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Phosphodiesterase-5 inhibitors oppose hyperoxic vasoconstriction and accelerate seizure development in rats exposed to hyperbaric oxygen

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Demchenko IT, Ruehle A, Allen BW, Vann RD, Piantadosi CA. Phosphodiesterase-5 inhibitors oppose hyperoxic vasoconstriction and accelerate seizure development in rats exposed to hyperbaric oxygen. *J Appl Physiol* 106: 1234–1242, 2009. First published January 29, 2009; doi:10.1152/jappphysiol.91407.2008.—Oxygen is a potent cerebral vasoconstrictor, but excessive exposure to hyperbaric oxygen (HBO₂) can reverse this vasoconstriction by stimulating brain nitric oxide (NO) production, which increases cerebral blood flow (CBF)—a predictor of O₂ convulsions. We tested the hypothesis that phosphodiesterase (PDE)-5 blockers, specifically sildenafil and tadalafil, increase CBF in HBO₂ and accelerate seizure development. To estimate changes in cerebrovascular responses to hyperoxia, CBF was measured by hydrogen clearance in anesthetized rats, either control animals or those pretreated with one of these blockers, with the NO inhibitor *N*^ω-nitro-L-arginine methyl ester (L-NAME), with the NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP), or with a blocker combined with L-NAME. Animals were exposed to 30% O₂ at 1 atm absolute (ATA) (“air”) or to 100% O₂ at 4 or 6 ATA. EEG spikes indicated central nervous system CNS O₂ toxicity. The effects of PDE-5 blockade varied as a positive function of ambient Po₂. In air, CBF did not increase significantly, except after pretreatment with SNAP. However, at 6 ATA O₂, mean values for CBF increased and values for seizure latency decreased, both significantly; pretreatment with L-NAME abolished these effects. Conscious rats treated with sildenafil before HBO₂ were also more susceptible to CNS O₂ toxicity, as demonstrated by significantly shortened convulsive latency. Decreases in regional CBF reflect net vasoconstriction in the brain regions studied, since mean arterial pressures remained constant or increased throughout. Thus PDE-5 blockers oppose the protective vasoconstriction that is the initial response to hyperbaric hyperoxia, decreasing the safety of HBO₂ by hastening onset of CNS O₂ toxicity.

nitric oxide; oxygen seizures; phosphodiesterase-5 blockers; cerebral blood flow

NITRIC OXIDE (NO) is a critical factor in the development of central nervous system (CNS) oxygen toxicity (4, 6, 13, 15, 35), which limits the safety of breathing oxygen at elevated partial pressures, either by patients undergoing treatment with hyperbaric oxygen (HBO₂) or by underwater divers. Furthermore, the use of phosphodiesterase (PDE)-5 blockers, which act by potentiating the action of endogenous NO (10, 19, 29), has not been studied in hyperoxia. These agents, such as sildenafil and tadalafil, have been introduced for the treatment of erectile dysfunction and more recently for the treatment of pulmonary hypertension (45) and may have effects on the

NO-mediated responses in cerebral blood flow (CBF) during hyperoxia.

NO plays a major role in CNS O₂ toxicity by reversing the initial response to hyperoxia in the brain—the protective vasoconstriction that prevents increases in O₂ delivery to toxic levels (15, 43). The effect of NO is to increase CBF and raise brain Po₂ in experimental animals. Thus in vasodilator experiments a doubling of CBF in rats breathing room air, where hemoglobin is nearly saturated with O₂, increases brain Po₂ less than twofold. However, at 2–6 atm absolute (ATA) O₂, with hemoglobin fully saturated and a great deal of additional O₂ dissolved in the plasma, a doubling of CBF elevates brain Po₂ 13- to 64-fold (16).

