Hemoglobin causes both endothelium-dependent and endothelium-independent contraction of the pig coronary arteries, independently of an inhibition of EDRF effects

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Summary. Hemoglobin is widely used as an inhibitor of EDRF effects. Hemoglobin contracts pig coronary arteries in vitro. However, during this contraction, effects of substance P and bradykinin which act via the EDRF are not inhibited. This means that the hemoglobin contraction is not caused by inhibition of the EDRF. This contraction is caused by a substance released from the endothelium, and by eicosanoids released from the smooth muscles.

Key words. Coronary artery; oxyhemoglobin; endothelium; EDRF; electrophysiology; eicosanoids.

Oxyhemoglobin has been shown to be a vasoconstrictor for cerebral arteries. Consequently, oxyhemoglobin could be the cause of cerebral vasospasms which follow subarachnoid hemorrhage. The contraction caused by oxyhemoglobin is more pronounced for cerebral arteries, yet it also contracts other arteries, among them the coronary arteries. These observations were made before the discovery of the role played by the endothelium in vasodilation. This implies that the studies undertaken previously were done without considering the possible role of a functional endothelium. Since the discovery of the endothelial-derived relaxing factor (EDRF), oxyhemoglobin has been extensively used as an inhibitor of the vasodilation caused by EDRF. In this context, vasoconstriction caused by oxyhemoglobin on arterial rings was interpreted as a result of superimposition of the relaxation caused by the EDRF. Yet destruction of the endothelium in cerebral arteries does not inhibit the vasocostriction caused by oxyhemoglobin. This demonstrates that oxyhemoglobin may induce arterial constriction by at least two distinct mechanisms: an inhibition of the EDRF and a direct action on smooth muscles.

In the present study we investigate whether oxyhemoglobin contracts pig coronary arteries by inhibiting EDRF or by a direct action on smooth muscle, or both together. The endothelium-dependent relaxation in pig coronary arteries is characterized by a concomitant hyperpolarization. We used this hyperpolarization as an indication of endothelium-dependent relaxation. Thus measurement of smooth muscle membrane potential together with isometric tension were performed in this study.

We report here that oxyhemoglobin contracts pig coronary arterial strips in vitro in two ways: by an action on smooth muscles via eicosanoids and by the release of the endothelium of a contracting substance, and not by inhibiting the EDRF. The existence of both an endothelium-dependent and an endothelium-independent vasoconstriction of coronary arteries caused by oxyhemoglobin could be important in cardiac physiopathology.

Materials and methods. Preparation of tissues. The anterior descending branches of coronary arteries were obtained from freshly killed pigs. The coronary lumen was rinsed by injection of cold oxygenated Krebs solution (mM: NaCl 118.7, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, NaHCO₃ 24.8, MgSO₄ 1.2, glucose 10.1; 4°C; pH 7.3). Segments of the coronary artery were cleaned of all adherent fat and connective tissue. Then they were cut into 2-mm-wide rings which were cut to give strips of about 5 mm in length. For some experiments, the endothelium was removed by gently rubbing the internal face of the strip with a cotton-tip. To check that the endothelium was removed by this procedure, a 0.5% w/v solution of Evans blue in Krebs solution was applied for 10 s to the strip, which was then washed with Krebs solution. The luminal face of a strip with intact endothelium remained white whereas a de-endothelialized strip was colored blue. The response of the strip to substance P produces a marked endothelial-dependent relaxation of precontracted smooth muscle. The absence of such a response, plus the positive Evans blue staining, was taken as evidence for the complete removal of the endothelium. When intact and de-endothelialized strips were compared as in figure 1, they were from adjacent coronary rings.

Two types of in vitro experiment were performed. In each type of experiment, changes in tension were measured isometrically (Grass force displacement transducer FT03C) and amplified (Lectromed 3559). Contractile responses were recorded on chart paper with polygraphs (W + W Electronics).

In each experiment, Krebs solution was pumped to the preparation from a siliconized glass or plastic beaker with a peristaltic pump. Oxyhemoglobin and peptides were applied to the preparations by diluting them directly in the beaker containing the superfusion solution. To avoid the production of mechanical artifacts during the experiment all changes in perfusate were achieved without the introduction of bubbles into the tissue chamber. Pharmacological experiments. To measure the tension only, strips were suspended in a 85-μl bath using two silk threads attached to the edges of the strips in parallel with the circular smooth muscles. Strips were continuously superfused with oxygenated Krebs solution (1.250 μl/min) main-
No effects were observed in 4 independent trials.

0.1 IxM sodium dithionite was applied to the coronary strip. This could not be responsible for the phenomena we studied, because the oxyhemoglobin was frozen in aliquots and kept at 36 °C until use. The peptides were diluted subsequently with Krebs solution to the desired concentrations.

Oxyhemoglobin was prepared by oxidizing bovine methemoglobin (Fluka) with sodium dithionite (Na2S2O4), followed by dialysis to remove sodium dithionite. The solution of oxyhemoglobin was frozen in aliquots and kept at -20 °C until used. To check that traces of sodium dithionite could not be responsible for the phenomena we studied, 0.1 µM sodium dithionite was applied to the coronary strip. No effects were observed in 4 independent trials.

Preparation of peptides and chemicals. Substance P and bradykinin (Buchem Feinchemikalien, Switzerland) were prepared at a concentration of 1 mg/ml in 0.25% acetic acid. They were stored as aliquots or 50 µl and kept frozen at -20 °C until use. The peptides were diluted subsequently with Krebs solution to the desired concentrations.

Oxyhemoglobin was prepared by oxidizing bovine methemoglobin (Fluka) with sodium dithionite (Na2S2O4), followed by dialysis to remove sodium dithionite. The solution of oxyhemoglobin was frozen in aliquots and kept at -20 °C until used. To check that traces of sodium dithionite could not be responsible for the phenomena we studied, 0.1 µM sodium dithionite was applied to the coronary strip. No effects were observed in 4 independent trials.


d-endothelium-dependent contraction 7, we used this property to evaluate the effects of lesions caused by de-endothelialization we used. Thus, if the contraction caused by oxyhemoglobin contraction was accompanied by an inhibition of the EDRF, the effects of the two kinins would also be inhibited. Perfusion of oxyhemoglobin inhibited neither relaxations (fig. 2) nor hyperpolarizations caused by substance P and bradykinin. In the presence of oxyhemoglobin (10 µM) the hyperpolarization which accompanied contraction for a strip with an intact endothelium and 68 ± 8% (n = 4) of the oxyhemoglobin contraction when the strip was de-endothelialized. The endothelium-dependent relaxation is characterized by a hyperpolarization of the smooth muscle 8,12. Therefore we checked whether oxyhemoglobin contraction was accompanied by an inhibition of the EDRF hyperpolarization. Oxyhemoglobin did not significantly (p = 0.07) change the smooth muscle membrane potential of a strip with intact endothelium: the membrane potential was -44.6 ± 1.8 mV (n = 13) before application of oxyhemoglobin and - 43.5 ± 1.1 mV (n = 15) when oxyhemoglobin contracted the strip.