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Short communication

Nuclear factor-κB inhibitors abolish hypoxic vasoconstriction in sheep-isolated pulmonary arteries

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Abstract

The aim of this study was to determine the role of nuclear factor-κB (NF-κB) in hypoxic constriction of isolated pulmonary arteries. Rings were suspended in an organ bath filled with Krebs–Henseleit solution and isometric contractions were recorded continuously. Hypoxia (%95 N_2–%5 CO_2) had no marked effect on resting force in artery rings. However, hypoxia caused further contractions in serotonin-precontracted arteries. Hypoxia-induced vasoconstrictions were abolished by preincubation with NF-κB inhibitors, pyrrolidine dithiocarbamate (100 μM) or pyrithione (10 μM). These results suggest that reactive oxygen species and/or NF-κB activation may be involved in the hypoxia-induced vasoconstriction in sheep-isolated pulmonary arteries.

Keywords: Hypoxia; Pulmonary artery, sheep; Pyrrolidine dithiocarbamate; Pyrithione

1. Introduction

Nuclear factor-κB (NF-κB) is a protein transcription factor that is required for maximal transcription of many proinflammatory molecules thought to be important in the generation of inflammation (Christman et al., 2000). NF-κB is activated by the release of the inhibitory subunit IκB in response to various stimuli, including proinflammatory cytokines, viruses, activators of protein kinase C and reactive oxygen species. In experimental animals, NF-κB has been demonstrated in the lungs after ozone exposure or allergen challenge (Haddad et al., 1996; Liu et al., 1997). Animal studies have also demonstrated that endotoxemia, blood loss and hyperoxia result in increased NF-κB activation (Abraham, 2000). There are some clinical data relating NF-κB activation to the pathogenesis of acute lung injury, acute respiratory distress syndrome, systemic inflammatory response syndrome, asthma, respiratory viral infections, occupational and environmental lung disease and cystic fibrosis (Christman et al., 2000).

Despite intensive studies, the cellular mechanism of hypoxia-induced pulmonary vasoconstriction is still unclear and the role of NF-κB in hypoxia-induced vasoconstriction is not known. Additionally, the effects of NF-κB inhibitors on isolated pulmonary arteries have not been examined. In this study, we aimed to determine the contribution of NF-κB to hypoxia-induced vasoconstriction of large-diameter sheep pulmonary arteries by using NF-κB inhibitors.

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 optimum 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. The arteries were cut into rings 3–5 mm long. Segments were suspended in a water-jacketed organ

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bath (10 ml) filled with Krebs–Henseleit solution (at 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 75% N_2–20% O_2–5% CO_2 (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, Commat, Ankara, Turkey) under optimal resting force. Isometric contractions were recorded continuously with TDA 97 data acquisition system (Commat). Arterial rings were equilibrated in Krebs–Henseleit solution for an hour at their optimum resting force (3 g) (Uzun et al., 1998). The isometric contractions were calculated as force developed per cross-sectional area, as presented previously (Demiryurek et al., 1991; Uzun et al., 1998). 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) dissolved in disodium tetraborate (10 mM).

In artery rings precontracted with 6 μM serotonin (EC_{50}), hypoxia was induced by changing to a 95% N_2–5% CO_2 gas mixture ($n=13$) when serotonin contractions reached a maximum. pO_2 values (mm Hg) of 47 ± 7, 19 ± 3, 11 ± 0.9, 8 ± 0.6, 8 ± 0.7, 8 ± 0.7, 8 ± 0.7, 8 ± 0.7 were obtained at 1, 2, 3, 4, 5, 10, 20 and 30 min, respectively. After 30 min of hypoxia, oxygenated conditions were re-established by changing to a 75% N_2–20% O_2–5% CO_2 gas mixture to yield a pO_2 of 117 ± 4 mm Hg ($n=13$). Serotonin was washed out of the organ bath before the second period of hypoxia. Following the measurement of a control hypoxic contraction, pulmonary artery rings were incubated with pyrrolidine dithiocarbamate (100 μM, Kim et al., 2000) or pyrithione (10 μM, Kim et al., 1999) for 1 h. Then, in the continued presence of the drugs, a second hypoxic contraction was induced in the presence of serotonin.

2.1. Drugs

5-Hydroxytryptamine creatinine sulfate complex (serotonin), pyrithione, pyrrolidine dithiocarbamate (all dissolved in distilled water) were obtained from Sigma (St Louis, MO, USA).

