Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 494 (2004) 73–76

www.elsevier.com/locate/ejphar

Short communication

Growth factor-induced contraction of human bronchial smooth muscle is Rho-kinase-dependent

Reinoud Gosens[a,*,1], Dedmer Schaafsma[a,1], Mechteld M. Grootte Bromhaar[a], Bart Vrugt[b], Johan Zaagsma[a], Herman Meurs[a], S. Adriaan Nelemans[a]

[a] *Department of Molecular Pharmacology, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands*
[b] *Department of Pathology and Pulmonology, Martini Hospital, P.O. Box 30033, 9700 RM Groningen, The Netherlands*

Received 15 April 2004; accepted 26 April 2004

Abstract

Growth factors have been implicated in the pathophysiology of asthma. However, the putative effects of these growth factors on human airway smooth muscle tone are still largely unknown. We performed contraction experiments using human bronchial smooth muscle ring preparations. The growth factor insulin-like growth factor-1 (IGF-1) induced a slowly developing sustained contraction, which was dependent on Rho-kinase, since contraction was almost completely inhibited by (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexane carboxamide (Y-27632; 1 μM). Angiotensin II, a G_q-coupled receptor agonist which can act as a growth factor as well, induced a biphasic contraction, the sustained phase of which was also almost completely inhibited by Y-27632. We conclude that angiotensin II and IGF-1 induce a Rho-kinase-dependent sustained contraction of human bronchial smooth muscle. Since growth factors are associated with pathophysiologiocal conditions such as asthma, inhibition of Rho-kinase could be effective under these conditions.

Keywords: Growth factor; Bronchial smooth muscle; Rho-kinase

1. Introduction

Growth factors are involved in proliferation and differentiation of smooth muscle cells originating from a variety of tissues, including the vasculature and the airways (Bayes-Genis et al., 2000; Hirst, 2000). They are potential contributors to the increased airway smooth muscle mass, as found in patients suffering from persistent severe asthma, by stimulating airway smooth muscle proliferation (Hirst, 2000).

However, in vascular smooth muscle, several growth factors have been shown to induce a concentration-dependent contraction and to be potential inducers of contractile mediator release (Berk et al., 1986; Berk and Alexander, 1989). The mechanism by which growth factors induce contraction has only been partly elucidated. Recent evidence shows that growth factor receptors, such as the insulin-like growth factor-1 (IGF-1)-receptor, can activate the Rho/Rho-kinase pathway directly (Taya et al., 2001) and may be involved in smooth muscle contraction via Rho-kinase (Fukata et al., 2001).

As regards airway smooth muscle, growth factors have been found to have long-term effects on bovine tracheal smooth muscle contractile phenotype (Gosens et al., 2002) and acute contractile effects on guinea-pig tracheal smooth muscle (Nasuhara et al., 1996). Thus far, however, the acute contractile effects of growth factors on human bronchial smooth muscle and the potential contribution of Rho-kinase have not been described. Therefore, we investigated human bronchial smooth muscle contraction induced by the growth factor IGF-1 and by angiotensin II which can transactivate different growth factor receptors

* Corresponding author. Tel.: +31-50-363-3321; fax: +31-50-363-6908.
E-mail address: R.Gosens@farm.rug.nl (R. Gosens).
[1] Both authors contributed equally.

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.04.035

(Eguchi and Inagami, 2000) and was shown to be a hypertrophic growth factor for human airway smooth muscle cells (McKay et al., 1998). In addition, we evaluated the possible involvement of Rho-kinase in growth factor-induced contraction.

