with the relaxation of rat tracheal rings (*r* = 0.88, *P* < 0.0001).

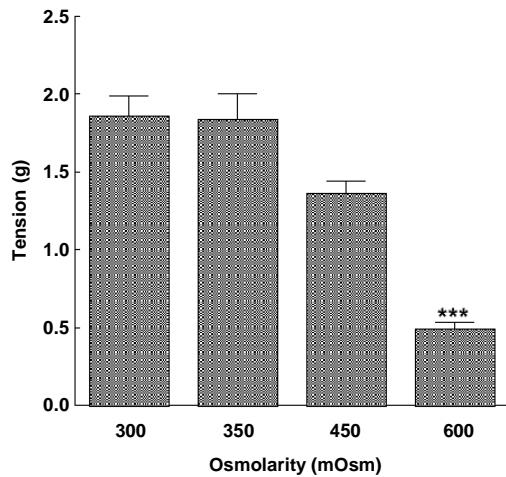


Figure 2. Effects of hyperosmolarity on 0.55 μ M carbachol-induced contraction of the rat trachea (mean \pm SEM, $n = 6-9$). *** $P < 0.001$ vs 300 mOsm. Hyperosmolarity solutions of 350, 450 and 600 mOsm were made by adding 50, 150 and 300 mM glucose, respectively, into K-H solution.

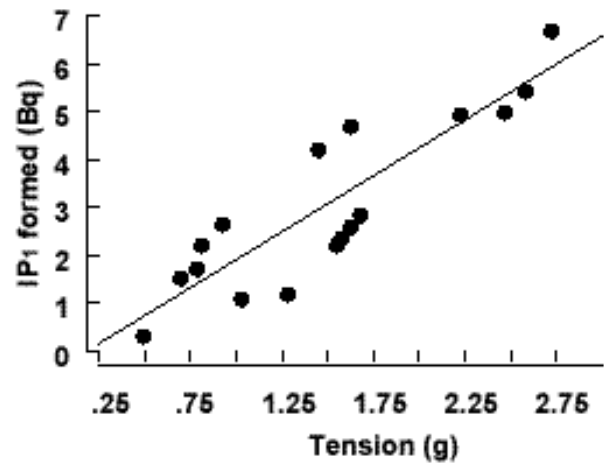


Figure 4. Simple correlation analysis between tension of the tracheal rings and inositol monophosphate (IP₁) accumulation. The contractile and phosphatidylinositol (PI) responses were measured using the tracheas isolated from the rats of same week of age. The attenuation by hyperosmolarity of CCh-induced IP₁ accumulation was correlated with the relaxation of rat tracheal rings ($r = 0.88$, $P < 0.0001$).

