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Effects of vasopressors on contractile and phosphatidylinositol responses of rat trachea

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Abstract

Purpose. Vasopressors, such as dopamine (DA), norepinephrine (NE), and phenylephrine (Phe), are commonly used during anesthesia to increase blood pressure through α_1-adrenoceptors. The present study was designed to examine the effects of DA, NE, and Phe on the contractile and phosphatidylinositol (PI) responses of the rat trachea induced by a muscarinic agonist, carbachol (CCh).

Methods. A rat tracheal ring was suspended between two stainless-steel hooks in Krebs-Henseleit (K-H) solution. Contraction was induced with 0.55 μM CCh, and 30 min later DA, NE, or Phe was added. The tracheal slices were incubated in K-H solution containing LiCl, ^{3}[H]*myo*-inositol, and CCh in the presence or absence of DA, NE, or Phe. The ^{3}[H]inositol monophosphate (IP_1) formed was measured.

Results. CCh caused tracheal ring contraction. NE attenuated CCh-induced contraction at a dose of 1 μM or greater and had a maximal effect at 3 μM. DA and Phe did not affect CCh-induced contraction. CCh-induced IP_1 accumulation was potentiated significantly by NE and Phe, but not by DA.

Conclusion. Although NE and Phe potentiated CCh-induced IP_1 accumulation, they could not potentiate CCh-induced contraction, suggesting that in clinical settings, vasopressors such as NE, DA, and Phe might be safely used in patients with asthma.

Key words α_1-Agonist · α_1-Adrenoceptors · Phosphatidylinositol response · Tracheal smooth muscle

Introduction

Vasopressors, such as dopamine (DA), norepinephrine (NE), and phenylephrine (Phe), are commonly used during anesthesia to increase blood pressure through α_1-adrenoceptors. Although α_1-adrenoceptors, as well as β-adrenoceptors, exist in airway smooth muscle [1], and α_1-adrenoceptor agonists are reported to stimulate human airway smooth-muscle contraction [2,3], vasopressors are used without concern for their effects on airway smooth muscle in patients with asthma.

When α_1-adrenoceptors in smooth muscle are stimulated to activate the phosphatidylinositol (PI) response, increased inositol 1,4,5-trisphosphate (IP_3) mobilizes Ca^{2+} from the sarcoplasmic reticulum [4]. Subsequently, the increase in cytoplasmic Ca^{2+} concentration causes smooth-muscle contraction.

It is not clear whether vasopressors can stimulate the PI response or induce additional airway smooth-muscle contraction in patients with vagotonic asthma. The present study was designed to examine the effects of vasopressors on the contractile and PI responses of the rat trachea induced by a muscarinic agonist, carbachol (CCh).

Materials and methods

The studies were conducted under guidelines approved by the Animal Care Committee of the Nagasaki University School of Medicine. Thirty-six male Wistar rats (Charles River, Yokohama, Japan) weighing 250–350 g were used for the experiments. The rats were anesthetized with pentobarbital sodium (50 mg·kg^{-1} intraperitoneal), and the trachea was rapidly isolated.

Contractile response

The trachea was cut into 3-mm-wide ring segments with a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, England). A tracheal ring was suspended between two stainless-steel hooks and placed in a 5-ml water-jacketed organ chamber (Kishimotoika, Kyoto, Japan) containing Krebs-Henseleit (K-H) solu-

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tion (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11, Na_2-EDTA 0.05). The solution was continuously aerated with 95%O_2/5%CO_2 at a temperature of 37°C. Isometric tensions were measured with an isometric transducer (Kishimotoika, Kyoto, Japan), and changes in isometric force were recorded by a MacLab system (Milford, MA, USA). The resting tension was adjusted periodically to 1.5 g during the equilibration period. The ring was washed every 15 min and re-equilibrated to the baseline tension for 60 min (time 0).

First, at time 0, CCh at submaximal contraction (0.55 μM in final concentration) was added, and 30 min later, DA, NE, or Phe was added stepwise cumulatively to final concentrations in the range of 0.1–100 μM.