2. Materials and methods

Macroscopically normal sections of human lungs, obtained from patients undergoing surgery as a consequence of lung carcinoma, were transported to our laboratory in Krebs–Henseleit (KH) buffer (composition (mM): NaCl 117.5, KCl 5.60, $MgSO_4$ 1.18, $CaCl_2$ 2.50, NaH_2PO_4 1.28, $NaHCO_3$ 25.00 and glucose 5.50, pregassed with O_2/CO_2 95:5% v/v, pH = 7.4). Human bronchi, typically 3–4 mm in diameter, were dissected free from connective tissue and cut into rings of approximately 3 mm in width. Bronchial rings were transferred into Dulbecco's modification of Eagle's medium, supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml) and maintained overnight at 37 °C in a humidified atmosphere containing 5% CO_2. The next day, bronchial rings were transferred into organ baths containing KH-buffer (37 °C), continuously gassed with O_2/CO_2 95:5% v/v. Rings were connected to an isometric force displacement transducer, using surgical wire. After equilibration for 90 min with three washings, rings were contracted using iso-osmotic KCl-solutions (20 and 40 mM) followed by washing. Finally, after re-equilibration in KH-buffer, rings were contracted with 40 mM KCl as a reference. After two additional washouts, rings were pre-incubated for 30 min with (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexane carboxamide (Y-27632; 1 μM), a selective inhibitor of the Rho-kinase pathway (Uehata et al., 1997) or vehicle, followed by the administration of growth factors or histamine.

2.1. Data analysis

All data represent means ± s.e.m. from *n* separate experiments. The statistical significance of differences between data was determined by one-way analysis of variance. Differences were considered to be statistically significant when $P < 0.05$.

2.2. Materials

Dulbecco's modification of Eagle's Medium was obtained from ICN Biomedicals (Costa Mesa, CA, USA). Penicillin/streptomycin solution (5000 U/ml; 5000 μg/ml) was obtained from Gibco BRL Life Technologies (Paisley, UK). Insulin-like growth factor (IGF-1, human recombinant) and angiotensin II were obtained from Sigma (St. Louis, MO, USA). Y-27632 was from Tocris Cookson (Bristol, UK). All other chemicals were of analytical grade.

3. Results

IGF-1 (10 ng/ml) induced a slow rise of myogenic tone, reaching 17.3 ± 5.7% of the 40 mM KCl response at $t = 30$ min (Fig. 1A). This sustained contraction induced by IGF-1 was prevented almost completely in the presence of Y-27632 (1 μM, $P < 0.001$; Fig. 1A). In contrast to IGF-1, angiotensin II (1 μM) induced a biphasic contraction in time, typically reaching its first transient peak approximately at 2 min after administration, which averaged 18.9 ± 6.0% of the KCl reference, followed by a slower and more sustained rise in myogenic tone, reaching a maximum of 24.5 ± 8.3% at $t = 10$ min (Fig. 1B). Interestingly, in the presence of the Rho-kinase inhibitor Y-27632 (1 μM), which lowered basal tone by itself, angiotensin II still induced the transient early-phase rise in contraction at

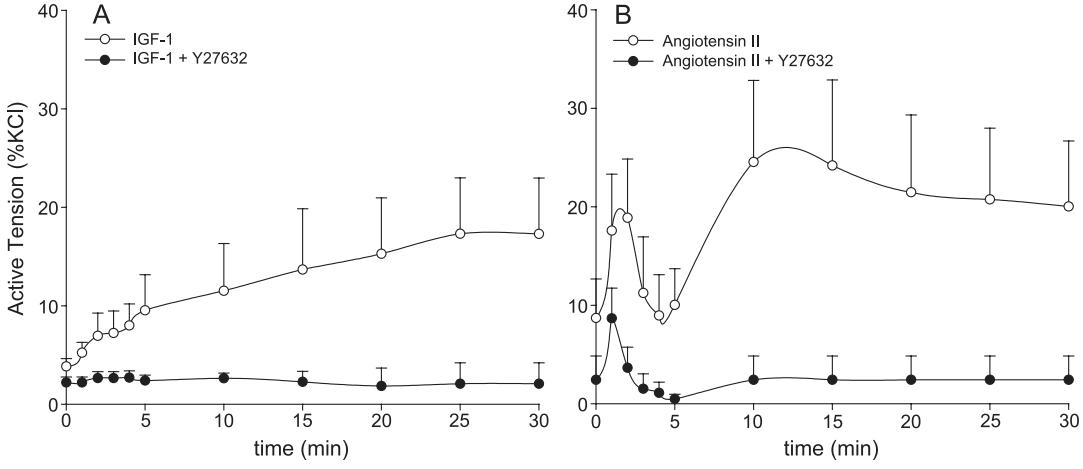

Fig. 1. Time-dependent IGF-1 (A) and angiotensin II (B)-induced contraction of isolated human bronchi. Contractile responses were measured both in the absence (open symbols) and presence (closed symbols) of 1 μM Y-27632. Shown are the means ± s.e.m. of three to four experiments each performed in duplicate.

