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EFFECTS OF HYPOXIA ON PULMONARY VASCULAR REACTIONS AND ON LUNG cGMP AND cAMP IN PIGS

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Abstract—Hypoxia leads to an elevation of the pulmonary vascular resistance (pvR) in pigs. This was accompanied by an increase of intraparenchymatous concentrations of cyclic GMP (cGMP), while cyclic AMP (cAMP) levels were not different from values measured during normoxia. The inhibition of the hypoxia-induced vasoconstriction by a β_2-receptor stimulating agent was associated with a remarkable increase in intraparenchymatous cAMP-concentrations and a decrease in cGMP levels. The correlation analysis between pvR versus the ratio cGMP/cAMP revealed a significant linearity. These data may suggest that cAMP and cGMP are involved in hypoxia induced pulmonary vasoconstriction and its pharmacological inhibition.

The mechanism by which a lowered alveolar PO_2 or increased PCO_2 leads to pulmonary vasoconstriction in the area of hypoventilation is still unknown [1–3]. β-receptor stimulating agents and methylxanthine derivatives are potent inhibitors of this mechanism while β-receptor blocking agents augment this effect [4–6]. While these pharmacological actions are associated with alterations of pulmonary cAMP levels, the hypoxia induced vasoconstriction itself was not accompanied by alterations of this cyclic nucleotide [7].

Observations of Dunham *et al.* [10], Goldberg *et al.* [9, 10], Schultz *et al.* [11] and Andersson *et al.* [1, 2] strongly suggest, that the contraction of smooth muscles is associated with, or mediated by, an accumulation of endogenous cGMP. In this study we wanted to clarify whether cGMP is involved in the hypoxia induced pulmonary vasoconstriction and its inhibition by β_2-receptor stimulating drugs.

MATERIALS AND METHODS

Pigs of 19.5 kg average wt were used. Anesthesia was performed with an initial dose of 30 mg/kg body wt pentobarbital-Na (Nembutal^R) administered intraperitoneally (i.p.) and additional injection of a maximum of 16 mg/kg body wt Nembutal^R 10 min before onset of hypoxia. The thorax was opened by a complete sternotomy. Intubated animals were mechanically ventilated during normoxia with a gas mixture containing N_2O and O_2. Hypoxia was obtained by reduction of the O_2 content. Tidal volume was adjusted to 10 ml/kg body wt, respiratory rate to 18–20/min. For further experimental details see Fig. legends.

Hemodynamics. The cardiac output (CO) was measured with an electromagnetic flowmeter (Hellige SQ 401), a wet-calibration using a dialysis hose filled with isotonic NaCl-solution preceded the recordings. The flowmeter probe (Statham Flow-Probe^R i.d. 12 mm) was fixed to the pulmonary artery after opening of the pericardium. The average pulmonary artery pressure (PAP_m) was measured with a microcatheter (Ygon, i.d. 0.6 mm. o.d. 0.95mm, length 50 cm).

The left ventricular end diastolic pressure (LVEDP) was measured with a steel cannula (length 82 mm, i.d. 1.4 mm, o.d. 2.1 mm) which was placed in the left ventricle. The femoral artery pressure (AOP) was also recorded with the aid of a plastic catheter.

The pulmonary vascular resistance was calculated according to the formula:

$$PVR = \frac{PAPm - LVEDP}{CO:60} \times 1332 \text{ dyn sec cm}^{-5}$$

Pressure transducer: Statham 23 Db: amplifier and differentiator: Hellige.

Drugs were administered continuously into the right ventricle over a period of 3 min (min 21–24).

Measurement of cAMP and cGMP. Following the opening of the parietal pleura, approx. 1–2 cm^3 pieces of peripheral lung tissue were clamped, ligated and removed. The lung material was immediately frozen between two aluminum prongs that were precooled in liquid nitrogen. The time between clamping the tissue and freezing was 4–7 sec. The frozen slices were broken and homogenized in 5% ice-cold trichloroacetic acid with a homogenizer (Ultraturrax, Janke u. Kunkel, Staufen). The samples were kept in ice for 10 min and centrifuged at 6000 rpm (Christ, Osterode). The further workup of the samples has been previously described [13]. Aliquots of the TCA supernatant were extracted five times with 3.5 volumes of water-saturated diethylether. Remaining ether was evaporated by heating the samples to 95°. The evaluation of cAMP was performed according to the method of Gilman [14], cGMP according to Steiner [15].