Second, to examine the effects of α_2- and β-adrenoceptors on the relaxation by NE of CCh-induced contraction, we used an α_2-adrenoceptor antagonist, yohimbine, and a β-adrenoceptor antagonist, propranolol. At time 0, yohimbine or propranolol (10 μM final concentration) was added; 15 min later, CCh was added; another further 30 min later, ring relaxation was induced by cumulative addition of NE.

PI response

Inositol 1,4,5-trisphosphate (IP_3) is rapidly degraded into inositol monophosphate (IP_1), which is recycled back to phosphatidylinositol via free inositol (Fig. 1). Lithium inhibits the conversion of IP_1 to inositol. Thus, in the presence of Li^+, the accumulation rate of IP_1 reflects the extent of PI response. We measured [^{3}H]IP_1 in tracheal slices incubated with [^{3}H]*myo*-inositol (Amersham, Tokyo, Japan) [5,6]. The trachea was cut longitudinally and chopped into 1-mm-wide pieces with a McIlwain tissue chopper. Three pieces of the tracheal slice were placed in small flat-bottomed tubes and preincubated for 15 min in K-H solution containing 5 mM LiCl. The solution was continuously aerated with 95%O_2/5%CO_2. An aliquot of 0.5 μCi [^{3}H]*myo*-inositol was then added to each tube (final concentration, 0.1 μM in a 300-μl incubation volume), and the tubes were flushed with 95%O_2/5%CO_2, capped, set in a shaking bath at 37°C, and incubated for 30 min (time 0).

We examined the effect of DA, NE, or Phe on CCh-induced IP_1 accumulation in rat tracheal slices. At time 0, varying doses (0, 1, 10, and 100 μM final concentration) of DA, NE, or Phe were added to the suspension of tracheal slices, the tubes were flushed with 95%O_2/5%CO_2, and 15 min later, CCh (0.55 μM final concentration) was added. After an additional 60 min, the reaction was stopped with 940 μl chloroform:methanol (1:2 v/v). Chloroform and water were then added (310 μl each), and the phases were separated by centrifugation at 90*g* for 5 min. [^{3}H]IP_1 was separated from [^{3}H]*myo*-inositol in the water phase of 750 μl by column chromatography using Dowex AG 1-X8 resin (Bio Rad, Richmond, CA, USA) in the formate form. The [^{3}H]IP_1 formed in the tracheal slices was counted with a liquid scintillation counter and presented in becquerels (Bq).

Data were expressed as mean ± SE. The results of repeated measures and multiple groups were analyzed by two-way analysis of variance. Multiple pairwise comparisons between groups were assessed by Scheffé's test. A *P* value less than 0.05 was considered significant.

Results

Figure 2 shows that CCh at a dose of 0.1 μM or greater caused tracheal ring contraction. The CCh concentra-

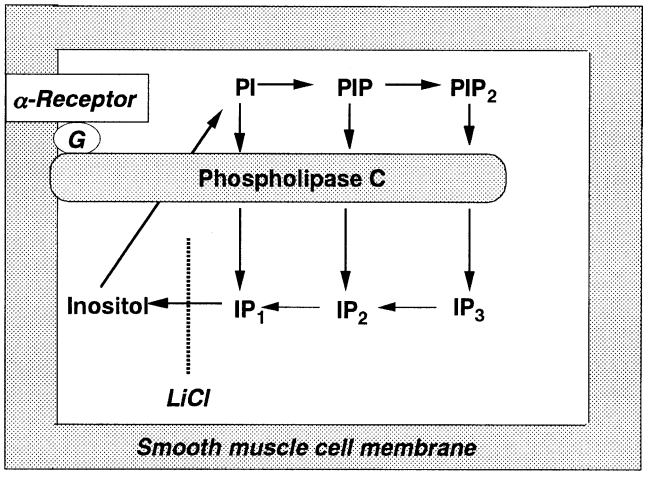

Fig. 1. PI response. *PI*, Phosphatidylinositol; *PIP*, phosphatidylinosltol monophosphate; PIP_2, phosphatidylinositol bisphosphate; IP_3, inositol 1,4,5-trisphosphate; IP_2, inositol bisphosphate; IP_1 inositol monophosphate; *G*, G protein