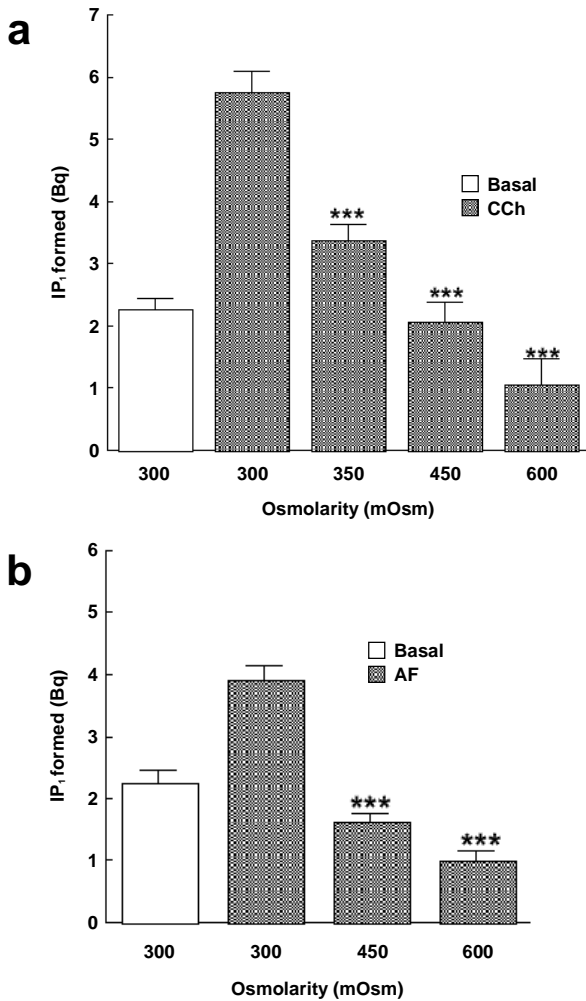


Figure 3. a, b. Effects of osmolarity on carbachol- and AF-induced IP₁ accumulation (mean \pm SEM, $n = 6-8$, *** $P < 0.001$ vs 300 mOsm). Three pieces of the tracheal slice were placed in small flat-bottomed tubes containing various osmolarity of K-H solution (300, 350, 450 and 600 mOsm).

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 (PIP₂) is hydrolyzed into IP₃ and diacylglycerol. IP₃ mobilizes Ca²⁺ from sarcoplasmic reticulum and, at the same time, an influx of Ca²⁺ occurs from the extracellular space. In the present study, hyperosmolarity attenuated CCh-induced IP₁ accumulation, and the attenuation of CCh-induced IP₁ 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 IP₃ formation.^{7,8} 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.⁹⁻¹¹ Munakata et al.¹² 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.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.¹⁴ demonstrated that the metabolites of arachidonic acid, most likely to be prostaglandin E₂, were partially responsible for the relaxation induced by the increased osmolarity with NaCl. Thus, in the present study, prostaglandin E₂ 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.

Acknowledgments

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References

1. Caplin I. Diabetes mellitus and insulin in an aspirin-sensitive asthmatic. *Ann Allergy* 36: 193-202, 1976
2. Douglas NJ, Campbell IW, Ewing DJ et al. Reduced airway vagal tone in diabetic subjects with autonomic neuropathy. *Clin Sci* 61: 581-584, 1981
3. Heaton RW. Diminished bronchial reactivity to cold air in diabetic patients with autonomic neuropathy. *Br Med J* 289: 149, 1984
4. Belmonte KE, Jacoby DB, Fryer AD et al. Increased function of inhibitory neuronal M₂ muscarinic receptor in diabetic rat lung. *Br J Pharmacol* 121: 1287-1294, 1997
5. Jongejan RC, De Jongste JC, Raatgeep RC, et al. Effects of change in osmolarity on isolated human airways. *J Appl Physiol* 64: 1568-1575, 1990
6. Meurs H, Roffel AF, Postema JB, Timmermans A. Evidence for a direct relationship between phosphoinositide metabolism and airway smooth muscle contraction induced by muscarinic agonists. *Eur J Pharmacol* 156: 271-274, 1988
7. Sternweis PC, Gilman AG. Aluminium: a requirement for activation of the regulatory component of adenylate cyclase by fluoride. *Proc Natl Acad Sci* 79:4888-4891, 1982
8. Wood SF, Szuts EZ, Fein A. Inositol trisphosphate production in squid photoreceptor. Activation by light, aluminum fluoride, and guanine nucleotides. *J Bio Chem* 264: 12970-12976, 1989
9. Boucher RC. Human airway ion transport. Part two. *Am J Respir Crit Care Med* 150: 581-593, 1994
10. Morrison KJ, Gao Y, Vanhoutte PM. Epithelial modulation of airway smooth muscle. *Am J Physiol* 258: 254-262, 1990
11. Rocha G, Bucher B, Tschopl M, et al. Hyperosmolarity enhances smooth muscle contractile responses to phenylephrine and partially impairs nitric oxide production in the rat tail artery. *J Vasc Res* 32: 58-65, 1995
12. Munakata M, Mitzner W, Menkes HA et al. Osmotic stimuli induce epithelial-dependent relaxation in the guinea pig trachea. *J Appl Physiol* 64: 466-471, 1988
13. Munakata M, Masaki Y, Sakura I et al. Pharmacological differentiation of epithelium-derived relaxing factor from nitric oxide. *J Appl Physiol* 69: 665-670, 1990
14. Hjoberg J, Folkerts G, van Gessel SBE et al. Hyperosmolarity-induced relaxation and prostaglandin release in guinea pig trachea in vitro. *Eur J Pharmacol* 398: 303-307, 2000