OXYGEN MODULATES CONTRACTILE RESPONSES TO POTASSIUM AND PROSTAGLANDIN F$_{2\alpha}$ IN HUMAN PIAL ARTERIES

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SUMMARY

Oxygen may modulate cerebrovascular resistance, but its direct influence on human pial arteries is unknown. We have investigated the effects of varying oxygen tension (73, 30 and 8 kPa) in depolarized (potassium) and receptor stimulated (prostaglandin F$_{2\alpha}$) isolated human pial arteries. Control responses were obtained at an oxygen tension of 30 kPa. Contractions induced by prostaglandin F$_{2\alpha}$ and potassium showed no significant difference in potency (unaffected EC$_{50}$ values) at the different oxygen concentrations. In contrast, the maximum contractions (Emax) were dependent on the oxygen tension. Potassium-induced contractions were enhanced (Emax = 107 (SE 3)% of control contractions (P < 0.01)) at an oxygen tension of 73 kPa, whereas a reduction in tension to 8 kPa had no significant effect (97 (2)%). Prostaglandin F$_{2\alpha}$-induced contractions were enhanced at 73 kPa (115 (6)% (P = 0.02) and depressed at 8 kPa (96 (2)% (P = 0.02). Reduction in oxygen tension induced a relaxation in depolarized and in receptor stimulated arteries, regardless of whether or not oxygen was replaced by nitrogen or by helium. Low oxygen tension relaxed arteries despite pretreatment with 2,4-dinitrophenol, an agent which blocks oxidative phosphorylation. It is concluded that a reduction in oxygen tension exerted a direct, although small, depressant effect on human pial arteries, and that this effect was not mediated exclusively by hyperpolarization or by inhibition of oxidative phosphorylation.

KEY WORDS


Cerebral blood flow (CBF) is controlled by the tone of the smooth muscle in the cerebral resistance arteries [1, 2]. Arterial tone is modified by the plasticity of the vessels (length–tension relationship), by direct influence of different chemical–hormonal actions and by nerve stimulation [1]. One of the factors which modulates local blood flow is oxygen [2].

In isolated vascular smooth muscle, the response to oxygen depends on the species and anatomical origin of the preparations [3–5]. An increase in oxygen tension has been shown to enhance vascular smooth muscle contraction in several animal species [3, 4, 6–11], an effect which probably represents a direct action of oxygen on the vascular smooth muscle membrane [1]. In man, it has been demonstrated that CBF decreases with increased oxygen tensions [12, 13]. However, little is known of the effects of oxygen in human cerebral arteries, which to the best of our knowledge have not been studied previously.

The present study was performed in order to describe the influence of different oxygen tensions on the contractability of isolated human cerebral arteries. This quantitative evaluation of the effects of oxygen is important when different acid–base regimens or the effects of drugs are studied in vitro.

MATERIALS AND METHODS

Preparation

Human pial arteries were removed from macroscopically normal parts of the brain in seven patients (mean age 52 yr; range 42–70 yr) without any history of cardiovascular disease, undergoing lobectomy because of underlying glioma. Anaesthesia was performed with fentanyl, thiopentone, suxamethonium, pancuronium and nitrous oxide. The vessels, together with adjacent normal cortex, were transferred immediately into cooled Krebs solution and subsequently freed from surrounding tissues under the microscope. If the arteries were not used for experiments immediately after removal, they were stored in a + 4 °C Krebs solution for no more than 24 h.

Ring segments, 500–1000 µm wide (o.d.) and 2–3 mm long, were suspended between two L-shaped metal prongs in organ baths each containing 5 ml of a Krebs solution and subsequently freed from surrounding tissues under the microscope. If the arteries were not used for experiments immediately after removal, they were stored in a + 4 °C Krebs solution for no more than 24 h.

