Relaxing Effect of Vesnarinone (OPC-8212) on the Tracheal Muscle Strips Isolated from Guinea Pigs

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ABSTRACT—The effect of vesnarinone (OPC-8212), an orally active positive inotropic agent was studied in tracheal muscle isolated from guinea pigs, and the mechanism of its action was analyzed. Vesnarinone (10^-6 –10^-4 M) caused a concentration-dependent relaxation of tracheal muscle pre-contracted by 10^-4 M histamine. The potency of the relaxing effect of vesnarinone was greater than that of theophylline; the pD2 values for vesnarinone and theophylline were 4.9 and 4.5, respectively. Vesnarinone reduced the high-K+ induced contracture of depolarized tracheal muscle non-competitively (pD'^2=3.7). Vesnarinone at the low concentration of 3 × 10^-6 M shifted the concentration-response curve for isoproterenol in a parallel fashion to the left. Vesnarinone additively acted on the relaxing effect of isobutyl methyl xanthine. Propranolol (10^-5 M) and reserpine pre-treatment (5 mg/kg, i.p., 24 hr) had no effect on the relaxing effect of vesnarinone. These results suggested that vesnarinone elevated the intracellular cyclic AMP level via phosphodiesterase inhibition, resulting in the tracheal muscle relaxation.

Keywords: Vesnarinone, Tracheal muscle, Cyclic AMP

Vesnarinone (OPC-8212) is an orally active positive inotropic drug that was synthesized by Tominaga et al. (1). Yamashita et al. (2) reported that it had a useful profile in the treatment of heart failure because it caused a selective positive inotropic effect with no obvious positive chronotropic and vascular effects. We previously studied the mechanism of the positive inotropic effect of vesnarinone in guinea pig papillary muscle (3). We found that vesnarinone strengthened the slow membrane action potential, whereas the muscarinic agonist carbachol inhibited the positive inotropic effect of vesnarinone. Taira and colleagues (4–6) reported that vesnarinone inhibited the cyclic AMP phosphodiesterase and elevated the cyclic AMP level, thereby producing its positive inotropic effect. Rapundalo et al. (7) also showed that vesnarinone inhibited the low-Km phosphodiesterase. These results suggest the involvement of cyclic AMP in the positive inotropic effect of vesnarinone. As for the lack of a positive chronotropic effect, Iijima and Taira (8) showed that vesnarinone decreased the outward potassium current, and Yanagisawa et al. (9) suggested that the absence of the chronotropic effect was related to the effect on the potassium current.

In general, c-AMP-phosphodiesterase inhibitors relax tracheal smooth muscle (10). We first observed the relaxing effect of vesnarinone on the tracheal muscle strips (11). However, there have been no other reports on the effect of vesnarinone in tracheal muscle. The present experiments were carried out to extend our previous study on the relaxing effect of vesnarinone in the airway smooth muscle, and to examine whether the mechanism of its action was dependent upon the phosphodiesterase inhibition in the same manner as methylxanthines.

MATERIALS AND METHODS

Tracheal muscle preparations

Guinea pigs of either sex, weighing from 300 to 450 g, were used. The tracheae were excised from the animals killed by a sharp blow on the skull. The muscle strips were prepared from the excised trachea by cutting it spirally with a sharp scalpel (12, 13) into strips of 20 mm length with a width of 2 to 3 mm. The muscle strip was mounted vertically and suspended at a resting tension of 1.0 g in a chamber containing a modified Tyrode solution of 50 ml.

Bathing solution

Two kinds of bathing solution were prepared: The composition of the Tyrode solution was as follows: 137 mM NaCl, 12 mM NaHCO3, 0.42 mM NaH2PO4, 1.8 mM
CaCl₂, 2.7 mM KCl, 0.52 mM MgCl₂ and 5.6 mM glucose (pH = 7.4 at 37°C). In the experiments to examine the extracellular calcium concentration dependence of high-K⁺-induced contractions, a K⁺-rich, Ca²⁺-free Tyrode solution was used. The total K⁺ concentration was raised to 40 mM by replacing equivalent amounts of NaCl with that of KCl, thereby bringing the muscle strips to a partially depolarized state. The bathing solution was constantly gassed with a mixture of 95% O₂ and 5% CO₂ and kept at a temperature of 37°C.

