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cAMP DEPENDENT AND cAMP INDEPENDENT ALTERATIONS OF LUNG HEMODYNAMIC IN THE PIG

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Abstract—*In vivo* hypoxemia leads to an elevation of pulmonary vascular resistance (pvR) in pig lungs. Orciprenaline aminophylline and Ca^{2+} are potent inhibitors of this effect, while propranolol augments the alveolo–vascular reflex. Evidence is presented that the effects of orciprenaline aminophylline and propranolol on pvR are mediated by intracellular alterations of cyclic AMP concentration. In contrast, the pvR-increasing effect of hypoxemia and its supression by Ca^{2+} seem to be cyclic AMP independent mechanisms.

There are only a few reports available[1–3] concerning the effects of agents which alter lung hemodynamic in correlation to intraparenchymatous cAMP levels *in vivo*. Alterations of cyclic nucleotide concentration induced by different drugs in isolated perfused lungs, lung slices and homogenates are well established[4–7].

In order to find correlations between physical and biochemical parameters we used pigs in our experiments. Hypoxia in humans, as well as in pigs, induces the alveolo-vascular reflex, the so called von Euler–Liljestrand (ELM) reflex, which leads to an elevation of pulmonary vascular resistance (pvR). This reflex is of well known clinical importance[8, 9], but the mechanism by which a lowered alveorlar PO_2, or increased PCO_2, leads to this reflex is still unknown.

β-adrenergic agents and methylxanthine derivatives are potent inhibitors of this mechanism, while β-receptor blocking substances augment this effect[11–14]. Since these substances are well known to influence the intracellular cyclic AMP level[15–18] it is probable that the hypoxia induced vasoconstriction (ELM) is mediated by alterations of endogenous cAMP.

MATERIAL AND METHODS

Four or five pigs of 19·5 kg average weight were used. Anaesthesia was performed with an initial dose of 30 mg/kg b.w. pentobarbital–Na Nembutal®) applied intraperitoneally and additional injection of a maximum of 16 mg/kg Nembutal® during the experiment. The thorax was opened by a complete sterniotomia, the intercostal muscles in the second ICS were incised and the internal thoracic artery and vein were ligated.

Hemodynamics. The cardiac output (HMV) 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-Flo-Probe® i.d. 12 mm) was fixed to the pulmonary artery after opening of the pericardium. The pulmonary artery pressure (PAP) was measured with a microcatheter (Vygon, i.d. 0·6 mm, o.d. 0·95 mm, length 50 cm). The left ventricle-pressure and the end diastolic pressure in the left ventricle (LVP, LVEDP) were measured with a steel canula (length 82 mm, i.d. 1·4 mm, o.d. 2·1 mm) which was placed in the left ventricle of the heart apex. The pulmonary vascular resistance was calculated according to the formula:

$$PVR = \frac{PAP_m - LVEDP}{HMV: 60} \times 1332 \text{ dyn sec cm}^{-5}.$$

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

Drugs were applied directly into the right ventricle.

Measurement of cAMP. 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 flat 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 TCA with a homogenizer (Ultraturrax, Janke u. Kunkel, Staufen). The samples were kept in ice for 10 min and centrifuged at 6000 rev/min (Christ, Osterode). The further workup of the samples has been previously described[19]. Aliquots of the TCA supernatant were extracted 5 times with 3·5 vol. 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[20].

DNA-determination was performed according to [21].

RESULTS

As demonstrated in Fig. 1a, hypoxemia led to a drastic elevation of pvR from 967 ± 287 to 2880 ± 1233 dyn sec cm^{-5}. During this phase of the experiments no alteration of cAMP concentration of peripheral lung tissue was detectable (Fig. 1b).

Orciprenaline (0·01 mg/kg b.w.) caused a decrease of the pvR within 3 min to 1706 ± 744 dyn sec cm^{-5} (Fig. 1a), while simultanously cAMP increased from 0·250 ± 0·038 to 0·617 ± 0·227 pmole/μg DN\. Both effects were of short duration. Continually increasing pvR was accompanied by a decrease of cAMP levels.

