ELSEVIER

European Journal of Pharmacology 358 (1998) 41–47

ejp

The role of tyrosine kinase in hypoxic constriction of sheep pulmonary artery rings

Özge Uzun, A. Tuncay Demiryürek *, İlker Kanzık

Department of Pharmacology, Faculty of Pharmacy, Gazi University, Etiler, TR-06330 Ankara, Turkey

Received 9 March 1998; revised 4 August 1998; accepted 11 August 1998

Abstract

Complex and incompletely understood mechanisms underline the vascular responses to hypoxia. Recent studies showed that the tyrosine kinase pathway is involved in vasoconstriction of vascular smooth muscle. Therefore, the aim of this study was to determine the tyrosine kinase pathway for the hypoxic contraction in large-diameter sheep pulmonary artery rings in vitro by studying the effects of selective inhibitors of tyrosine kinase and of a protein tyrosine kinase inhibitor. Lowering the pO_2 from 96 to 5 mm Hg caused a contraction in arteries precontracted with 5-hydroxytryptamine (5-HT) but not under resting force. Preincubation of arteries with the tyrosine kinase inhibitors, genistein and tyrphostin, abolished the hypoxic contraction without affecting 5-HT contractions. Inhibition of tyrosine phosphatase activity by sodium orthovanadate increased the hypoxic vasoconstriction in 5-HT-precontracted arteries. These results suggest that the tyrosine kinase pathway is involved in hypoxic pulmonary vasoconstriction in sheep isolated pulmonary artery rings.

Keywords: Hypoxia; Genistein; Tyrphostin; Sodium orthovanadate

1. Introduction

Severe hypoxia has been shown to cause contraction of isolated pulmonary artery rings from dogs (Rorie and Tyce, 1983), pigs (Miller et al., 1989), rats (Rodman et al., 1989), cats (Madden et al., 1985) and humans (Demiryürek et al., 1993). We have also demonstrated that such a hypoxia-induced vasoconstriction occurs in pulmonary arteries isolated from sheep and is endothelium-dependent (Demiryürek et al., 1991a,b). Although K^+ channels (Post et al., 1992) and endothelium-derived vasoconstrictor and dilator substances (Amatya et al., 1989; Demiryürek et al., 1994; Wadsworth, 1994) have been shown to be involved in hypoxic vasoconstriction, inositol triphosphate has been reported not to be involved in the pulmonary hypoxic contractile response (Jin et al., 1993). Despite intensive study, the cellular mechanism of hypoxic pulmonary vasoconstriction remains unclear.

There is increasing evidence that protein–tyrosine phosphorylation is involved, not only in proliferation and transformation, but also in vasoconstriction of vascular smooth muscle (Tsuda et al., 1991; Di Salvo et al., 1993b, 1994; Laniyonu et al., 1994a,b; Hollenberg, 1994). In this pathway, the phosphorylation of proteins on tyrosine residues by tyrosine kinases has been suggested to contribute to signaling processes that lead to contraction. Two groups of tyrosine kinase inhibitors have been described: compounds interacting with the ATP binding site, such as genistein, an isoflavone compound (Akiyama et al., 1987) and those which interact with the substrate binding site, such as the tyrphostins, which are synthetic analogues of erbstatin (Levitzki and Gilon, 1991). Structurally different tyrosine kinase inhibitors inhibit contraction of visceral and vascular smooth muscle elicited with a variety of neurohumoral agents (Di Salvo et al., 1993a,b, 1994; Hollenberg, 1994). Contraction of visceral smooth muscle evoked with vanadate, a potent inhibitor of protein tyrosine phosphatases, is associated with enhanced tyrosine phosphorylation of several endogenous substrates (Di Salvo et al., 1993a).

Although receptor- and non-receptor-mediated stimuli such as 5-Hydroxytryptamine (5-HT), noradrenaline, protein kinase C-activating phorbol esters and the Ca^{2+} ionophore stimulate tyrosine phosphorylation (Tsuda et al., 1991; Abebe and Agrawal, 1995), there is currently no evidence for activation of tyrosine kinase pathway by hypoxia. The aim of this study was to determine the role of the tyrosine kinase pathway, using selective tyrosine ki-

* Corresponding author. Tel.: +90-532-4161032; Fax: +90-312-2235018; E-mail: dtuncay@neuron.ato.org.tr

0014-2999/98/$19.00
PII: S0014-2999(98)00592-5

nase inhibitors, genistein and tyrphostin, and a tyrosine phosphatase inhibitor, sodium orthovanadate, on hypoxic vasoconstriction in sheep isolated pulmonary artery rings.