**Measurement and recording of tension**

The muscle tension was measured isometrically by means of a strain-gauge type transducer (SB-1T-H, Nihon Kohden, Tokyo) and an amplifier (RP-5, Nihon Kohden), and recorded on the graph paper of the recording device (VP-6521, Matsushita Communication Industrial, Yokohama). The resting force of the preparation was kept constant at 1.0 g. All test preparations were allowed to equilibrate for at least 40 min in a normal Tyrode solution before drug application.

**Drugs and chemicals**

Vesnarinone and its solvent sulfolane were supplied from the Research Institute of Otsuka Pharmaceutical Co. (Osaka). The following materials were purchased from the indicated sources: histamine·2HCl and theophylline (Wako Pure Chemical Industry, Osaka), l-propranolol·HCl (Rheinpharma, Heidelberg, Germany), reserpine (Daiichi Seiyaku Co., Tokyo), carbachol chloride (Tokyo Kasei Co., Tokyo), papaverine·HCl (Houei Yakuko Co., Osaka), l-isoproterenol·HCl (Nikkien Kagaku Co., Tokyo), and 3-isobutyl-1-methylxanthine (IBMX; Aldrich Chemical Co., Milwaukee, WI, U.S.A.)

**Analysis of concentration-response curves and statistical analysis**

The dose needed for 50% relaxation was determined by the concentration-relaxation curves, and the negative logarithm of this dose, pD₂, was calculated. In the experiments of high-K⁺-induced contraction, the negative logarithmic concentration of vesnarinone needed for a twofold parallel shift of the Ca²⁺-concentration-tension curve to the right was designated as pA₂ and that for a 50% downward shift of the curve was designated as PD₂.

Student's t-tests were carried out to evaluate the difference of the results from two groups. In all Figures and Tables, measured values are expressed as the means ± S.E.

**RESULTS**

**Relaxing effect of vesnarinone on histamine-induced contraction**

Vesnarinone produced a relaxation of the tracheal muscle that was pre-contracted with 10⁻⁴ M histamine in Tyrode solution. Vesnarinone (in sulfolane: final concentration kept constant at 0.3%) caused a relaxation concentration-dependently in a range from 10⁻⁶ to 10⁻⁴ M. Sulfolane, the solvent of vesnarinone, had a weak relaxing potency at 0.3% (final concentration); At a final concentration of 0.3%, it relaxed the tracheal muscle by 16.8% (Fig. 1). Accordingly, all experiments to observe the relaxing effect of vesnarinone and theophylline for histamine contraction were carried out in the presence of 0.3% sulfolane. Each concentration of compound with 0.3% sulfolane was administered in a bolus single shot and then washed out 3 times by drug-free Tyrode solution. Histamine (10⁻⁴ M) caused a reversible contraction of almost the same extent. Therefore, the concentration-relaxation relationship for each compound was plotted after subtracting the relaxing effect of 0.3% sulfolane alone. Since the maximum relaxation occurred with 3 x 10⁻⁴ M theophylline plus 0.3% sulfolane, this response was plotted as 100% relaxation in Fig. 2. The relaxing potencies of theophylline and vesnarinone expressed by the pD₂ values were 4.47 ± 0.02 (n = 6) and 4.94 ± 0.03 (n = 4), respectively. The pD₂ value of vesnarinone was significantly larger than that of theophylline (P < 0.01).

**Influence of l-propranolol and reserpinization on the relaxant action of vesnarinone**

In our previous study (3), we reported that the positive...
inotropic effect of vesnarinone developed with or without β1-receptor blocking. Experiments were carried out to examine the role of β2-receptors in the relaxing effect of vesnarinone on histamine-induced contractures of guinea pig tracheal muscle. The concentration-response curve for vesnarinone was not affected by the presence of 10^-5 M β-propranolol (Fig. 3). Also, reserpine pre-treatment (5 mg/kg, i.p., 24 hr) had no effect on the relaxing effect of vesnarinone (data not shown).