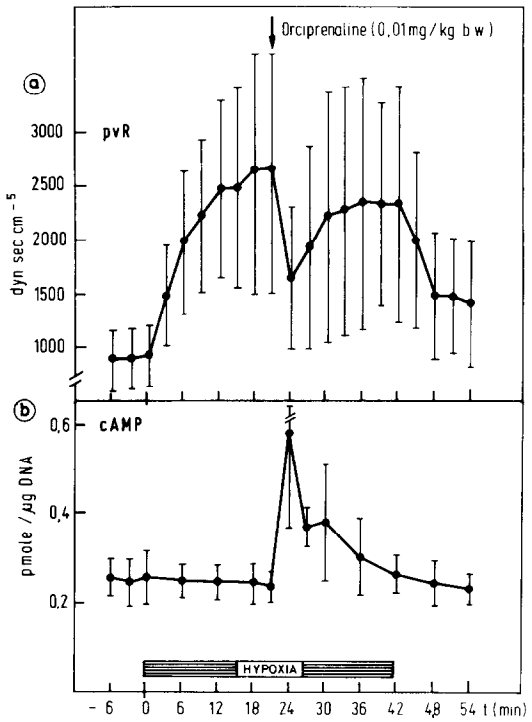

Fig. 1. Pulmonary vascular resistance and cAMP content during hypoxemia ± orciprenaline (each point represents in (a) the mean value ± S.D. of four measurements in four pigs, in (b) the mean value ± S.D. of four determinations in duplicate).

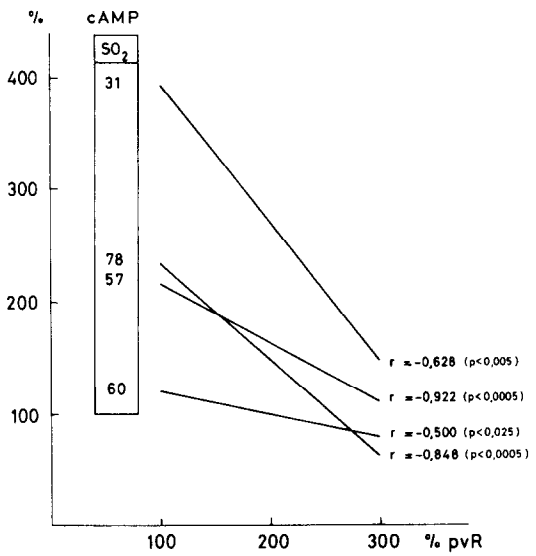

Fig. 2. Individual regression lines and their correlations to the experiments shown in Fig. 1. Percentage cAMP content and corresponding pvR (12th–24th min) in reference to normoxemic values = 100%, SO_2 = mean values of the arterial oxygen saturation during the time mentioned above.

The correlation between the percentage (in reference to basal values during normoxia) pvR alteration and endogenous cAMP content for each experiment during the 12th–21th min (steady state of the hypoxia-induced pvR elevation) and the 24th min (maximal inhibition of the alveolo-vascular reflex by orciprenaline) are presented in Fig. 2. In all experiments the linear negative correlation was statistically significant.

The injection of propranolol (0·2 mg/kg b.w.) during normoxia led to a significant ($P < 0{\cdot}0025$, see Fig. 4) elevation of pvR from 1008 ± 340 to 1200 ± 353 dyn sec cm^{-5} (Fig. 3a).

During this treatment cAMP levels are found to be significantly ($P < 0{\cdot}0025$, see Fig. 4) below the basal concentration in the lung tissue (Fig. 3b).

The subsequent hypoxia provoked an additional increase of pvR up to 4248 ± 1861 dyn sec cm^{-5}, which exceeded the values seen without prior application of propranolol (cf. Figs. 1, 5, 6).

As demonstrated in Fig. 5a, aminophylline (6 mg/kg b.w.) strikingly decreased the hypoxia-induced pulmonal vasoconstriction from 2448 ± 871 to 1728 ± 673 dyn sec cm^{-5}. This effect was accompanied by an elevation of endogenous cAMP from $0{\cdot}233 \pm 0{\cdot}055$ to $0{\cdot}414 \pm 0{\cdot}154$ pmole/µg DNA (Fig. 5b) [$r_1 = -0{\cdot}966$ ($P < 0{\cdot}0005$); $r_2 = -0{\cdot}458$ ($P < 0{\cdot}025$); $r_3 = -0{\cdot}888$ ($P < 0{\cdot}0005$); $r_4 = -0{\cdot}651$ ($P < 0{\cdot}005$); $r_5 = -0{\cdot}432$ ($P < 0{\cdot}05$)] (cf. Fig. 2). Here again the vasodilatory and cAMP-increasing effect was only transient.