2.2. Data analysis

All results are expressed as means ± S.E.M. *n* refers to number of lungs used in the organ bath assay. The significance between 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

Under oxygenated conditions, serotonin caused a contraction, which was repeatable after a second challenge. Lowering the pO_2 of the bathing solution from 97 to 5 mm Hg at the peak of the serotonin-induced contraction produced a further contraction (from 0.02 ± 0.01 mN mm^{-2}, $n=6$, to 2.1 ± 0.5 mN mm^{-2}, $n=6$, $P<0.05$). The hypoxia-induced contraction was measured as the difference between the contraction obtained just prior to hypoxia (i.e., 5 min of the serotonin contraction) and that obtained at the peak of and again at the end of the hypoxic response (i.e., at 35 min of the serotonin-induced contraction). In control experiments, the first and second (after 1 h) serotonin-induced contractions at 5 min were 5.8 ± 1.6 mN mm^{-2} ($n=5$) and 6.2 ± 1.3 mN mm^{-2} ($n=5$), respectively. There were also no significant differences between first (2.2 ± 0.5 mN mm^{-2}, $n=5$) and second (after 1 h) hypoxia-induced contractions (2.0 ± 0.2 mN mm^{-2}, $n=5$) in the presence of serotonin in control group.

Pretreatment with the NF-κB inhibitor pyrrolidine dithiocarbamate (100 μM) itself had no effect on the resting force, but markedly attenuated the contraction induced by serotonin (from 6.2 ± 1.3 mN mm^{-2}, $n=6$, to 2.9 ± 0.9 mN mm^{-2}, $n=6$) prior to the introduction of hypoxia (Fig. 1A). How-

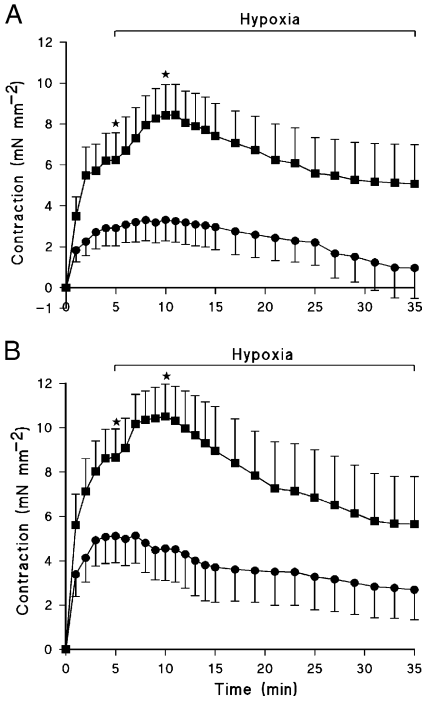

Fig. 1. Effects of NF-κB inhibitors on hypoxia-induced contraction of serotonin-precontracted pulmonary arteries. Contraction was measured at various time points up to 35 min after the administration of serotonin. Serotonin (6 μM) + hypoxia ($n=6$) (■) and effects of hypoxia in pyrrolidine dithiocarbamate (100 μM)-pretreated pulmonary artery rings ($n=6$) (●) (A), and serotonin (6 μM) + hypoxia ($n=7$) (■) and effects of hypoxia in pyrithione (10 μM)-pretreated pulmonary artery rings ($n=7$) (●) (B). *$P<0.05$, when compared to before treatment.

ever, pyrrolidine dithiocarbamate abolished the hypoxia-induced contraction (from 2.2 ± 0.6 mN mm^{-2}, $n=6$, to 0.3 ± 0.4 mN mm^{-2}, $n=6$, Fig. 1A). There was also no marked difference in the contraction between 5 and 35 min (without pyrrolidine dithiocarbamate; -1.2 ± 1.2 mN mm^{-2}, $n=6$, with pyrrolidine dithiocarbamate; -2 ± 1.4 mN mm^{-2}, $n=6$). Incubation with another NF-κB inhibitor pyrithione (10 μM) itself had no effect on the resting force, but markedly depressed the contraction induced by serotonin (from 8.7 ± 1.3 mN mm^{-2}, $n=7$, to 5.1 ± 1.2 mN mm^{-2}, $n=7$) prior to the introduction of hypoxia (Fig. 1B). Similar to pyrrolidine dithiocarbamate, pyrithione abolished the hypoxia-induced contraction (from 1.84 ± 0.4 mN mm^{-2}, $n=7$, to -0.6 ± 0.9 mN mm^{-2}, $n=7$), as shown in Fig. 1B. The difference in the contraction between 5 and 35 min in the absence and presence of pyrithione was -3.0 ± 1.25 mN mm^{-2} ($n=7$) and -4.0 ± 2.2 mN mm^{-2} ($n=7$, $P>0.05$), respectively.