2. Materials and methods

Lungs of freshly slaughtered sheep were obtained from a local abattoir and delivered in cooled oxygenated physiological salt solution to the laboratory within 10 min of excision. Pulmonary arteries (3–5 mm outer diameter at their optimal resting force) were isolated from lungs mainly from the second branch of the main pulmonary artery. Arteries were cleared of fat and adhering connective tissue. Care was taken to avoid stretching and damage to the luminal surface. In some preparations the endothelium was removed by gentle rubbing of the internal surface with a wooden stick. The removal of endothelium was confirmed by the lack of relaxation in response to bradykinin. The arteries were cut into rings 4–5 mm long. Segments were suspended in a water-jacketed organ bath (10 ml) filled with Krebs–Henseleit solution (37°C) of the following composition (in mM): NaCl 119; $NaHCO_3$ 25; KCl 4.6; $MgCl_2$ 1.2; KH_2PO_4 1.2; $CaCl_2$ 2.5; glucose 11. The solution was aerated with room air (normoxic) or a gas mixture containing 95% N_2–5% CO_2 (hypoxic). The rings were suspended on a pair of stainless-steel hooks, one of which was fixed to an L-shaped rod inside the chamber and the other to an isometric transducer (May FDT10-A, Ankara, Turkey) under optimal resting force. A stainless-steel hook was used to connect to the force displacement transducer. Isometric contractions were recorded continuously with an amplifier system (Tümel, Izmir, Turkey) on a computer, using the Labsys computer program.

Arterial rings were equilibrated in Krebs–Henseleit solution gassed with room air for an hour at their optimum resting force. The optimum resting force of the pulmonary rings was found to be 3 g by comparison the with tension developed with 20 mM KCl (EC_{50}) under different resting forces. The isometric contractions were calculated as force developed per cross-sectional area (Demiryürek et al., 1991a,b). The cross sectional-area (A) of the artery was calculated by using the equation: A = blotted weight of the artery$/h \times \beta$ where h = the distance (mm) between the stainless steel hook and rod with the artery ring under optimum resting and β the density of the artery ring which has been shown to be 1.05 g cm^{-3} in sheep carotid artery (Keatinge, 1968). The artery rings were exposed repeatedly (generally three times) to 20 mM KCl until two consecutive identical responses were observed before the start of the experimental protocol. The oxygen tension of the bathing medium was measured using an oxygen electrode (Jenway 9071 England). This electrode was calibrated to zero using sodium sulfite 100 mM in dissolved disodium tetraborate 10 mM.

2.1. Experimental protocol

2.1.1. Effects of hypoxia on artery rings precontracted with 5-HT or resting force

In artery rings, under resting force or precontracted with 6 μM 5-HT (EC_{50}), hypoxia was induced by changing to a 95% N_2–5% CO_2 gas mixture. pO_2 values ($n = 26$) (mmHg) of 47 ± 6, 21 ± 3, 10 ± 1, 7 ± 0.6, 6 ± 0.5, 5 ± 0.5, 5 ± 0.5, 5 ± 0.5 were obtained at 1, 2, 3, 4, 5, 10, 20 and 30 min, respectively. After 30 min of hypoxia, oxygenated conditions were reestablished by changing to room air to yield a pO_2 of 96 ± 2 mmHg ($n = 26$). In control experiments, two consecutive responses to 5-HT under oxygenated conditions and two consecutive responses to hypoxia were obtained.

2.1.2. Effects of tyrosine kinase inhibitors and tyrosine phosphatase inhibitor on hypoxic contraction in 5-HT precontracted arteries

Following the measurement of a control hypoxic contraction, either under resting force or in a 5-HT-precontracted artery, the tyrosine kinase inhibitors, genistein (30 μM, 20-min incubation, Laniyonu et al., 1994a,b) tyrphostin (50 μM, 15-min, Di Salvo et al., 1993b) and a tyrosine phosphatase inhibitor, orthovanadate (100 μM, 30 min incubation, Jin et al., 1996) were added to the bath and in the continued presence of the drugs a second hypoxic contraction was induced.