Under the same experimental conditions, Ca^{2+} (30 mg/kg b.w.) led to a marked inhibition of the hypoxia-induced vasoconstriction from 3161 ± 293 to 2121 ± 301 dyn sec cm^{-5} (Fig. 6a). This effect was of longer duration than that caused by orciprenaline and aminophylline as demonstrated above.

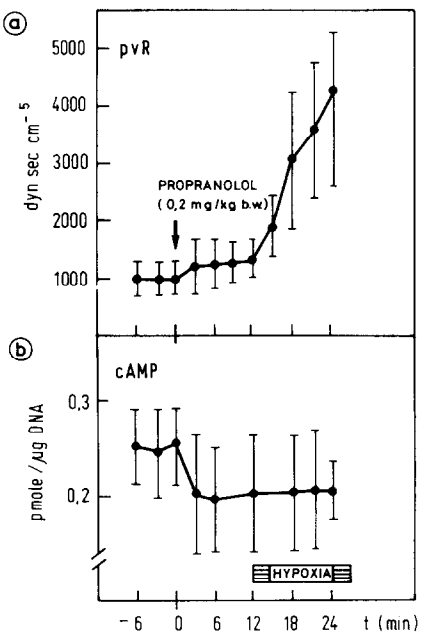

Fig. 3. Influence of propranolol on the pulmonary vascular resistance and cAMP concentration in lung tissue during normoxemia and hypoxemia (each point represents in (a) the mean values ± S.D. of four measurements in four pigs, in (b) the mean values ± S.D. of 4 determinations in duplicate).

In contrast to the β-receptor stimulating or cAMP-phosphodiesterase inhibiting agents used, Ca^{2+} caused no alteration of the cAMP-levels in peripheral lung tissue (Fig. 6b).

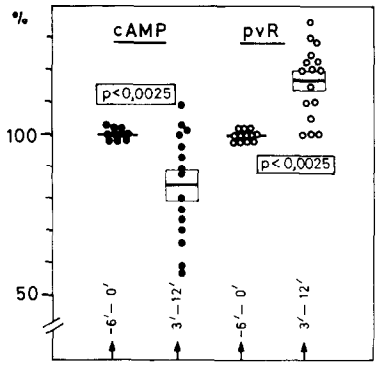

Fig. 4. Percentage alterations of pvR and cAMP content during propranolol application (cf. Fig. 3).

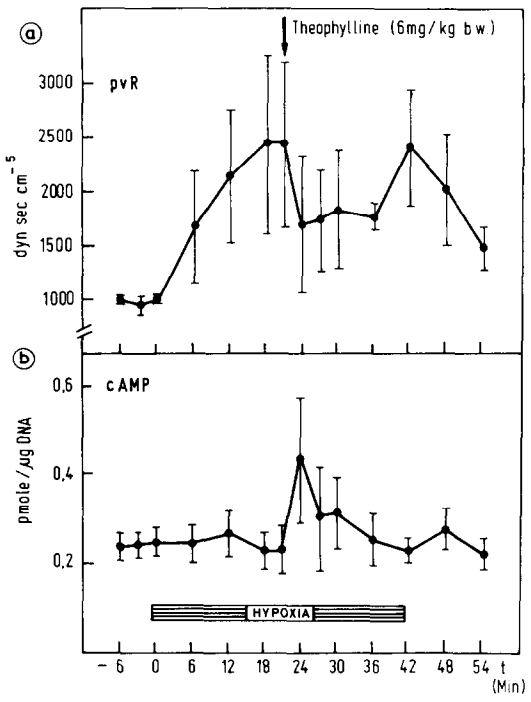

Fig. 5. Influence of aminophylline on pvR and cAMP concentration during hypoxemia (each point represents in (a) the mean values ± S.D. of five measurements in five pigs, in (b) the mean values ± S.D. of five determinations in duplicate).

