Induction of Dyspnea by Aerosol of Endothelin-1/Histamine or /Methacholine in the Conscious Guinea Pig

Tomoyuki KOSHI,*[,a] Yoshinori ETOH,[a] Takahiro TORII,[a] Yasushi WADA,[a] Mitsuteru HIRATA,[a] Masao OHKUCHI,[a] and Tetsuro OKABE[b]

Tokyo Research Laboratories, Kowa Co., Ltd.,[a] 2-17-43 Noguchi-cho, Higashimurayama, Tokyo 189, Japan and The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo,[b] 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. *Received September 7, 1991*

The inhalation of aerosol of endothelin-1 (ET-1) induced dyspneal behavior in conscious guinea pigs pretreated with histamine or methacholine inhalation, although it was not observed by the inhalation of ET-1 alone. The dyspneal response to the inhalation of ET-1/histamine was potently inhibited by indomethacin, but it was not affected by nifedipine or diphenhydramine.

Keywords endothelin-1; histamine; indomethacin; dyspnea; conscious guinea pig

Introduction

It has generally been recognized that endothelin-1 (ET-1), composed of 21-amino acid residues with two intramolecular disulfide linkages, is produced by various cells (vascular endothelial cells, lung epithelial cells, *etc.*) and that this mediator shows a contracting effect on vascular,[1,2] bronchial,[3,4] and cardiac smooth muscle.[5] It was also reported that ET-1 showed the contracting effect on isolated tracheal strips from guinea pigs and was the most potent bronchoconstrictor hithero known.[3] In addition, ET-1 administered intravenously increased the pulmonary inflating pressure in anesthetized guinea pigs.[6] In this study, the induction of dyspneal behavior by exposure to ET-1 aerosol and effects of various compounds on the response were investigated in conscious guinea pigs.

Materials and Methods

Materials were obtained from the following commercial sources and used according to the procedures given by the respective suppliers: Male Hartley strain guinea pigs weighing 350 to 500 g from Clean Experiment Animals Center Inc. (Tokyo); histamine dihydrochloride, methacholine chloride, indomethacin and nifedipine from Sigma Co. (St. Louis); diphenhydramine hydrochloride from Kowa Co., Ltd. (Tokyo); and ET-1 from Peptide Institute Inc. (Osaka). ET-1 was dissolved in phosphate buffered saline (pH 7.4) containing 0.1% bovine serum albumin.

Guinea pigs were placed in an exposure chamber of 5 l volume and exposed to histamine or methacholine aerosol for 30 s. The aerosol was produced by placing the histamine solution (1 mg/ml) or methacholine solution (1 mg/ml) in a nebulizer (Azma-mist Nephron Co., U.S.A.) attached to the side of the exposure chamber. This exposure treatment was repeated 3 times at intervals of 30 min. Thirty minutes after the pretreatment, the guinea pig was exposed to ET-1 aerosol for 60 s and was observed for 15 min for the appearance of dyspnea. The intensity of dyspnea behavior was graded by 3 observers unaware of the treatment, as follows: 0; no symptom, 1; light abdominal respiration, 2; heavy abdominal respiration, 3; systemically heavy respiration accompanied with convulsion.

The test drug was injected into the ear vein 5 min before nebulization of ET-1. The doses used in this study clearly showed pharmacological action (data not shown).

Statistics of all data were carried out by a Mann-Whitney U-test.

Results and Discussion

The induction of dyspnea by ET-1 aerosol exposure was investigated in conscious guinea pigs. Lagente *et al.*[7] recently demonstrated that a bronchopulmonary response (changes in volume of excess air) was induced by aerosol administration of 5 or 10 μg/ml ET-1 for 1 min in anesthetized and ventilated guinea pigs. However, in this study, dyspnea in conscious guinea pigs was not induced even by the aerosol inhalation of 300 μg/ml ET-1 for 1 min as shown in Table I. The aerosol exposure of 1 mg/ml histamine or methacholine induced dyspneal behavior but the response immediately disappeared. On the contrary, the exposure to ET-1 after the recovery from dyspnea by pretreatment of animals with histamine or methacholine caused dose-dependently heavy dyspnea, a syndrome similar to an asthmatic attack. The background of the discrepancy in the two results is thought to be as follows. First: the amount of ET-1 inhaled freely in the chamber by a conscious guinea pig may have been less than that inhaled forcibly in Lagente's experiment.[7] Second: the sensitivity to ET-1 in the conscious guinea pig trachea might be lower than that in an anaesthetized one.