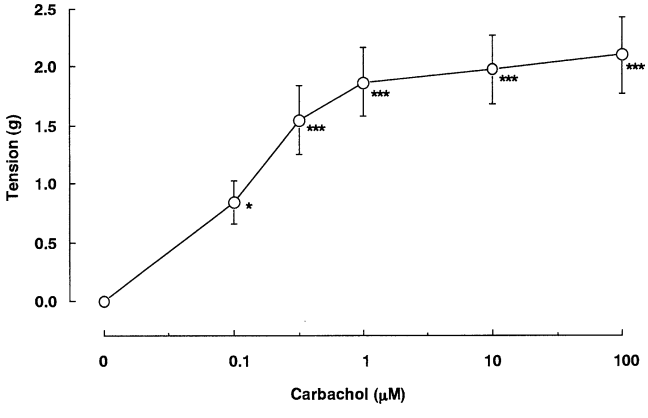

Fig. 2. Effects of carbachol on the resting tension of the rat trachea (mean ± SE; $n = 8$). $^*P < 0.05$, $^{***}P < 0.001$ vs 0

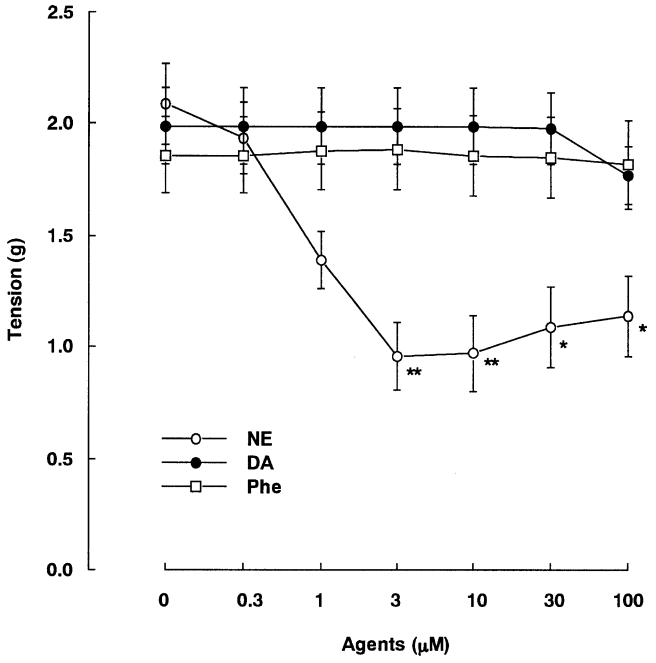

Fig. 3. Effects of norepinephrine (*NE*), dopamine (*DA*), and phenylephrine (*Phe*) on 0.55 μM carbachol carbachol-induced contraction of the rat trachea (mean ± SE; $n = 6$–8). $^{*}P < 0.05$, $^{**}P < 0.01$ vs 0

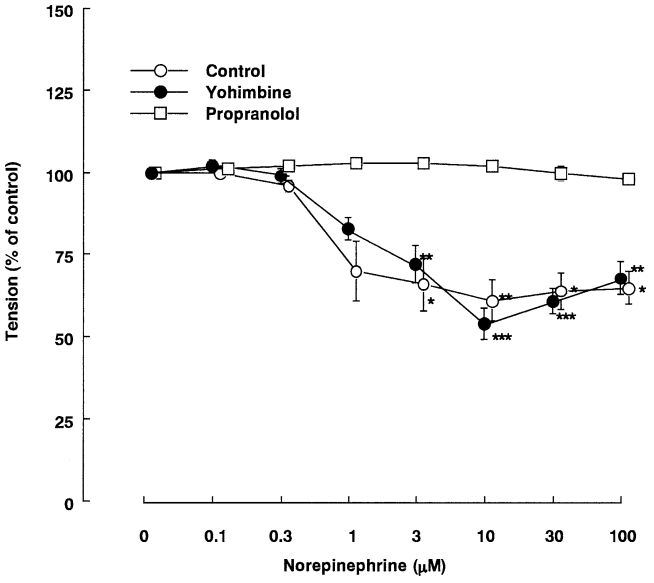

Fig. 4. Effects of norepinephrine on 0.55 μM carbachol-induced contraction of the rat tracheal rings in the presence and absence of yohimbine or propranolol (mean ± SE; $n = 6$–9). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs 0