2.2. Drugs

5-Hydroxytryptamine creatine sulfate complex, potassium chloride, sodium orthovanadate bradykinin (all dis-

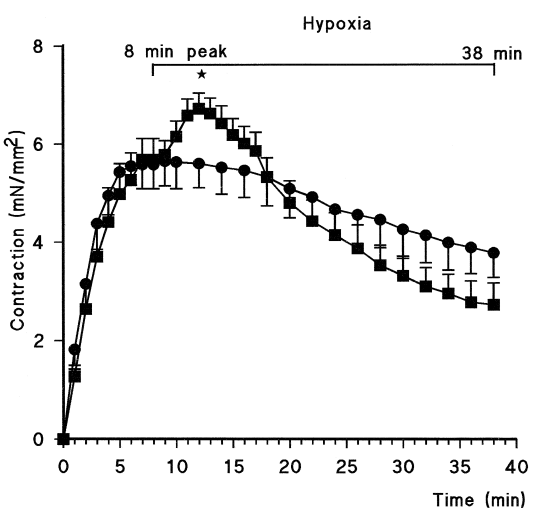

Fig. 1. The effect of hypoxia on sheep endothelium intact pulmonary artery rings precontracted with 5-HT at its EC_{50}. Contraction was measured at various time points up to 38 min after the administration of 5-HT. (●) 5-HT (6 μM) contraction during normoxia ($n = 7$). (■) The effect of hypoxia on 5-HT contraction ($n = 7$). $* P < 0.05$, when compared to normoxic values.

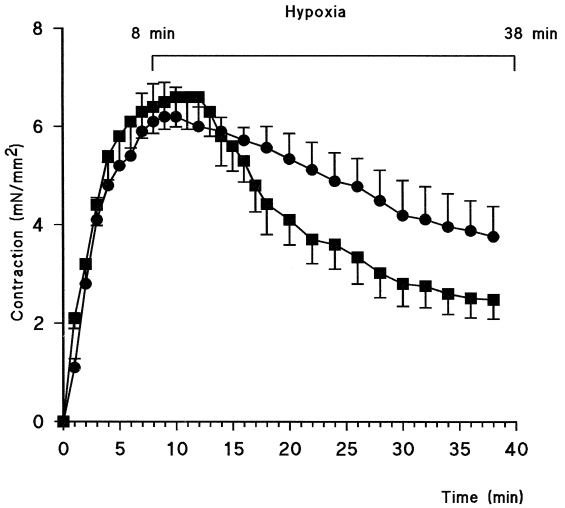

Fig. 2. The effect of hypoxia on sheep endothelium denuded pulmonary artery rings precontracted with 5-HT at its EC_{50}. Contraction was measured at various time points up to 38 min after the administration of 5-HT. (●) 5-HT (6 μM) contraction during normoxia ($n = 7$). (■) The effect of hypoxia on 5-HT contraction ($n = 6$).

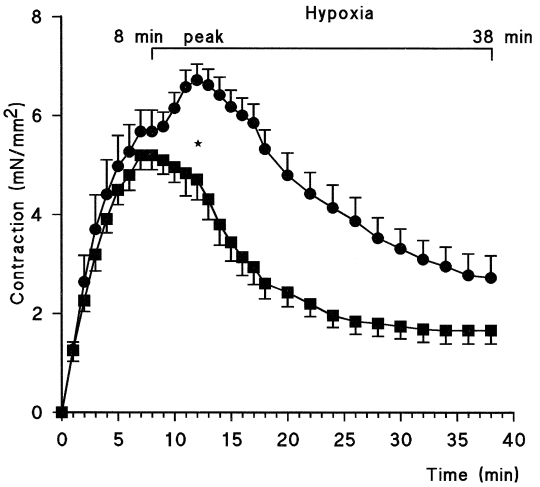

Fig. 3. The effect of genistein on hypoxic contraction in 5-HT precontracted pulmonary arteries. Contraction was measured at various time points up to 38 min after the administration of 5-HT. (●) 5-HT (6 μM) + hypoxia ($n = 7$). (■) The effect of hypoxia in 30 μM genistein-pretreated pulmonary artery rings ($n = 7$). $* P < 0.05$, when compared to before treatment values.

solved in distilled water), genistein and tyrphostin I (both dissolved in dimethyl sulfoxide) were obtained from Sigma (St. Louis, MO, USA).

2.3. Data analysis

All results are expressed as means ± S.E.M. *n* refers to the number of lungs used in the organ bath assay. The significance of for two groups was determined with Student's paired or unpaired *t*-test as appropriate. *P* values of less than 0.05 were considered to denote statistical significance of differences.

3. Results

3.1. Effects of hypoxia on artery rings precontracted with 5-HT or under resting force

Under normoxic conditions, 5-HT at its EC_{50}, caused a contraction which was not sustained, so that at 8 and 38 min post-administration, it was 5.6 ± 0.6 and 3.8 ± 0.5 mN mm^{-2} ($n = 6$), respectively. A second challenge with 5-HT resulted in a similar vasoconstriction; measurements at 8 and 38 min were 5.8 ± 0.7 and 4.1 ± 0.4 mN mm^{-2} ($n = 6$), respectively. Lowering the pO_2 of the bathing solution from 96 to 5 mmHg at the peak of the 5-HT contraction (Fig. 1) produced a further contraction. As this contraction was not sustained in all of the artery rings studied, measurements were made at two time points as illustrated in Fig. 1. The hypoxic contraction was measured as the difference between the contraction obtained

Table 1
Effects of hypoxia in endothelium-intact 5-HT-precontracted isolated pulmonary artery rings

	n	Contraction (mN mm^{-2})	
		8 min–peak	8–38 min
Normoxia	7	0.02 ± 0.01	-1.80 ± 0.30
Hypoxia	7	1.21 ± 0.50[a]	-3.20 ± 0.48[a]

[a] $P < 0.05$, when compared to normoxia values.