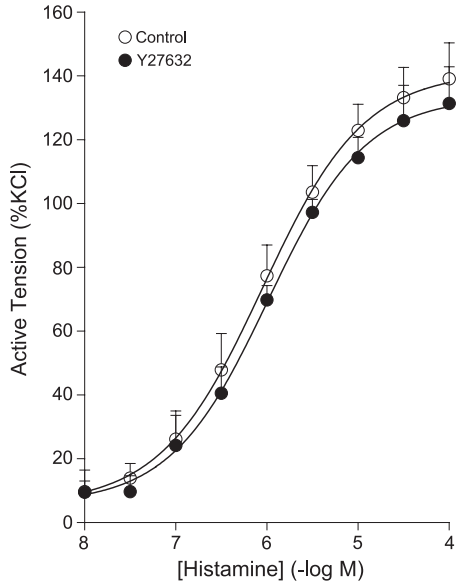

Fig. 2. Histamine-induced contraction of isolated human bronchi. Contractile responses were measured both in the absence (open symbols) and presence (closed symbols) of 1 μM Y-27632. Shown are the means ± s.e.m. of four experiments each performed in duplicate.

$t = 1$ min (8.6 ± 3.0%). The sustained rise, however, was no longer present ($P < 0.001$; Fig. 1B). Contractions induced by the G_q-coupled receptor agonist histamine were not affected at all by treatment with Y-27632 (Fig. 2).

4. Discussion

The results presented in this study demonstrate that the growth factors IGF-1 and angiotensin II induce Rho-kinase-dependent contractions of human bronchial smooth muscle. The concentrations of growth factor applied in this study are commonly used and represent submaximal to maximal concentrations with respect to other effects such as mitogenesis (Kelleher et al., 1995; Watanabe et al., 2001). Quantitatively, these contractions amounted approximately 20–25% of 40 mM KCl-induced contraction. These limited contractile effects may clearly have physiological implications as small changes in internal diameter may result in significant limitation of airflow, since flow is proportional to internal radius to the fourth power (Poiseuille's law).

Interestingly, histamine-induced contraction was not affected by this concentration of Y-27632, which indicates receptor-specificity for the contribution of Rho-kinase to contraction in the human bronchus. Similarly, methacholine- and KCl-induced contractions are differentially sensitive to Y-27632 as also observed in bovine tracheal smooth muscle (Gosens et al., 2004). Since Y-27632 predominantly affected the sustained angiotensin II-induced contraction, it seems likely that angiotensin II receptors couple to the Rho/Rho-kinase-pathway selectively for this phase of contraction. In addition to the G_q-coupled consequences of angiotensin AT_1-receptor activation, the angiotensin AT_1-receptor has been shown to be able of transactivating different growth factor receptors (Eguchi and Inagami, 2000). Such a transactivation leads to activation of downstream signaling molecules also used by growth factors themselves. Our results show that Rho-kinase is not involved in the transient contraction by angiotensin II, but only in the slowly developing sustained phase, indicating possible transactivation of growth factor receptors by angiotensin II. In accordance with our observation that IGF-1 induced responses are fully Rho-kinase-dependent, this phase is completely abolished by Y-27632.