It was further considered that the mechanism of this ET-1/histamine induced dyspnea is not due to direct histaminic action, but to the increase in sensitivity to

TABLE I. Dyspneal Response Induced by the Combination of ET-1 with Histamine or Methacholine

ET-1 (mg/ml)	Pretreatment		Number of animals	Dyspnea intensity			
	Drug	(mg/ml)		0	1	2	3
0.1	—		5	5	0	0	0
0.3	—		5	5	0	0	0
0.03	Histamine	1	5	5	0	0	0
0.1	Histamine	1	5	0	4	1	0
0.3	Histamine	1	5	0	0	0	5
0.03	Methacholine	1	5	5	0	0	0
0.1	Methacholine	1	5	2	3	0	0
0.3	Methacholine	1	5	0	0	2	3

TABLE II. Effects of Various Drugs on ET-1/Histamine Induced Dyspnea Response in Conscious Guinea Pigs

Drug	Dose (mg/kg i.v.)	Number of animals	Dyspnea intensity			
			1	2	3	4
Control		6	0	0	3	3
Diphenhydramine	0.3	6	0	0	2	4
Nifedipine	0.3	6	0	1	3	2
Indomethacin	5	6	4	2	0	0

There was significant difference ($p<0.01$) between control and indomethacin groups (Mann-Whitney U-test).

ET-1 since the dyspnea was not inhibited by i.v. injection of 300 μg/kg diphenhydramine, an anti-histamine drug (Table II).

It was reported recently that ET-1 leveles in bronchial exudate increased during acute asthmatic episodes,[8] and that ET-1 was not produced only by endothelial cells but also by lung epithelial cells.[9] Endogenous ET-1 released from the tracheal epithelium could be a candidate for an ET-1 induced asthma attack under such hypothetical preparatory conditions as those induced by endogenous histamine or cholinergic stimulants of the nervous system.

On the other hand, pretreatment with 5 mg/kg indomethacin, a cyclooxygenase inhibitor, inhibited the dyspnea in our experiments, but 300 μg/kg of nifedipine, a calcium channel blocker, was somewhat effective. These observations were similar to the results of Lagente's experiment on the bronchopulmonary response in anaesthetized guinea pig.[7]

In contrast, it has been shown that ET-1 induced contraction of guinea pig isolated tracheal strips was slightly reduced by the administration of indomethacin, but was inhibited by nifedipine.[4] These results indicate that the mechanism of ET-1/histamine-induced dyspnea *in vivo* is different from that *in vitro*. The former seems to be induced *via* cyclooxygenase metabolites such as thromboxane A_2, and the latter due to calcium influx.

References

1) M. Yanagisawa, H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto, and T. Masaki, *Nature* (London), **332**, 411 (1988).
2) Y. Tomobe, T. Miyauchi, A. Saito, M. Yanagisawa, S. Kimura, K. Goto, and T. Masaki, *Eur. J. Pharmacol.*, **152**, 373 (1988).
3) Y. Uchida, H. Ninomiya, M. Saotome, A. Nomura, M. Ohtsuka, M. Yanagisawa, K. Goto, T. Masaki, and S. Hasegawa, *Eur. J. Pharmacol.*, **154**, 227 (1988).
4) C. A. Maggi, R. Patacchini, S. Giuliani, and A. Meli, *Eur. J. Pharmacol.*, **160**, 179 (1989).
5) T. Ishikawa, M. Yanagisawa, S. Kimura, K. Goto, and T. Masaki, *Am. J. Physiol.*, **255**, H970 (1988).
6) A. N. Payne and B. J. R. Whittle, *Eur. J. Phamacol.*, **158**, 303 (1988).
7) V. Lagente, P. E. Chabrier, J. M. Mencia-Huerta, and P. Braquet, *Biochem. Biophys. Res. Commun.*, **158**, 625 (1989).
8) A. Nomura, Y. Uchida, M. Kameyama, M. Saotome, K. Oki, and S. Hasegawa, *Lancet*, ii, 747 (1989).
9) P. N. Black, M. A. Ghatei, K. Takahashi, D. Bretherton-Watt, T. Krausz, C. T. Dollery, and S. R. Bloom, *FEBS Lett.*, **225**, 129 (1989).