As the degradation of lung tissue cAMP and cGMP by phosphodiesterase was complete after 30 min, recovery of cAMP was 96 per cent, of cGMP 98 per cent, and serial dilutions of the tissue extracts revealed linearity, thus an interference of tissue

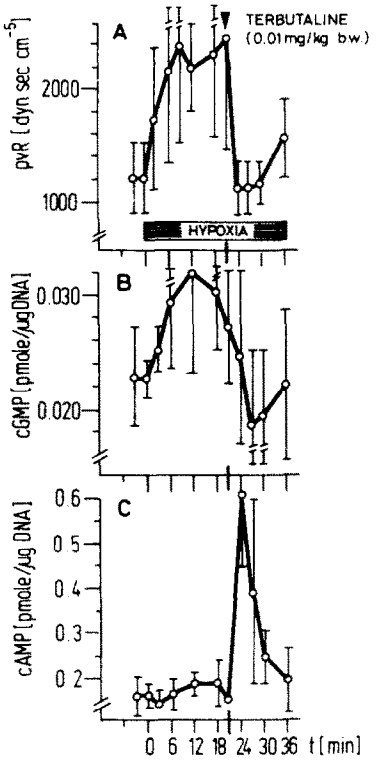

Fig. 1. Pulmonary vascular resistance (pvR) (Fig. 1a), cGMP content (Fig. 1b) and cAMP content (Fig. 1c) during hypoxemia ± terbutaline. (Each point represents mean values ± S.D.) The number of animals, in which pvR, intraparenchymatous cAMP and cGMP levels have been determined simultaneously, is indicated in parenthesis in Fig. 2a. For further experimental details see methods.

components with the cGMP-radioimmunoassay could be excluded, no purification of tissue extracts was performed.

DNA determination was performed according to Burton [16].

RESULTS

As demonstrated in Fig. 1a pulmonary vascular resistance increased from an average of 1200 dyn sec cm^{-5} during normoxic conditions (arterial $pO_2 = 214$ mmHg, pH 7.497 · $pCO_2 = 38$ mmHg) to 2405 dyn sec cm^{-5} within 21 min after onset of hypoxia (arterial $pO_2 = 43$–49 mmHg). After a 3 min continuous administration of terbutaline (0.01 mg/kg body wt) from min 21–24, the pvR decreased to 1120 dyn sec cm^{-5} at min 24 and gradually increased in the next few minutes to 1584 dyn sec cm^{-5} at min 36. This time course was highly significant ($P < 0.0005$, Friedman-test).

Under these experimental conditions intraparenchymatous cGMP (Fig. 1b) ($P < 0.009$, Friedman-test) increased significantly from 0.0223 ± 0.0033 pmole/µg DNA under normoxia to reach a maximum of 0.0319 pmole/µg DNA 12 min after onset of hypoxia. The injection of terbutaline led to a decrease in cGMP levels; reaching the minimum level of 0.0185 pmole/µg DNA at min 27 it gradually rose to 0.220 pmole/µg DNA at min 36.

In Fig. 1c the alterations of intraparenchymatous cAMP levels under the same experimental conditions are demonstrated. Until min 21 no significant (Friedman-test) alterations of cAMP-levels (in comparison to values observed during normoxia = 0.162 ± 0.035 pmole/µg DNA) were measured. The injection of terbutaline led to a remarkable elevation of cAMP up to 0.610 ± 0.160 pmole/µg DNA at min 24. In the following minutes cAMP decreased to 0.197 pmole/µg DNA at min 36.

The analysis for linear correlation of pvR vs cGMP was in all experiments ($n = 9$) positive, ($r_1 = 0.7992$, $r_2 = 0.2252$, $r_3 = 0.6037$, $r_4 = 0.5827$, $r_5 = 0.2455$, $r_6 = 0.5646$, $r_7 = 0.7945$, $r_8 = 0.8855$, $r_9 = 0.1938$; $P < 0.05$, signtest). The correlation-analysis of pvR vs cAMP from min 21 (beginning of the terbutaline induced pulmonary vasodilation, cf. Fig. 1a) to min 36 revealed a negative linear correlation in all experiments ($r_1 = -0.3547$, $r_2 = -0.6552$, $r_3 = -0.8542$, $r_4 = -0.9104$, $r_5 = -0.6862$). The curves of the mean values of the pvR (Fig. 2a) and the mean values of the ratio cGMP/cAMP (Fig. 2b) revealed with $r = 0.9557$ (inset of Fig. 2b) a strong similarity. As eight of nine correlation coefficients ($r_1 = 0.8983$, $r_2 = 0.9104$, $r_3 = 0.8773$, $r_4 = 0.6957$, $r_5 = 0.7752$, $r_6 = 0.0399$, $r_7 = 0.5560$, $r_8 = -0.6038$, $r_9 = 0.4380$) of the pvR vs the ratio cGMP/cAMP were positive from the sign-test follows a significance of $P < 0.05$.