**Relaxing effect of vesnarinone on carbachol-induced contraction**

The relaxing effect of vesnarinone on the contraction of tracheal muscle strip induced by the muscarinic agonist carbachol (10^-5 M) was examined in same manner as in Fig. 1. Vesnarinone (in sulfolane: final concentration kept constant at 1%) caused a concentration-dependent relaxation in a range from 10^-5 to 3 x 10^-4 M. Sulfolane (1%) alone relaxed the tracheal muscle by 21.8%. Accordingly, all experiments to observe the relaxing effect of papaverine, vesnarinone and theophylline for carbachol contraction were carried out in a condition of constant presence of 1% sulfolane. The results are shown in Fig. 4. The concentration-relaxation curve for each compound was plotted after subtracting the relaxing effect of 1% sulfolane alone. The relaxing response to 10^-4 M papaverine was taken as 100% relaxation. Because of the restriction of water solubility of vesnarinone or theophylline, we could not examine the relaxing effect in the higher dose range of these compounds. The pD2 value of vesnarinone and theophylline was 3.63 ± 0.04 (n=4) and 3.22 ± 0.04 (n=4), respectively. The pD2 value of vesnarinone was significantly larger than that of theophylline (P < 0.01).

**The relaxing effect of vesnarinone on high-K⁺-induced contracture of tracheal muscle**

Figure 5 shows the influence of vesnarinone on the contractile effect of Ca²⁺ in partially depolarized tracheal smooth muscle. The tracheal muscle strips were first incubated in normal Tyrode solution, washed 3 times with the

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**Fig. 2.** Dose-dependent relaxation of guinea pig tracheal muscle pre-contracted with 10^-4 M histamine by vesnarinone (●) and theophylline (○). The extent of relaxation was plotted after subtracting the relaxing effect of the vehicle (sulfolane, 0.3%). Circles and bars represent the mean ± S.E. Numbers of experiments were 4 (vesnarinone) and 6 (theophylline), respectively.

**Fig. 3.** Relaxing effect of vesnarinone on guinea pig tracheal muscle in the absence (○) and presence (●) of 10^-3 M propranolol. Circles and bars represent the mean ± S.E. Numbers of experiments were 6, respectively.

**Fig. 4.** Comparison of the relaxing potency of vesnarinone, papaverine and theophylline on 10^-5 M carbachol-induced contraction. The extent of relaxation with 10^-4 M papaverine was taken as 100% relaxation. Symbols: (▲) papaverine, (●) vesnarinone, (○) theophylline. Symbols and bars represent the mean ± S.E. Four experiments were performed for each drug.
Ca²⁺-free Tyrode solution and then incubated in the Ca²⁺-free and K⁺-rich (40 mM) solution for an hr. In the control experiments, we added CaCl₂ cumulatively into the bathing solution in the absence of drugs. The second control experiments were carried out in the presence of either 0.03, 0.09 or 0.30% sulfolane, since sulfolane itself relaxed the tracheal muscle. Then the muscle strip was washed again with the Ca²⁺-free Tyrode solution and treated for 10 min with either 10⁻⁵, 3×10⁻⁵ or 10⁻⁴ M of vesnarinone in 0.03, 0.09 or 0.3% sulfolane (Fig. 5: A, B, C). Subsequently, CaCl₂ was added to the bathing solution by the same procedure as used in the control experiments. The Ca²⁺ concentration-response curve with 10⁻⁵ M vesnarinone was shifted to the right in a parallel fashion (Fig. 5A). The pAₘ value of competitive antagonism of vesnarinone with regards to the high-K⁺-induced contraction (in presence of sulfolane) was 5.22±0.05 (n=5). At the higher concentrations of 3×10⁻⁵ M and 10⁻⁴ M vesnarinone, a downward shift of the curve was observed (Fig. 5: B and C). Vesnarinone non-competitively antagonized the high-K⁺-induced contraction (in presence of sulfolane) with a pDₘ value of 3.71±0.05 (n=5). Reserpine pre-treatment (5 mg/kg, i.p., 24 hr) did not affect pDₘ value for the relaxing effect of vesnarinone (3.73±0.04, n=5).