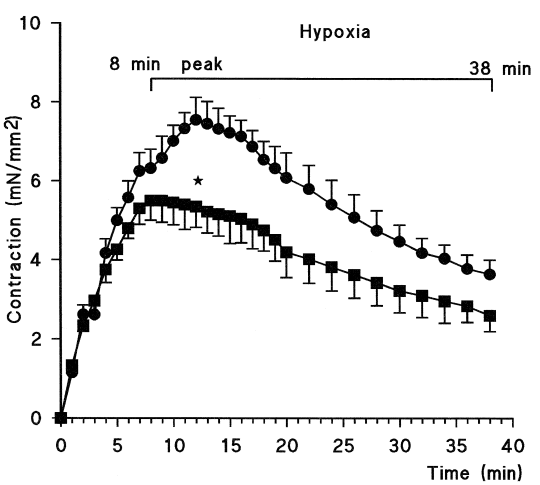

Fig. 4. The effect of tyrphostin on hypoxic contraction in 5-HT precontracted pulmonary arteries. Contraction was measured at various time points up to 38 min after the administration of 5-HT. (●) 5-HT (6 μM) + hypoxia ($n = 6$). (■) The effect of hypoxia in 50 μM tyrphostin-pretreated pulmonary artery rings ($n = 6$). $* P < 0.05$, when compared to before treatment values.

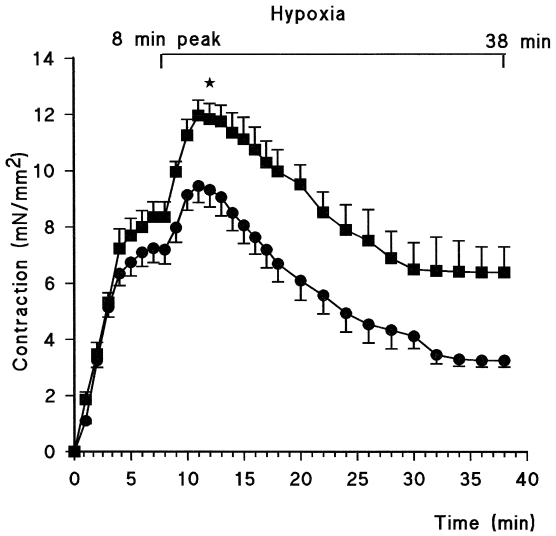

Fig. 5. The effect of orthovanadate on hypoxic contraction in 5-HT precontracted pulmonary arteries. Contraction was measured at various time points up to 38 min after the administration of 5-HT. (●) 5-HT (6 μM) + hypoxia ($n = 6$). (■) The effect of hypoxia in 100 μM orthovanadate-pretreated pulmonary artery rings ($n = 6$). $*$ $P < 0.05$, when compared to before treatment values.

just prior to hypoxia (i.e., 8 min of the 5-HT contraction) and that obtained at the peak of and again at the end of the hypoxic response (i.e., at 38 min of the 5-HT contraction). It can be seen that there were no significant differences between two consecutive 5-HT plus hypoxia-induced contractions. Hypoxic vasoconstriction was not observed in endothelium-denuded pulmonary arteries (Fig. 2). Table 1 shows the mean data obtained following two hypoxic challenges in 5-HT-precontracted endothelium-intact arteries. In consecutive 5-HT contractions under normoxic conditions, the difference in the contraction between 8 and 38 min was -1.8 ± 0.3 mN mm^{-2} ($n = 6$) and -1.7 ± 0.4 mN mm^{-2} ($n = 7$), respectively. Comparison of this data with that given in Table 1 shows that hypoxia did not cause a contraction even at the end of the hypoxic challenge.

Under optimum resting force, hypoxia for 30 min caused a slight, but significant, decrease in the baseline tone of the pulmonary artery rings from -0.28 ± 0.17 to -1.4 ± 0.35 mN mm^{-2} ($n = 6$). There was no difference in this hypoxic response with the second hypoxic exposure -1.77 ± 0.37 mN mm^{-2} ($n = 6$).