Ring segments, 500–1000 µm wide (o.d.) and 2–3 mm long, were suspended between two L-shaped metal prongs in organ baths each containing 5 ml of a Krebs solution, the composition of which is presented below. The solution was maintained at
37 °C. One of the two metal prongs was connected to a force transducer (Grass FT 03C) for recording of isometric tension and the other prong was fixed to a displacement device. Mechanical activity was recorded on a polygraph (Grass Model 7A). Each vessel was given a tension of approximately 1 mN mm⁻¹ (length) and allowed to accommodate for 30-90 min, during which time the fluid was replaced every 15 min [14]. The Krebs solution was bubbled continuously with gas mixtures composed of carbon dioxide, oxygen and nitrogen controlled with flowmeters calibrated with a Tiemeter RT-200 calibration analyser. The gas mixture was analysed constantly with a Datex Normocap CD 102-24-02. Partial pressures of oxygen and carbon dioxide and the pH of the Krebs solution were controlled using a Radiometer ABL 300. Samples were obtained in glass syringes and kept in water at 4 °C until analysed.

Solutions and drugs

The composition of the Krebs solution was (mmol litre⁻¹): NaCl 119; NaHCO₃ 20; KCl 4.6; MgCl₂ 1.2; CaCl₂ 1.5; NaH₂PO₄ 1.2; glucose 11.0. Solutions containing different concentrations of potassium were obtained by exchanging NaCl in the Krebs solution with equimolar amounts of KCl. Prostaglandin F₂α (PGF₂α) was supplied as an aqueous solution (Amoglandin, Astra, Sweden) and dilutions were made up with saline immediately before use. 2,4-Dinitrophenol (Sigma, St Louis, U.S.A.) was dissolved in and diluted with water.

Experimental procedures

During the accommodation period, the Krebs solution was equilibrated with 5 % carbon dioxide in oxygen. Contractions were induced by K⁺ 11-124 mmol litre⁻¹ and experiments were not continued unless two reproducible contractions were obtained. Contractions were considered reproducible when maximum tension differed by less than 10 %. The carbon dioxide content in the gas mixture was adjusted to maintain constant PCO₂ 4 kPa and pH 7.4. The experiments were excluded when the pH deviated ±0.1 from this value.

The effects of oxygen were studied under two different conditions.

(1) Cumulative concentration–response curves [15] were obtained by inducing contractions by stepwise increments with either K⁺ 11-124 mmol litre⁻¹ or PGF₂α 2 x 10⁻⁸-1 x 10⁻⁹ mol litre⁻¹. The concentration–response relationship was determined at different concentrations of oxygen (95, 30 and 0 %) in the gas mixture, attained by exchanging oxygen with nitrogen in a randomized way. The vessels were allowed to adapt for approximately 20 min at each oxygen concentration before concentration–response curves were elicited. The carbon dioxide content in the gas mixture was kept constant at 5 %. Oxygen concentrations of 95, 30 or 0 % in the
gas mixture used to bubble the contents of the organ baths resulted in partial pressures for oxygen \( (P_{O_2}) \) in the Krebs solutions of 73.4 (2.9) kPa, 29.8 (1.6) kPa and 7.7 (0.5) kPa, respectively \((n = 7)\). The partial pressure for carbon dioxide \( (P_{CO_2}) \) was 4.10 (0.08) kPa and the pH was 7.430 (0.009) \((n = 21)\).

(2) In separate experiments, the arteries were contracted with either potassium 29 mmol litre\(^{-1} \) or PGF\(_{2\alpha} \) 2 \( \times 10^{-6} \) mol litre\(^{-1} \). When the contractile responses were stable at 95% oxygen and 5% carbon dioxide in the gas mixture, relative "hypoxia" was induced by exchanging oxygen in the gas mixture with either 95% nitrogen or 95% helium. About 20 min later, the contents of the baths were reoxygenated with 95% oxygen. Some of the arteries, in which stable contractions were obtained with K\(^+\) 29 mmol litre\(^{-1} \) or PGF\(_{2\alpha} \), were exposed to 2,4-dinitrophenol \( (2-4 \times 10^{-5} \) mol litre\(^{-1} \)), which uncouples the oxidative phosphorylation [16]. During this exposure, decrease in oxygen tension and reoxygenation were performed according to the procedure described above.

Calculations and statistical analysis

All results are expressed as mean \((\text{SE})\): \( n \) denotes the number of observations (all from different patients).

The maximum response (Emax) elicited in each preparation with either K\(^+\) or PGF\(_{2\alpha} \) at an oxygen concentration of 30% in the gas mixture was defined as 100%. All subsequent contractions were related to this response. The Emax values induced by K\(^+\) and PGF\(_{2\alpha} \) with 30% oxygen were 8.8 (1.7) mN \((n = 7)\) and 6.0 (1.2) mN \((n = 7)\), respectively.