**Potentiation of the relaxing effect of isoproterenol by vesnarinone**

From the reported evidence (3, 5) that vesnarinone potentiated the positive inotropic effect of isoproterenol in guinea pig papillary muscle, similar experiments were performed using guinea pig tracheal muscle. Six tracheal muscle preparations were divided into two groups. One group was treated with 3×10⁻⁹ M vesnarinone in 0.009% sulfolane, and the other group was treated with sulfolane alone in an equivalent dose. The preparations of both groups were equilibrated for 10 min. The following experiments were carried out in the presence of 0.009% sulfolane. During the time of incubation, vesnarinone produced a slight relaxation of the tracheal muscle (9.8±1.1% of maximum contraction produced by histamine). Next, all the tracheal muscle strips were contracted by adding 10⁻⁴ M histamine. Then isoproterenol was cumulatively added into the bathing solution in concentrations ranging from 10⁻¹⁰ to 3×10⁻⁷ M. After the maximum response of relaxation, each group of muscle strips was washed with normal Tyrode solution at least 3 times. Subsequently, the experimental protocols were exchanged between the two groups. The maximum extent of relaxation by 3×10⁻⁷ M isoproterenol was taken as 100% relaxation. Thus the concentration-response curves for isoproterenol were depicted as shown in Fig. 6. The pDₘ values for isoproterenol determined from the curves in the absence and presence of vesnarinone were 7.91±0.03 and 8.48±0.06 (n=6), respectively. Vesnarinone significantly (P<0.01) shifted the concentration-response curve for isoproterenol to the left in a parallel fashion, but the maximum relaxing effect of isoproterenol was not significantly affected by vesnarinone.
Influence of vesnarinone on the relaxing effect of IBMX

The potentiation of the relaxing effect of isoproterenol on tracheal muscle by vesnarinone lead to the next experiment to assess the phosphodiesterase inhibition. If the mechanism by which vesnarinone relaxed tracheal muscle originated from phosphodiesterase inhibition, there must exist an additive correlation between this drug and IBMX, a well-established phosphodiesterase inhibitor (14, 15).

Figure 7 shows the influence of vesnarinone on the concentration-relaxation curves for IBMX in tracheal muscle contracted by $10^{-4}$ M histamine. Tracheal muscle preparations were divided into 4 groups, and vesnarinone at a concentration of 0 ($0.03\%$ sulfolane alone), 3 $\mu$M, 6 $\mu$M and 10 $\mu$M were added into the bathing solution in a state of histamine-induced contracture. The final concentration of sulfolane was kept at 0.03%. Vesnarinone relaxed the tracheal muscle by 9, 32, 45% of the maximum relaxation produced by IBMX. The extent of the maximum relaxation by IBMX was almost the same as the maximum response by papaverine or vesnarinone. IBMX was added cumulatively to the tracheal muscle preparations of the respective group, from which four concentration-relaxation curves were obtained. Figure 7A shows the theoretical curves calculated from the assumption that IBMX and vesnarinone additively acted on the same mechanism. Comparing the pD$_2$ values (4.94 and 5.92) obtained from the data in Figs. 2 and 7, vesnarinone at the concentration of 3, 6 and 10 $\mu$M will be equipotent to IBMX concentrations of 0.3, 0.6 and 1 $\mu$M, since the potency of vesnarinone was 1/10 of that of IBMX. The expected curves could be drawn, like those in Fig. 7A, that show the concentration-relaxation curve of IBMX in the presence of any drug having the same mechanism of action. Figure 7B shows the concentration-relaxation curves of IBMX on guinea pig tracheal muscle in the presence of vesnarinone. Vesnarinone shifted the concentration-response curve for IBMX upwardly, rather than in a parallel fashion. The maximum relaxing effect of IBMX was not significantly

**Fig. 6.** Potentiation by vesnarinone of the relaxing effect of isoproterenol on $10^{-4}$ M histamine-induced contraction. Symbols: (○) presence of $3 \times 10^{-6}$ M vesnarinone and sulfolane ($0.009\%$), (□) presence of sulfolane alone ($0.009\%$). Circles and bars represent the mean±S.E. The number of experiments was 6.