4. Discussion

We have obtained experimental evidence that inhibitors of NF-κB have an inhibitory effect on hypoxia-induced contractions of isolated pulmonary arteries. Our observation may support the results of a previous study with Hep3B cells showing that hypoxia activates transcription via a mitochondria-dependent signalling process including increased reactive oxygen species (Chandel et al., 1998). The underlying mechanism of this inhibition is not entirely clear, although it has been demonstrated that these agents can suppress basal NF-κB activity as early as 1 h (Kim et al., 1999).

It is known that serotonin constricts the pulmonary artery by releasing Ca^{2+} from intracellular stores and by promoting Ca^{2+} influx through Ca^{2+} channels in pulmonary artery smooth muscle cells (Yuan et al., 1997). Increases in intracellular Ca^{2+} can lead to enhanced phosphorylation, degradation of IκB and NF-κB activation (Sen et al., 1996). Although we observed a reduction in the serotonin-induced contraction in the presence of NF-κB inhibitors in the present study, the mechanism of this inhibition is not known and requires further investigation. It has been reported that pyrrolidine dithiocarbamate applied acutely does not alter the tone elicited by phenylephrine in rat aortic rings and has no effect on the subsequent relaxation induced by acetylcholine or SIN-1 (Schini-Kerth et al., 1994). In a study with canine basilar cerebral arteries, preincubation of arterial rings with pyrrolidine dithiocarbamate has been shown to attenuate the contractions produced by alcohol, but not KCl or prostaglandin $F_{2\alpha}$ (Li et al., 2001). Pyrrolidine dithiocarbamate was shown to abolish hypoxic contractions without affecting contractions induced by U46619 in cultured pulmonary artery myocytes (Waypa et al., 2001). The reasons for the differences in these responses are unknown.

Reactive oxygen species have been shown to be involved in NF-κB activation in many cells, through a mechanism that is not yet understood (Gius et al., 1999). At least one potential mechanism for the interaction between reactive oxygen intermediates and NF-κB activation is through reactive oxygen species potentiating the activity of IκB phosphorylating kinases, such as IκB kinase-1 (IKK-1) or IKK-2, which would then lead to enhanced degradation of IκB-α, followed by NF-κB translocation to the nucleus (Abraham, 2000). In certain cell types, H_2O_2 was shown to be an effective inducer of NF-κB activation (Schreck et al., 1991). NF-κB is rapidly activated in endothelial cells treated with H_2O_2 (Barchowsky et al., 1995). However, pyrrolidine dithiocarbamate may inhibit NF-κB by other mechanisms related to its antioxidant or metal-chelating activities (Schreck et al., 1991). As an antioxidant, pyrrolidine dithiocarbamate appears to enhance H_2O_2 clearance by reducing oxidised glutathione (Schreck et al., 1991; Chandel et al., 1998; Waypa et al., 2001). There are several studies that show increased reactive oxygen species generation in response to hypoxia. NADPH-oxidase is activated to release superoxide under hypoxic conditions, which may be involved in the initiation of hypoxia-induced pulmonary vasoconstriction (Marshall et al., 1996). Pulmonary artery smooth muscle cells when acutely exposed to hypoxia exhibit a marked increase in intracellular reactive oxygen or nitrogen species production (Killilea et al., 2000). In a recent study, Waypa et al. (2001) demonstrated in isolated lungs and pulmonary arterial myocytes that reactive oxygen species generated by mitochondria appear to function as second messengers during hypoxia and that pyrrolidine dithiocarbamate can attenuate hypoxia-induced pulmonary vasoconstriction.

There is evidence that an altered activity of endothelium-derived constrictor mediators may play an important role in hypoxia-induced pulmonary vasoconstriction (Wadsworth, 1994). Recently, hypoxia-induced 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). Our results suggest that hypoxia-induced contractile factor(s) may be released from the endothelium, response can be blocked by pyrrolidine dithiocarbamate or pyrithione in a way that is independent of NF-κB inhibition.

Although further studies are required to determine the mechanism underlying the inhibition, the results of the present study show that pyrithione and pyrrolidine dithiocarbamate, two structurally unrelated compounds, abolish hypoxia-induced contractions in isolated pulmonary arteries. Reactive oxygen species and/or NF-κB activation may be involved in hypoxia-induced pulmonary hypertension.

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