Since growth factors are involved in tissue repair processes, growth factor-induced contraction may protect damaged areas in the airways from the outside air throughout the repair process. In asthma, however, the repair process is usually not restricted to a single segment of the airways and growth factors may then contribute to airflow obstruction. The growth factors used in the present study may be relevant for asthma for a number of reasons. The growth factor IGF-1 is known to be secreted in the airways from various inflammatory cells and airway smooth muscle (Johnson and Knox, 1997). In addition, plasma exudation may increase their tissue concentration during allergen exposure. Also, increased levels of the IGF-binding protein protease matrix metalloproteinase-1 (MMP-1) have been demonstrated in human asthmatic airways, which could increase the bio-availability of IGF-1 (Rajah et al., 1999). Angiotensin II has also been implicated in asthma as its serum levels are increased in patients with acute severe asthma (Millar et al., 1994). Moreover, intravenous administration of angiotensin II in concentrations similar to those endogenously observed in severe asthmatics causes acute bronchoconstriction in mild asthmatics (Millar et al., 1995). In addition, antigen-induced airway hyperresponsiveness in guinea pigs has been found in part angiotensin AT_1-receptor-dependent (Myou et al., 2002).

Another Rho-kinase inhibitor, fasudil, is being tested in clinical trials and has been shown to be very effective in inhibiting symptoms associated with hypertension (Masumoto et al., 2001) and coronary artery spasm (Masumoto et al., 2002). Rho-kinase inhibitors may surpass other drugs considering their selectivity for the pathophysiological condition. Such a pathophysiology-primed role may also be relevant in the airways, as repeated allergen challenge is known to increase the role of Rho-mediated Ca^{2+}-sensitization in antigen-induced airway hyperresponsive rats (Chiba et al., 1999). In addition, Y-27632 is known to suppress airway hyperresponsiveness induced by ovalbumin and respiratory syncytial virus in mice (Hashimoto et al., 2002). Therefore, the contraction induced by growth factors, which is completely dependent on Rho-kinase for IGF-1 and angiotensin II, may be more pronounced in inflamed areas and Rho-kinase inhibitors may relieve airflow limitations more pronouncedly in these areas when compared to control segments.

In conclusion, this study showed that the growth factors angiotensin II and IGF-1 induce a sustained contraction of human bronchial smooth muscle, which is completely dependent on Rho-kinase, in contrast to the histamine-induced contraction. Since growth factors are associated with pathophysiological conditions such as asthma, growth factor-induced contraction may be pathophysiologically relevant. Under these conditions, inhibition of Rho-kinase may be effective.

Acknowledgements

This work was financially supported by the Netherlands Asthma Foundation, grants NAF 99.53 (R.G.) and 01.83 (D.S.). The authors wish to acknowledge Dr. Lex Groote (Martini Hospital, Groningen) for collaboration.

References

Bayes-Genis, A., Conover, C.A., Schwartz, R.S., 2000. The insulin-like growth factor axis: a review of atherosclerosis and restenosis. Circ. Res. 86, 125–130.

Berk, B.C., Alexander, R.W., 1989. Vasoactive effects of growth factors. Biochem. Pharmacol. 38, 219–225.

Berk, B.C., Alexander, R.W., Brock, T.A. , Gimbrone Jr., M.A., Webb, R.C. 1986. Vasoconstriction: a new activity for platelet-derived growth factor. Science 232, 87–90.

Chiba, Y., Takada, Y., Miyamoto, S., MitsuiSaito, M., Karaki, H., Misawa, M., 1999. Augmented acetylcholine-induced, Rho-mediated Ca^{2+} sensitization of bronchial smooth muscle contraction in antigen-induced airway hyperresponsive rats. Br. J. Pharmacol. 127, 597–600.

Eguchi, S., Inagami, T., 2000. Signal transduction of angiotensin II type 1 receptor through receptor tyrosine kinase. Regul. Pept. 91, 13–20.

Fukata, Y., Amano, M., Kaibuchi, K., 2001. Rho–Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. Trends Pharmacol. Sci. 22, 32–39.

Gosens, R., Meurs, H., Bromhaar, M.M., McKay, S., Nelemans, S.A., Zaagsma, J., 2002. Functional characterization of serum- and growth factor-induced phenotypic changes in intact bovine tracheal smooth muscle. Br. J. Pharmacol. 137, 459–466.