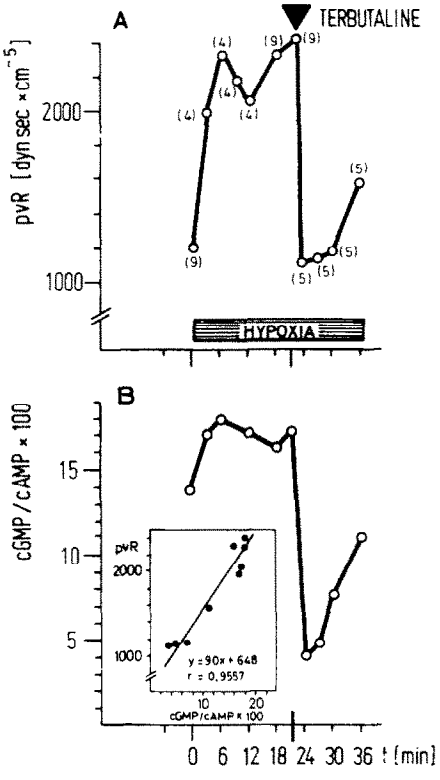

Fig. 2. Alterations of the pvR (Fig. 2a) and the ratio cGMP/cAMP (mean values) of animals in which these parameters have been determined simultaneously (numbers in parenthesis). Inside of Fig. 2b the correlation line of these values is plotted.

DISCUSSION

Recent observations suggest a causal relation between cAMP and smooth muscle relaxation [12, 17–21]. As previously reported, the inhibition of the hypoxia induced pulmonary vasoconstriction by β_2-receptor stimulating agents and aminophylline is accompanied by an increase of intraparenchymatous levels of cAMP, whereas propranolol leads to an amplification of the hypoxia induced pulmonary vasoconstriction and an associated decrease of intraparenchymatous concentration of cAMP [7]. However, the hypoxia induced pulmonary vasoconstriction itself was found to be an cAMP-independent mechanism [7]. The data presented here show that alveolar hypoxia is associated with an increase in pulmonary cGMP concentrations. This observation is in accord with findings of Dunham *et al.* [8], Goldberg *et al.* [9, 10], Andersson *et al.* [12] and Clyman *et al.* [22] indicating a causal relation between cGMP and smooth muscle contraction.

These observations conflict however with recent reports that no causal relation exists between cGMP and smooth muscle contraction [23, 24]. According to these authors cGMP may act as a negative feedback inhibitor of hormonally stimulated calcium influx. From this view, the observed increase in cGMP during hypoxia may be interpreted as a counter-regulatory phenomenon in order to diminish the vasoconstrictive effect of hypoxia.

As postulated by Goldberg *et al.* [10], the absolute values of cGMP and cAMP in cell specific reactions are not as important as the relation of one to the other. Our data sustain this hypothesis as well as observations of Dunham *et al.* [8] who found comparable alterations of the ratio cGMP/cAMP in veins exposed to the vascular smooth muscle relaxants PGE_1 or isoproterenol.

Because of the heterogenicity of the tissue examined in our experiments it should be pointed out that one should deal with the possibility, that the alterations of cyclic nucleotide concentrations may relate to other cellular events rather than only to vasoconstriction or vasodilation. However, the linear correlation between the pvR and cAMP after inhibition of the hypoxia induced pulmonary vasoconstriction by terbutaline, the significant linear correlation between pvR versus cGMP, as well as the significant linear correlation between the pvR vs the ratio cGMP/cAMP during the whole experimental period may indicate that our data reflect at least partly pulmonary vascular metabolic alterations. This interpretation is sustained by data of Kadowitz *et al.* [21], who found similar alterations of cyclic nucleotide metabolism occurring in isolated pulmonary vessels after treatment with PGE_1 or PGF_2.

The mechanism by which the β_2-receptor stimulating agent terbutaline caused the demonstrated decrease in cGMP and subsequently the decrease of the ratio cGMP/cAMP below values measured during normoxia is unclear. An explanation may be the cAMP-induced increase in Ca^{2+}-efflux with subsequent inactivation of a guanylatecylase [25–27]. However this interpretation conflicts with data of Kakiushi *et al.* [28] who found a Ca^{2+}-dependent activation of cGMP-phosphodiesterase.

Conclusively, our data are consistent with the concept of the antagonistic regulatory effects of cAMP and cGMP [10] and (2) indicate that cGMP and cAMP may be involved in the hypoxia induced pulmonary vasoconstriction and its pharmacological inhibition.

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