Table 3
Effects of genistein, tyrphostin and orthovanadate on hypoxic dilatation in pulmonary arteries under optimum resting force

	n	Tension (mN mm^{-2})	
		Before treatment (30 min)	After treatment (30 min)
Control	7	-1.40 ± 0.35	-1.77 ± 0.37
Genistein	7	-1.10 ± 0.40	-1.52 ± 0.35
Tyrphostin	6	-1.40 ± 0.42	-1.50 ± 0.40
Orthovanadate	7	-1.71 ± 0.39	-0.60 ± 0.21[a]

[a] $P < 0.05$, when compared to before treatments values.

3.2. Effects of genistein, tyrphostin and orthovanadate on hypoxic contraction in 5-HT precontracted arteries

As can be seen in Fig. 3, pretreatment with genistein (30 μM) had no effect on the contraction produced by 5-HT prior to the introduction of hypoxia. This figure also shows that genistein abolished the hypoxic contraction measured at the peak of the contraction. In the presence of the structurally different tyrosine kinase inhibitor, tyrphostin, 5-HT-induced contractions were unchanged (Fig. 4). Similarly to genistein, tyrphostin abolished hypoxic contractions in 5-HT-precontracted arteries (Fig. 4). As shown in Fig. 5 and Table 2, orthovanadate significantly augmented the hypoxic contraction in 5-HT-precontrated arteries.

3.3. Effects of genistein, tyrphostin and orthovanadate on hypoxic dilatation in pulmonary arteries under resting force

Inhibition of tyrosine kinase activity by genistein or tyrphostin did not change the hypoxic dilatation under resting force as shown in Table 3. However, pretreatment with tyrosine phosphatase inhibitor, orthovanadate, significantly inhibited hypoxic relaxation (Table 3).

Table 2
Effects of genistein, tyrphostin and orthovanadate on hypoxic contraction in endothelium-intact 5-HT-precontracted pulmonary arteries

Treatment	n	Hypoxic contraction (mN mm^{-2})			
		Before treatment		During treatment	
		8 min—peak	8–38 min	8 min—peak	8–38 min
Control	6	1.21 ± 0.50	-3.20 ± 0.48	0.97 ± 0.23	-3.20 ± 0.48
Genistein	7	1.07 ± 0.33	-2.94 ± 0.66	-0.50 ± 0.27[a]	-3.55 ± 0.27
Tyrphostin	6	1.15 ± 0.26	-2.75 ± 0.37	-0.16 ± 0.07[a]	-2.91 ± 0.49
Orthovanadate	7	2.11 ± 0.27	-3.95 ± 0.45	3.58 ± 0.56[a]	-2.53 ± 0.69

[a] $P < 0.05$, when compared to before treatment values.

4. Discussion

In the present study, we found that hypoxia-induced contraction was abolished by removal of endothelium in sheep pulmonary arteries. These observation are in agreement with the results of previous studies showing that hypoxic vasoconstriction is endothelium-dependent in sheep pulmonary arteries (Demiryürek et al., 1991a). It is likely that the profile of mediators released during hypoxia depends on the species and to some extent on the experimental conditions, such as the level of precontraction (Wadsworth, 1994).

5-HT induces tyrosine phosphorylation of several proteins in cultured rat aortic smooth muscle cells (Tsuda et al., 1991). The tyrosine kinase inhibitors, genistein and tyrphostin, decreased the potency of 5-HT and reduced maximal contraction in response to 5-HT in rat arterial strips denuded of endothelium (Watts et al., 1996), implying that tyrosine kinase activation may partially mediate contractility in response to 5-HT in arterial smooth muscle. However, in sheep pulmonary arteries, both genistein and tyrphostin were incapable of attenuating the contractile responses to 5-HT, suggesting lack of involvement of tyrosine kinases in these responses. These observations imply that species-related differences apparently exist. The reason for the discrepancy between responses is unknown.

Two structurally and functionally distinct tyrosine kinase inhibitors, genistein and tyrphostin, both prevented hypoxic contraction. No report is available in the literature concerning the effect of tyrosine kinases inhibitors on hypoxic vasoconstriction in pulmonary arteries. Therefore, we believe that our study is the first to provide evidence that the tyrosine kinase pathway is involved in the hypoxic vasoconstriction.