Estimation of the concentrations of the drugs producing half-maximum effect (EC\(_{50}\)) was based on the geometrical means of the sigmoid concentration–response curves. The Wilcoxon rank sum test was used for determination of statistical significance between groups of data. \( P \leq 0.05 \) was considered to indicate statistical significance.

RESULTS

The effects of oxygen were negligible in unstimulated arteries.

Effects of oxygen on contractions induced by depolarization with potassium

A stepwise depolarization of the smooth muscle membranes, attained with increasing K\(^+\) concentrations at 30% oxygen, induced concentration-dependent contractions. A decrease in oxygen tension depressed Emax of the concentration–response curves for potassium and an increase in oxygen tension enhanced the contractions (fig. 1A). The increase in Emax (fig. 2) was significant only at an oxygen tension of 73 kPa \((P = 0.01)\). The threshold concentrations for contraction and the EC\(_{50}\) values were not significantly affected. The Emax and EC\(_{50}\) values are presented in table I.

A sudden decrease from 95% to 0% oxygen

![Diagram](https://academic.oup.com/bja/article-abstract/68/2/187/298260/2620061)

**Fig. 3. Tracings demonstrating the effect of decreased oxygen tension (0% oxygen in the gas mixture) induced at \( \Delta \) and subsequent reoxygenation induced at \( \triangle \) on arteries contracted by (A) K\(^+\) 29 mmol litre\(^{-1} \) \((n = 3)\) or (B) PGF\(_{2\alpha} \) \(2 \times 10^{-6} \) mol litre\(^{-1} \) \((n = 3)\) applied at \( \bullet \).**
The contractions are characterized by the EC$_{50}$ (mean), −log (EC$_{50}$) (mean (SE)) and Emax (mean (SE)) values. n = Number of experiments. *P = 0.02

<table>
<thead>
<tr>
<th>Oxygen (%)</th>
<th>EC$_{50}$ (mmol litre$^{-1}$)</th>
<th>−log (EC$_{50}$) (%)</th>
<th>Emax (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>2.6 × 10$^{-6}$</td>
<td>5.58 (0.15)</td>
<td>115 (6)*</td>
<td>7</td>
</tr>
<tr>
<td>30</td>
<td>3.1 × 10$^{-6}$</td>
<td>5.50 (0.14)</td>
<td>100 (0)</td>
<td>7</td>
</tr>
<tr>
<td>0</td>
<td>3.7 × 10$^{-6}$</td>
<td>5.43 (0.18)</td>
<td>96 (2)*</td>
<td>7</td>
</tr>
</tbody>
</table>

During partial depolarization with K$^+$ 29 mmol litre$^{-1}$ (approximately representing the EC$_{50}$ values), a sudden decrease from 95% to 0% oxygen induced an immediate relaxation (fig. 3B). The decrease in arterial tension was independent of whether the decreased oxygen tension was achieved with nitrogen or helium. Subsequent reoxygenation generated a contraction which exceeded the tension before oxygen depletion. The effects of 2,4-dinitrophenol on arteries contracted by PGF$_{2\alpha}$ $2 \times 10^{-6}$ mol litre$^{-1}$ were qualitatively similar to the effects in arteries contracted by excess K$^+$. Induction of a decreased oxygen tension induced an immediate relaxation in dinitrophenol-exposed PGF$_{2\alpha}$-contracted arteries and subsequent reoxygenation failed to restore the contraction (fig. 4B).

**Discussion**

In human pial arteries a decrease in oxygen tension from 73 kPa to 8 kPa diminished the vascular smooth muscle contraction in response to K$^+$ and PGF$_{2\alpha}$. This observation is in accordance with studies on cerebral arteries from the cat [9-11], rabbit [4], cow [17] and dog [8, 18]. In our experiments, the variation in contractile force between arteries exposed to oxygen 73 and 8 kPa was no more than 20%. Although this difference may seem small, it was consistently reproducible. We have not tried to induce a more pronounced hypoxia which can be
expected to induce more profound effects [8], some of which, however, may be the result of an irreversible injury to the arteries. Indeed, at a very small oxygen tension (1.3 kPa) the relaxant effect may even be reversed to a contraction, as described by Nakagomi and colleagues [8] in dog cerebral arteries.