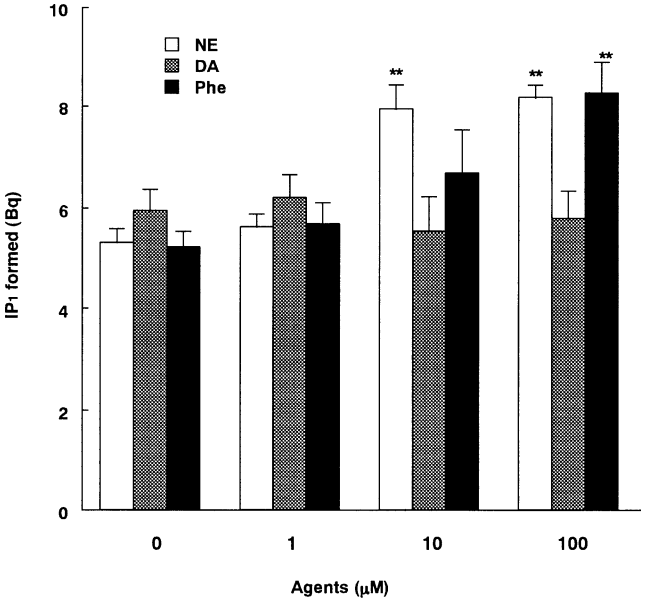

Fig. 5. Effects of norepinephrine (*NE*), dopamine (*DA*), and phenylephrine (*Phe*) on 0.55 μM carbachol-induced IP_1 accumulation in the rat trachea (mean ± SE; $n = 6$–8). $^{**}P < 0.01$ vs 0

tion needed to cause 80% of maximal contraction was 0.55 μM.

Figure 3 shows the effects of NE, DA, and Phe on CCh-induced contraction of the rat trachea. NE attenuated CCh-induced tracheal ring contraction at a dose of 1 μM or greater and had its maximal effect at 3 μM. DA and Phe did not affect CCh-induced tracheal ring contraction.

Figure 4 shows the effects of NE on CCh-induced contraction of the rat trachea in the presence and absence of yohimbine or propranolol. Attenuation by NE of CCh-induced contraction was completely inhibited by propranolol but was not affected by yohimbine.

Figure 5 shows the effects of NE, DA, and Phe on CCh-induced IP_1 accumulation in the rat trachea. NE and Phe potentiated CCh-induced IP_1 accumulation at doses of 10 μM and 100 μM, respectively.

Discussion

The present results show that NE attenuates CCh-induced contraction.

Preuss et al. [7] reported significant regional variation regarding α-adrenoceptor-mediated contraction of rat tracheal rings. They found that α-adrenoceptor stimulation-induced contraction of tracheal rings prepared from the laryngeal end of the trachea was much larger than that of rings prepared from the carinal end. We mainly used the middle and carinal regions of the rat trachea.

The relaxant effects of NE may be mediated via airway epithelium, α_2-adrenoceptors, and β-adrenoceptors. First, epithelial cells release mediators that can inhibit bronchoconstriction by relaxing the underlying smooth muscle. Fedan et al. [8] reported that the epithelium

reduced access of bronchoactive agents such as isoproterenol, whereas an immediate relaxant effect of epithelium-derived relaxing factors released by agonists could not be demonstrated. Barnes et al. [9] reported that the relaxant response to isoproterenol was enhanced in the presence of epithelium, although this was significant only in the case of precontraction with 5-HT. Thus, it is unlikely that the relaxant effects of NE on CCh-induced contraction are mediated via airway epithelium. Second, we previously reported that the clonidine attenuated the CCh-induced contractile and PI responses of rat trachea [10]. In addition, Kamikawa et al. [11] reported that the inhibitory effect of NE on electrical field stimulation-induced contraction of guinea pig bronchial rings was prevented by both propranolol and yohimbine. These findings suggest that the relaxant effects of NE might be mediated via both β- and α_2-adrenoceptors. However, in the present study, yohimbine did not affect the relaxant effects of NE, whereas propranolol completely inhibited the relaxant effects of NE on rat tracheal rings. Thus, the relaxant effect of NE on CCh-induced contraction of rat tracheal rings would be mediated via β-adrenoceptors.