A tyrosine kinase pathway is involved in the regulation of Ca^{2+} entry since inhibitors of tyrosine kinase are able to attenuate presumed Ca^{2+} entry following agonist-mediated Ca^{2+} store depletion (Low, 1996). This observation is consistent with the hypothesis that the release of Ca^{2+} activates a tyrosine kinase that, in turn, stimulates Ca^{2+} entry (Huckle et al., 1990, 1992; Hollenberg, 1994) and hypoxic vasoconstriction is reduced by Ca^{+2} channel blockers (McMurtry et al., 1976; Harder et al., 1985). Inhibition of the tyrosine kinase causes tyrosine dephosporylation, which may lead to the inhibition of Ca^{2+} entry (Laniyonu et al., 1994a) and the abolition of hypoxic contraction. Additionally hypoxia may activate the tyrosine kinase pathway through an increase in the intracellular Ca^{2+} levels, which induces tyrosine kinase activity in vascular smooth muscles cells (Tsuda et al., 1991).

We demonstrated that potent tyrosine kinase inhibitors, genistein and tyrphostin, inhibited the contractile responses of sheep pulmonary arteries to hypoxia, which suggests that the hypoxic response of pulmonary artery is mediated by activation of tyrosine kinases. That similar effects were produced by the two structurally unrelated tyrosine kinase inhibitors, which act through different mechanisms (Akiyama et al., 1987; Gazit et al., 1989), confirms that the inhibition was the result of a specific action on tyrosine kinases. In our study, additional support for the involvement of tyrosine kinase in hypoxic pulmonary vasoconstriction was provided by the ability of sodium orthovanadate to potentiate the effects of the hypoxia. Sodium orthovanadate, by inhibiting the activity of tyrosine-specific phosphatases, has been shown to result in the enhanced expression of tyrosine kinase-mediated responses (Chao et al., 1992). Our results confirm the previous observation that vanadate causes potentiation of the hypoxic vasoconstriction in rat isolated lungs (Voelkel and Czartolomna, 1991). Since a protein kinase C inhibitor, staurosporine, blocked the potentiation of hypoxic contraction, it was concluded that activation of protein kinase C was involved in the mechanism of vanadate-induced potentiation of hypoxic constriction (Voelkel and Czartolomna, 1991). Vanadate not only inhibits tyrosine phosphatase activity (Swarup et al., 1982), but also stimulates the activity of tyrosine kinases as demonstrated in Kupffer cells (Chao et al., 1992) or cell-free preparations (Elberg et al., 1994). It has been widely accepted that vanadate causes its contractile effects via the inhibition of a tyrosine phosphatase pathway, thereby amplifying constitutive tyrosine kinase activity, so as to facilitate the calcium entry through voltage-sensitive calcium channels (Di Salvo et al., 1993a,b; Laniyonu et al., 1994a,b). Additionally, vanadate is also able to inhibit calcium ATPases (Raeymeakers et al., 1983). Inhibition of Ca^{+2}-ATPase and consequent intracellular calcium release may contribute to vanadate-induced contraction of smooth muscle (Sanchez-Ferrer et al., 1988). In contrast to results obtained with genistein and tyrphostin, the protein tyrosine phosphatase inhibitor, vanadate, enhanced hypoxic contraction in pulmonary artery. Together, these results provide evidence for the involvement of tyrosine kinase pathway in hypoxic contraction.

Although both genistein and tyrphostin in the concentrations used in this study can inhibit the activity of tyrosine kinases (Di Salvo et al., 1994; Laniyonu et al., 1994b), they have no significant effect on other enzymes, including myosin light chain kinase (Di Salvo et al., 1993b), protein kinase A (Akiyama et al., 1987; Gazit et al., 1989; Di Salvo et al., 1993b), or protein kinase C (Akiyama et al., 1987; Gazit et al., 1989). In addition, sodium orthovanadate has been shown to inhibit protein tyrosine phosphatases involved in agonist-induced responses selectively (Laniyonu et al., 1994a; Abebe and Agrawal, 1995). Therefore, the effects of the inhibitors observed in the current study were probably the result of selective inhibition of tyrosine kinases or tyrosine phosphatases.

There is an interrelationship between activation of the tyrosine kinase pathway and the myosin light chain kinase pathway in vascular smooth muscle (Jin et al., 1996). Myosin light chain phosphorylation have been shown to

correlate with the initiation of pulmonary arterial smooth muscle contraction in response to hypoxia (Zhao et al., 1996). Hypoxia may act on pulmonary arterial smooth muscle cells by increasing tyrosine kinase activity, leading to phophorylation of myosin light chain and smooth muscle contraction.

Although the role of endothelium-derived vasodilators in the mediation or modulation of hypoxic pulmonary vasoconstriction is under intense investigation, altered activity of endothelium-derived constrictor mediators may play an important role in hypoxia (Wadsworth, 1994). Recently, hypoxic contraction of porcine pulmonary arteries has been demonstrated to be mediated by a diffusible, contractile factor distinct from endothelin released from hypoxic endothelial cells (Gaine et al., 1998). Additionally, our results may suggest that hypoxia-induced contractile factor(s) may be released from the endothelium via a pathway that is dependent on tyrosine kinase activity.