In previous investigations, hypoxia has been induced by decreasing the oxygen:nitrogen ratio. However, it cannot be excluded that nitrogen in itself may have an effect on smooth muscle contractions. In our study, the effects of reduced oxygen tension were independent of whether this condition was attained through addition of nitrogen or helium, which supports the concept that the observed effects were indeed caused by a decrease in oxygen tension and not increased nitrogen tension.

Resting arteries showed a hardly measurable tendency to relaxation when the oxygen tension was decreased. Similar findings have been described in rabbit common carotid arteries and rabbit aortas [3, 6]. These results indicate that oxygen modulates only the active tone, which may imply, for instance, a sodium–potassium, calcium or metabolic influence, or any combination of these [19, 20].

We observed that a reduction in \( P_O_2 \) diminished the contraction elicited by \( K^+ \), which does not support the hypothesis that the relaxation induced by decreased oxygen tension was caused by hyperpolarization [21]. A relaxation induced by hyperpolarization is unattainable in \( K^+ \)-contracted arteries, as the Na–K ATPase is maximally activated when the extracellular \( K^+ \) concentration is increased to greater than the threshold for contraction [21]. In contrast, hypoxia-induced stimulation of the Na–K ATPase may contribute to the effects in PGF\(_{2\alpha}\)-contracted arteries.

Oxygen is known to affect ATP production in mitochondria and, theoretically, this may fit with the hypothesis of hypoxia-induced metabolic depression. However, the critical \( P_O_2 \) for mitochondria, before oxygen becomes the limiting factor for electron transport and oxidative phosphorylation, seems to be less than 7 kPa [22].JOBiS [23] and Detar [21] implied that energy metabolism does not become restricted until the oxygen concentration diminishes to 1.3 kPa or less. Coburn, Grubb and Aronson [24] and Ebeigbe, Pickard and Jannet [3] observed arterial relaxation when oxidative metabolism (cytochrome \( a_0 \)) was blocked with cyanide, but there was no correlation between the effects of cyanide and oxygen regarding the rapidity and magnitude of relaxation. In the present study, 2,4-dinitrophenol showed the same mode of action, corroborating the conclusions drawn by Coburn’s group [24] that the relaxation induced by hypoxia was not caused by metabolic depression. We observed also that the relaxant effect of reducing the oxygen tension was preserved in dinitrophenol-pretreated arteries, further supporting the concept that the effect of a moderately reduced oxygen tension is at least partially independent of mitochondrial respiratory chain activity.

The present study was not designed to evaluate closely the mechanisms behind the relaxant effect of reduced oxygen tension. It is well known that vascular smooth muscle contraction is dependent on the free intracellular concentration of calcium [20]. However, studies on femoral, renal and saphenous rabbit arteries failed to demonstrate a simple relationship between the relaxing effect of hypoxia and the ability to contract in calcium-free medium [25]. Hence, it seems less likely that oxygen directly influences intracellular calcium homeostasis in smooth muscle cells. Instead, hypoxia may be linked to effects on smooth muscle via an intermediate step involving events in, for example, endothelium [26]. In support of this concept, it is known that removal of the vascular endothelium abolishes the vasodilatory response to hypoxia in canine femoral and coronary arteries and rat tail arteries [5]. The vascular endothelium is able to produce the potent vasodilator, endothelium-derived relaxing factor (EDRF) [27]. EDRF consists of two or more substances [28, 29]; nitric oxide is probably one [30–32]. Nitric oxide is labile and inactivated by superoxides, whereas hypoxia and free radical scavengers prolong its half-life [31, 33, 34]. Hyperoxia, and ischaemia followed by re-perfusion, could be associated with an increased local production of superoxides [35], and in turn an increased inactivation of nitric oxide leading to enhancement of contractions.

In conclusion, our results demonstrate a direct action of oxygen on human pial arterial contractility. While there are many theories on the modulation of contracted arteries by oxygen, our data suggest that hyperpolarization of the arteries or inhibition of oxidative phosphorylation are not likely to be important.

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REFERENCES