DISCUSSION

As demonstrated in earlier reports[13, 14] orciprenaline and methylxathine inhibited the alveolovascular reflex. As previously reported[2], this effect is accompanied by an elevation of cAMP in peripheral lung tissue. Elevation of endogenous cAMP by inhibition of the cAMP-phosphodiesterase with aminophylline led to relaxation of pulmonary vessels, counteracting the hypoxia-induced vasoconstriction. Under normoxic conditions propranolol causes an elevation of the pvR, accompanied by an intracellular decrease of cAMP. Comparable observations have been made *in vitro* on smooth muscles by Robinson *et al.*[22], Bueding *et al.*[23] and Andersson *et al.*[24, 25] *in vivo* in the intact dog by Kadowitz *et al.*[3], suggesting a causal relation between cAMP

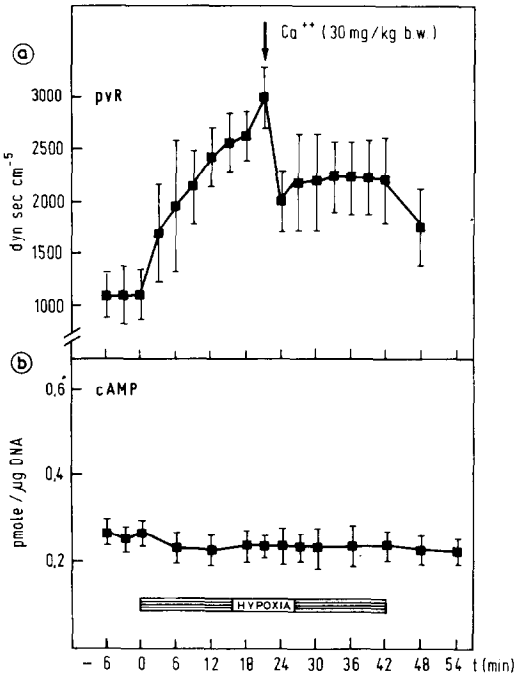

Fig 6. Pulmonary vascular resistance and cAMP content during hypoxemia ± Ca^{2+} (each point represents in (a) the mean values ± S.D. of five measurements in five pigs in (b) the mean values of five determinations in duplicate).

levels and smooth muscle contraction–relaxation cycle.

In contrast to these data, the hypoxia-induced vasoconstriction was not associated with alterations of intraparenchymatous cAMP levels, indicating a cAMP-independent mechanism of vasoconstriction under these conditions.

Whether the values of endogenous cAMP presented reflect the content of vascular smooth muscles is problematical because of the heterogenicity of the material examined in our experiments. However, the negative correlation between the pvR and cAMP contents during the application of orciprenaline and aminophylline, as well as findings reported by Kadowitz[3], suggest the specificity of our data.

The mechanism by which hypoxia induces pulmonary vasoconstriction may be explained by anaerobic metabolism with intracellular pH-decrease, which might cause an elevation of free cytoplasmatic Ca^{2+}. According to Ebashi[26] the inhibitory effect of the troponin–tropomyosin system on the actin–myosin system of skeletal muscles was abolished by saturation of the former with Ca^{2+} resulting in muscle contraction. This regulator system was confirmed in smooth muscles by Sparrow *et al.*[27] and Rueg[28].

In this respect our observations during the application of Ca^{2+} are somewhat difficult to interpret. Ca^{2+} caused a cAMP-independent inhibition of the hypoxia induced alvelolo-vascular reflex. These findings can hardly be explained by the mechanism cited above[26] and conflict with data of other authors[29–31] who found a contraction in response to Ca^{2+}. A possible explanation of the demonstrated Ca^{2+} effect may be a membrane

potential stabilizing effect of exogenous Ca^{2+} as described by Tomita[32] and Kuriyama[33].

Whether the observed cAMP-independent (hypoxia, Ca^{2+}) vascular reactions are accompanied by alterations of endogenous cGMP levels, must yet be established.

In summary, our data indicate two different mechanisms of action of vasoreagibility:

(1) a cAMP-dependent one, as indicated by the experiments with orciprenaline, propranolol and aminophylline.