In conclusion, hypoxic contraction, which is endothelium-dependent, was inhibited by tyrosine kinase inhibitors and potentiated by tyrosine phosphatase inhibitor in sheep isolated pulmonary arteries, providing evidence for a potential role of the tyrosine kinase pathway in hypoxic pulmonary hypertension.

Acknowledgements

This study was supported by a research grant from Gazi University (Project no:SBE 11/95-3).

References

Abebe, W., Agrawal, D.K., 1995. Role of tyrosine kinases in norepinephrine-induced contraction of vascular smooth muscle. J. Cardiovasc. Pharmacol. 26, 153–159.

Akiyama, T., Ishida, J., Nagakawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M., Fukami, Y., 1987. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J. Biol. Chem. 262, 5592–5593.

Amatya, M.P., Demiryürek, A.T., Kane, K.A., Wadsworth, R.M., Zeitlin, I.J., 1989. The role of prostacyclin and other mediators in hypoxic constriction of sheep isolated coronary artery rings. J. Vasc. Med. Biol. 1, 341–346.

Chao, W., Liu, H., Hanahan, D.J., Olson, M.S., 1992. Protein tyrosine phosphorylation and regulation of the receptor for platelet-activating factor in rat Kupffer cells. Effect of sodium vanadate. Biochem. J. 288, 777–784.

Demiryürek, A.T., Wadsworth, R.M., Kane, K.A., 1991a. Effects of hypoxia on isolated intrapulmonary arteries from the sheep. Pulm. Pharmacol. 4, 158–164.

Demiryürek, A.T., Wadsworth, R.M., Kane, K.A., 1991b. Pharmacological evidence for the role of mediators in hypoxia-induced vasoconstriction in sheep isolated intrapulmonary artery rings. Eur. J. Pharmacol. 203, 1–8.

Demiryürek, A.T., Wadsworth, R.M., Kane, K.A., Peacock, A.J., 1993. The role of endothelium in hypoxic constriction of human pulmonary artery rings. Am. Rev. Respir. Dis. 147, 283–290.

Demiryürek, A.T., MacLean, M.R., Wadsworth, R.M., Kane, K.A., 1994. Effects of hypoxia on endothelin-1 release from human, sheep and rabbit pulmonary artery rings. Endothelium 1, 251–258.

Di Salvo, J., Semenchuk, L.A., Lauer, J., 1993a. Vanadate-induced contraction of smooth muscle and enhanced protein tyrosine phosphorylation. Arch. Biochem. Biophys. 304, 386–391.

Di Salvo, J., Steusloff, A., Semenchuk, L., Satoh, S., Kolquist, K., Pfitzer, G., 1993b. Tyrosine kinase inhibitors suppress agonist-induced contraction in smooth muscle. Biochem. Biophys. Res. Commun. 190, 968–974.

Di Salvo, J., Pfitzer, G., Semenchuk, L., 1994. Protein tyrosine phosphorylation, cellular Ca^{+2} and Ca^{+2} sensitivity for contraction of smooth muscle. Can. J. Physiol. Pharmacol. 72, 1434–1439.

Elberg, G., Li, J., Shechter, Y., 1994. Vanadium activates or inhibits receptor and non-receptor protein tyrosine kinases in cell-free experiments, depending on its oxidation state. Possible role of endogenous vanadium in controlling cellular protein tyrosine kinase activity. J. Biol. Chem. 269, 9521–9527.

Gaine, S.P., Hales, M.A., Flavahan, N.A., 1998. Hypoxic pulmonary endothelial cells release a diffusible contractile factor distinct from endothelin. Am. J. Physiol. 274, L657–664.

Gazit, A., Yaish, P., Gilon, C., Levitzki, A., 1989. Tyrphostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. J. Med. Chem. 32, 2344–2352.

Harder, D.R., Madden, J.A., Dawson, C., 1985. Hypoxic induction of Ca^{2+}-dependent action potentials in small pulmonary arteries of the cat. J. Appl. Physiol. 59, 1389–1393.

Hollenberg, M.D., 1994. Tyrosine kinase pathways and the regulation of smooth muscle contractility. Trends Pharmacol. Sci. 15, 108–114.

Huckle, W.R., Procop, C.A., Dy, R.C., Herman, B., Earp, H.S., 1990. Angiotensin II stimulates protein–tyrosine phosphorylation in a Ca^{+2} dependent manner. Mol. Cell. Biol. 10, 6290–6298.