Gosens, R., Schaafsma, D., Meurs, H., Zaagsma, J., Nelemans, S.A., 2004. Role of Rho-kinase in maintaining airway smooth muscle contractile phenotype. Eur. J. Pharmacol. 483, 71–78.

Hashimoto, K. , Peebles Jr., R.S., Sheller, J.R., Jarzecka, K., Furlong, J., Mitchell, D.B., Hartert, T.V., Graham, B.S. 2002. Suppression of airway hyperresponsiveness induced by ovalbumin sensitisation and RSV infection with Y-27632, a Rho kinase inhibitor. Thorax 57, 524–527.

Hirst, S.J., 2000. Airway smooth muscle as a target in asthma. Clin. Exp. Allergy 30, 54–59.

Johnson, S.R., Knox, A.J., 1997. Synthetic functions of airway smooth muscle in asthma. Trends Pharmacol. Sci. 18, 288–292.

Kelleher, M.D., Abe, M.K., Chao, T.S.O., Jain, M., Green, J.M., Solway, J., Rosner, M.R., Hershenson, M.B., 1995. Role of MAP kinase activation in bovine tracheal smooth muscle mitogenesis. Am. J. Physiol. 268, L894–L901.

Masumoto, A., Hirooka, Y., Shimokawa, H., Hironaga, K., Setoguchi, S., Takeshita, A., 2001. Possible involvement of Rho-kinase in the pathogenesis of hypertension in humans. Hypertension 38, 1307–1310.

Masumoto, A., Mohri, M., Shimokawa, H., Urakami, L., Usui, M., Takeshita, A., 2002. Suppression of coronary artery spasm by the Rho-kinase inhibitor fasudil in patients with vasospastic angina. Circulation 105, 1545–1547.

McKay, S., de Jongste, J.C., Saxena, P.R., Sharma, H.S., 1998. Angiotensin II induces hypertrophy of human airway smooth muscle cells: expression of transcription factors and transforming growth factor-beta1. Am. J. Respir. Cell Mol. Biol. 18, 823–833.

Millar, E.A., Angus, R.M., Hulks, G., Morton, J.J., Connell, J.M., Thomson, N.C., 1994. Activity of the renin–angiotensin system in acute severe asthma and the effect of angiotensin II on lung function. Thorax 49, 492–495.

Millar, E.A., Nally, J.E., Thomson, N.C., 1995. Angiotensin II potentiates methacholine-induced bronchoconstriction in human airway both in vitro and in vivo. Eur. Respir. J. 8, 1838–1841.

Myou, S., Fujimura, M., Kita, T., Watanabe, K., Hirose, T., Tachibana, H., Ishiura, Y., Nakao, S., 2002. Effect of intranasal administration of CV-11974, a type 1 angiotensin II receptor antagonist, on airway hyperresponsiveness and airway inflammation induced by antigen inhalation in guinea pigs. Int. Arch. Allergy Immunol. 129, 86–92.

Nasuhara, Y., Munakata, M., Sato, A., Amishima, M., Homma, Y., Kawakami, Y., 1996. Mechanisms of epidermal growth factor-induced contraction of guinea pig airways. Eur. J. Pharmacol. 296, 161–168.

Rajah, R., Nachajon, R.V., Collins, M.H., Hakonarson, H., Grunstein, M.M., Cohen, P., 1999. Elevated levels of the IGF-binding protein protease MMP-1 in asthmatic airway smooth muscle. Am. J. Respir. Cell Mol. Biol. 20, 199–208.

Taya, S., Inagaki, N., Sengiku, H., Makino, H., Iwamatsu, A., Urakawa, I., Nagao, K., Kataoka, S., Kaibuchi, K., 2001. Direct interaction of insulin-like growth factor-1 receptor with leukemia-associated RhoGEF. J. Cell Biol. 155, 809–820.

Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M., Narumiya, S., 1997. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 389, 990–994.

Watanabe, T., Pakala, R., Katagiri, T., Benedict, C.R., 2001. Angiotensin II and serotonin potentiate endothelin-1-induced vascular smooth muscle cell proliferation. J. Hypertens. 19, 731–739.