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**Fig. 7.** Additive relaxing effect of vesnarinone and IBMX in guinea pig tracheal muscle contracture produced by $10^{-4}$ M histamine. Tracheal preparations were divided into 4 groups and vesnarinone at final concentrations of 0 ($0.03\%$ sulfolane alone (○)), 3 $\mu$M (●), 6 $\mu$M (■) and 10 $\mu$M (▲) were added into the bathing solution. Figure 7A shows the control concentration-relaxation curves of IBMX and the theoretical curves (dotted lines) calculated from the assumption that IBMX and vesnarinone additively acted on the same mechanism. Figure 7B shows the concentration-relaxation curves of IBMX on guinea pig tracheal muscle in the presence of vesnarinone. Symbols and bars represent the mean±S.E. Numbers of experiments were 6.
affected by vesnarinone. Comparing Fig. 7: A and B, we found that the two curves closely resembled each other. This is consistent with the assumption that vesnarinone and IBMX have the same inhibitory effect on the phosphodiesterase.

DISCUSSION

The present experiments clearly showed that vesnarinone relaxed the guinea pig tracheal muscle dose-dependently. The potency of this drug for the relaxation was 2 to 3 times stronger than that of theophylline either for histamine- or carbachol-induced contracture. For carbachol-induced contracture, the relaxing effect of vesnarinone was, however, less potent than the histamine-induced one as observed in the case of theophylline, nitroglycerin or isoproterenol for acetylcholine-induced contracture (16). Propranolol or reserpine pre-treatment had no effect on the relaxing effect of vesnarinone.

The following observations have been made about the positive inotropic effect of vesnarinone on the heart muscle: 1) The positive inotropic effect was decreased by carbachol (3, 5, 6). This suggested the involvement of cyclic AMP in the positive inotropic effect, since carbachol was generally accepted as a depressant for cyclic AMP-mediated intervention (17–19). 2) Vesnarinone potentiated the positive inotropic effects of isoproterenol and histamine, adenylate cyclase stimulators (3, 5), but did not affect the dose-response curves of cardiac glycoside (3). 3) Vesnarinone increased the force of rested-state contraction like β-agonists, phosphodiesterase inhibitors and dibutyl cyclic AMP (3, 20–22). 4) The slow action potentials elicited from partially depolarized guinea pig papillary muscle were increased as like β-agonists and phosphodiesterase inhibitors (3, 22–24). These results suggest that vesnarinone increases the cyclic AMP levels in the myocardium and produces the positive inotropic effect.

We also observed in another study that vesnarinone increased the cyclic AMP level significantly in guinea pig Langendorff heart (25). In analogy, the involvement of cyclic AMP will be assumed in guinea pig tracheal muscle relaxation. It is generally accepted that an increased cyclic AMP in tracheal smooth muscle is closely associated with the relaxation as suggested by Murad and Kimura (26). The present study showed that vesnarinone acted on the same mechanisms, like the action of IBMX, while the existence of a potentiative relationship between isoproterenol and vesnarinone on tracheal muscle, just like the case of its positive inotropic effect (3, 5), favors the view that intracellular cyclic AMP was increased by phosphodiesterase inhibition, not by adenylate cyclase stimulation. Taira et al. (4) showed that vesnarinone inhibits cyclic AMP phosphodiesterase. Rapundalo et al. (7) also reported that vesnarinone is a selective inhibitor of the low-K_m, c-GMP-inhibited phosphodiesterase (CGI-PDE).

The present study showed that vesnarinone is not only an inotropic drug with a moderately high potency (3) but also a potent relaxant of airway smooth muscle. The relaxing potency of vesnarinone was 3 times more potent than that of theophylline. The clinical application of vesnarinone for the treatment of heart failure is restricted from the incidence of agranulocytosis. However, Busch et al. (27) reported that the toxic effect of vesnarinone on bone-marrow progenitor cells was dose-dependent and not exhibited at very low drug concentration, with a threshold at the lower end of the therapeutic range. In this study, we found that the pD_2 value of vesnarinone on tracheal muscle relaxation (pD_2=4.94) was larger than that on the positive inotropic effect (pD_2=4.27) in guinea pig papillary muscle (3). Vesnarinone will act more selectively on tracheal smooth muscle than on cardiac muscle. These results suggest the possibility of another clinical application of vesnarinone for the treatment of obstructive attack of the respiratory way.

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