Huckle, W.R., Dy, R.C., Earp, H.S., 1992. Ca^{2+}-dependent increase in tyrosine kinase activity stimulated by angiotensin II. Proc. Natl. Acad. Sci. USA 89, 8837–8841.

Jin, N., Packer, C.S., English, D., Rhoades, R.A., 1993. Inositol triphosphate is involved in norepinephrine but not in hypoxia induced pulmonary arterial contraction. Am. J. Physiol. 264, L160–L164.

Jin, N., Siddiqui, R.A., English, D., Rhoades, R.A., 1996. Communication between tyrosine kinase pathway and myosin light chain kinase pathway in smooth muscle. Am. J. Physiol. 271, H1348–H1355.

Keatinge, N., 1968. Sodium flux and electrical activity of arterial smooth muscle. J. Physiol. 194, 183–200.

Laniyonu, A., Saifeddine, M., Ahmad, S., Hollenberg, M.D., 1994a. Regulation of vascular and gastric smooth muscle contractility by pervanadate. Br. J. Pharmacol. 113, 403–410.

Laniyonu, A.A., Saifeddine, M., Yang, S.G., Hollenberg, M.D., 1994b. Tyrosine kinase inhibitors and the contractile action of G-protein-linked vascular agonists. Can. J. Physiol. Pharmacol. 72, 1075–1085.

Levitzki, A., Gilon, C., 1991. Tyrphostins as molecular tools and potential antiproliferative drugs. Trends Pharmacol. Sci. 12, 171–173.

Low, A.M., 1996. Role of tyrosine kinase on Ca^{+2} entry and refilling of agonist-sensitive Ca^{+2} stores in vascular smooth muscles. Can. J. Physiol. Pharmacol. 74, 298–304.

Madden, J.A., Dawson, C.A., Harder, D.R., 1985. Hypoxia-induced activation in small isolated pulmonary arteries from the cat. J. Appl. Physiol. 59, 113–118.

McMurtry, I.F., Davidson, A., Reeves, J., Grover, R.F., 1976. Inhibition of hypoxic pulmonary vasoconstriction by calcium antagonists in isolated rat lungs. Circ. Res. 38, 99–104.

Miller, D.S., Hamilton, Y.A., Paterson, N.A.M., 1989. Role of aracidonic acid metabolites in hypoxic contraction of isolated porcine pulmonary artery vein. Exp. Lung Res. 15, 213–222.

Post, J., Hume, J., Archer, S., Weir, E.K., 1992. Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction. Am. J. Physiol. 262, C882–C890.

Raeymeakers, L., Wuytack, F., Eggermont, J., De Schutter, G., Casteels,

R., 1983. Isolation of plasma membrane fraction from gastric smooth muscle. Biochem. J. 210, 315–322.

Rodman, D.M., Yamaguchi, T., O'Brien, R., McMurty, I., 1989. Hypoxic vasoconstriction of isolated rat pulmonary artery. J. Pharmacol. Exp. Ther. 248, 952–959.

Rorie, D.K., Tyce, G.M., 1983. Effects of hypoxia on norepinephrine release and metabolism in dog pulmonary artery. J. Appl. Physiol. 55, 750–758.

Sanchez-Ferrer, C., Marin, J., Lluch, M., Valverde, A., Salaices, M., 1988. Action of vanadate on vascular tension and sodium pump activity in cat isolated cerebral and femoral arteries. Br. J. Pharmacol. 93, 53–60.

Swarup, G., Cohen, S., Garbers, D.L., 1982. Inhibition of membrane phosphotyrosyl-protein phosphatase activity by vanadate. Biochem. Biophys. Res. Commun. 107, 1104–1109.

Tsuda, T., Kawahara, Y., Shii, K., Koide, M., Ishida, Y., Yokoyama, M., 1991. Vasoconstrictor-induced protein–tyrosine phosphorylation in cultured vascular smooth muscle cells. FEBS Lett. 285, 44–48.

Voelkel, N.F., Czartolomna, J., 1991. Vanadate potentiates hypoxic pulmonary vasoconstriction. J. Pharmacol. Exp. Ther. 259, 666–672.

Wadsworth, R.M., 1994. Vasoconstrictor and vasodilator effects of hypoxia. Trends Pharmacol. Sci. 15, 47–53.

Watts, S.W., Yeum, C.H., Camphell, G., Webb, R.C., 1996. Serotonin stimulates protein tyrosyl phosphorylation and vascular contraction via tyrosine kinase. J. Vasc. Res. 33, 288–298.

Zhao, Y., Packer, S., Rhoades, R.A., 1996. Hypoxia-induced pulmonary arterial contraction appears to be dependent on myosin light chain phosphorylation. Am. J. Physiol. 15, L768–L774.