Although α_1-adrenoceptor agonists such as NE and Phe potentiated CCh-induced IP_1 accumulation, they could not potentiate CCh-induced contraction. Lal et al. [12] reported that Phe injected into the pulmonary artery produced selective increases in pulmonary perfusion pressure without affecting airway tone in the isolated, perfused rat lung. In the present study, Phe did not affect CCh-induced tracheal ring contraction, which is consistent with results reported by Lal et al. [12] However, it potentiated CCh-induced IP_1 accumulation in the rat trachea. The effect of NE on CCh-induced contraction can be explained by the β-adrenoceptor mechanisms. However, the inconsistency between the effects of Phe on the CCh-induced PI response and on CCh-induced tracheal ring contraction cannot be explained by the β-adrenoceptor mechanisms, because Phe is not a β-adrenoceptor agonist, but rather an α-adrenoceptor agonist. Agonists induce three breakdown systems of the PI cascade in airway smooth muscle, i.e., phosphatidylinositol (PI), phosphatidylinositol monophosphate (PIP), and phosphatidylinositol bisphosphate (PIP_2) (Fig. 1). In the presence of lithium, the accumulation rate of IP_1 reflects the extent of the PI response. In vascular smooth muscle, α_1-adrenoceptor agonists stimulate contraction by activating of the PI response. NE stimulates both contraction and IP_1 accumulation in the rat aorta [6]. To stimulate the contractile response, mobilization of Ca^{2+} from the sarcoplasmic reticulum by IP_3 and influx of Ca^{2+} from the extracellular space are indispensable conditions. Thus, NE-induced IP_1 in a vascular study would be a metabolite of IP_3 through hydrolysis of PIP_2. On the other hand, in the present study, Phe stimulated IP_1 accumulation, but it did not potentiate CCh-induced contraction. Thus, Phe-induced IP_1 may not be a metabolite of IP_3 through the hydrolysis of PIP_2, but through the direct hydrolysis of PIP or PI. Namely, α_1-adrenoceptor-coupled mobilization of Ca^{2+} from the sarcoplasmic reticulum by IP_3 and influx of Ca^{2+} from the extracellular space may not be induced in the rat trachea. The best experiment may be to measure IP_3 directly using a mass assay, but this method may not be sensitive enough to resolve the issue. Assessment of the relative accumulations of $[^3H]IP_1$ from PI/PIP versus IP_1 from PIP_2 by high-performance liquid chromatography (HPLC) methods is needed to give an indication of the routes of metabolism.

In the present study, DA did not change tracheal smooth muscle tone. Michoud et al. [13] reported that in guinea pig tracheal chains, DA produced relaxation that was completely blocked by propranolol, whereas in human and dog tracheal smooth muscle, DA induced a contraction that was entirely abolished by an α-adrenoceptor antagonist. These findings suggest that DA would indirectly affect β- and α-adrenoceptors. NE is an agonist of α- and β-adrenoceptors. Koga et al. [14] reported that DA appeared to cause the release of NE from noradrenergic nerves. Thus, the preparations used in the present study would not contain functional noradrenergic nerves, so that there would be no release of NE from noradrenergic nerves. Subsequently DA did not affect CCh-induced contraction of rat tracheal rings.

The plasma concentrations of NE and DA are 0.02 μM and 0.5 μM during infusion of $0.12\,\mu g \cdot kg^{-1} \cdot min^{-1}$ of NE and $6\,\mu g \cdot kg^{-1} \cdot min^{-1}$ of DA, respectively [15]. The concentrations used in the present study are outside the clinical range. However, it is well known that the density of adrenergic receptors and muscarinic receptors differs among the trachea and the small and large bronchus. In addition, the distribution of these receptors is different among species. Thus, we cannot conclude that these vasopressors can be safely used in patients with asthma.

In conclusion, although NE and Phe potentiated CCh-induced IP_1 accumulation, they could not potentiate CCh-induced contraction, suggesting that there is no direct relationship between the PI response induced by α_1-adrenoceptor agonists and airway smooth muscle tension, and that in clinical settings, vasopressors such as NE, DA, and Phe might be safely used in patients with asthma.

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