(2) a cAMP-independent one, as concluded from the data obtained during hypoxia and the suppression of the hypoxia-induced vasoconstriction by Ca^{2+}.

As propranolol and hypoxia led to additive effects on pvR, the two mechanism seem to work independently.

REFERENCES

1. P. J. Kadowitz, W. J. George, P. D. Joiner and A. L. Hyman, *Adv. Biosci.* **9**, 501 (1972).
2. V. Sill, E. Kaukel, N. Völkel and S. Siemssen, *Pneumonlogy* **150**, 377 (1974).
3. P. J. Kadowitz, P. D. Joiner, A. C. Hyman and W. J. George, *2nd Int. Conf. cAMP*, Vancouver 1974, abstracts p. 73. (1974).
4. G. C. Palmer, *Biochem. Pharmac.* **21**, 2907 (1972).
5. W. Schmutzler, G. Poblete-Freundt and R. Derwall, *Agents Actions* **3**, 192 (1973).
6. M. Collins, G. C. Palmer, G. Baca and H. R. Scott, *Res. Commun. Chem. Path. Pharmac.* **6**, 805 (1973).
7. M. Hitchcock, *Biochem. Pharmac.* **22**, 959 (1973).
8. U. S. von Euler, *Verh. dt. Ges. Kreis/Forsch.* **17**, 8 (1951).
9. U. S. von Euler and G. Liljestrand, *Acta physiol. scand.* **12**, 301 (1946).
10. E. D. Silove and R. F. Grover, *J. clin. Invest.* **47**, 274 (1968).
11. R. Felix, C. Winkler, A. Dux, P. Geisler, H. Rink and F. K. Schattauer, ———, p. 213. Stuttgart (1970).
12. R. Wettengel, W. Hartmann and H. Fabel, *Prog. Resp. Res.* **6**, 418 (1971).
13. V. Sill, H. C. Siemensen, S. Siemssen and W. Rothenberger, *Pneumologie* **147**, 52 (1972).
14. V. Sill, W. Greul, N. Völkel and S. Marwede *Arzneimittel-Forsch. (Durg Res.)* **23**, 1053 (1973).
15. T. V. Rall and E. W. Sutherland, *J. biol. Chem.* **232**, 1076 (1958).
16. E. W. Sutherland and T. W. Rall, *Pharmac. Rev.* **12**, 265 (1960).
17. E. W. Sutherland, G. A. Robison and R. W. Butcher, *Circulation* **37**, 279 (1968).
18. G. A. Robison, R. W. Butcher and E. W. Sutherland, ———, Academic Press, New York (1971).
19. E. Kaukel and H. Hilz, *Biochem. biophys. Res. Commun.* **46**, 1011 (1972).
20. A. G. Gilman, *Proc. natn. Acad. Sci., U.S.A.* **67**, 305 (1970).
21. W. C. Schneider, *Meth. Enzym.* **III**, 680 (1957).
22. G. A. Robison, R. W. Butcher and E. W. Sutherland, *Ann. N.Y. Acad. Sci.* **139**, 703 (1967).
23. E. Bueding and E. Bülbring, *Ann. N.Y. Acad. Sci.* **139**, 758 (1967).
24. R. Andersson and E. Mohme-Lundholm *Acta physiol. scand.* **77**, 372 (1969).
25. R. Andersson, *Acta physiol. scand.* Suppl. 382 (1972).
26. S. Ebashi and M. Eudo, *Prog. Biophys. mol. Biol.* **18**, 123 (1968).
27. M. P. Sparrow, L. C. Maxwell, J. C. Ruegg and D. F. Bohr, *Am. J. Physiol.* **219**, 1366 (1970).
28. J. C. Rueg, *Physiol. Rev.* **51**, 201 (1971).
29. Ch. L. Seidel and D. F. Bohr, *Circulat. Res.* Suppl. II **28/29**, 88 (1971).
30. K. A. Edman and H. O. Schild, *J. Physiol.* **161**, 424 (1962).
31. W. G. Nayler, *Am. Heart J.* **73**, 379 (1967).
32. T. Tomita, ———, Arnold, London (1970).
33. H. Kuriyama, ———, Arnold, London (1970).