PDE-5 inhibitors potentiate the effect of endogenously released NO by slowing the degradation of guanosine 3',5'-cyclic monophosphate (cGMP), the second messenger in the NO signaling pathway. Inhibition of PDE-5 present in the endothelium of the corpora cavernosa prolongs the relaxation of the pericavernous smooth muscle, sustaining its engorgement with blood (20). PDE-5 is also found in platelets, skeletal muscle, and visceral and pulmonary vascular smooth muscle, as well as in cerebral neurons and vessels (24, 31). Since NO dilates cerebral vessels through the activation of soluble guanylyl cyclase (sGC), causing increased cGMP levels, inhibition of PDE-5 in brain would enhance and prolong the local effect of NO. And because HBO₂ stimulates NO production in the brain, we hypothesized that the combination of PDE-5 inhibition and HBO₂ might work in synergy to accelerate delivery of toxic doses of oxygen and hasten the onset of seizures. Studies in air at sea level have assessed the effects of PDE-5 inhibition on cerebral hemodynamics in human subjects and have found no significant changes in brain blood flow or red blood cell velocity, unless endogenous NO production is stimulated or an NO donor is administered (1, 25–28). Because no previous studies have evaluated the effects of PDE-5 blockade in hyperbaric hyperoxia, it has not been established whether PDE-5 blockers diminish or abolish the initial, protective vasoconstriction or hasten the onset of seizures.

The hypothesis tested in this study is that the use of PDE-5 inhibitors before exposure to HBO₂ accelerates the development of CNS O₂ toxicity by increasing CBF through the activity of the NO/sGC signaling pathway. This proposition was prefigured by our demonstration that HBO₂ stimulates neuronal nitric oxide synthase (nNOS) activity in the brain (17), but the question remains unanswered as to whether the

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dilator effect of the resulting endogenous NO release is amplified or prolonged by PDE-5 inhibition. Specifically, we set out to determine whether in HBO₂ PDE-5 inhibition opposes the initial vasoconstriction and increases CBF or hastens the onset of oxygen seizures and, if so, whether this occurs through the NO/sGC/cGMP pathway.

METHODS

Experimental Animals

Male Sprague-Dawley rats (Charles River) weighing 327 ± 13 g were used, as approved by the Duke University Institutional Animal Care and Use Committee. Anesthesia was induced with urethane (750 mg/kg ip) and α -chloralose (75 mg/kg ip). Both femoral arteries and one femoral vein were catheterized to measure blood pressure, take samples, and infuse drugs. The trachea was intubated, and anesthetized animals were ventilated with 30% O₂ in N₂ (termed "air" in this report). The head of each rat was secured in a stereotaxic frame, and a midline incision was made in the scalp to expose the cranial surface between bregma and a point 4–5 mm posterior to lambda. A hole was drilled in the skull over either the left or right caudate putamen, 0.5 mm anterior and 2.5 mm lateral to bregma (38). A needle-type hydrogen-sensitive platinum electrode was inserted 5.4 mm below the dura mater and fixed in position by a stereotaxic manipulator. The anatomic location of the electrode was confirmed postmortem. A second hole in the skull (~2 mm in diameter) was drilled over the dural venous sinuses, taking lambda as the center of the confluence of the superior sagittal and transverse sinuses; care was taken not to rupture the venous sinuses, and a platinum disk electrode (~1 mm in diameter) was fixed on the intact dura mater with another micromanipulator. For EEG recording, two stainless steel screws were driven into the skull symmetrically over the left and right parietal cortex.

Anesthetized rats were given pancuronium bromide (0.5 mg/kg iv) to prevent voluntary respiratory movements and to permit the maintenance of arterial P_{CO₂} (Pa_{CO₂}) at 35–40 Torr by adjusting tidal volume. Anesthesia and immobility were maintained by intravenous administration of one-quarter of the initial doses of anesthetic each hour, or as necessary. Adequacy of anesthesia was verified by observing blood pressure responses to toe pinch. Control studies indicated that this regimen of supplemental anesthesia is adequate for the HBO₂ exposures.

Physiological Measurements

Arterial blood pressure was measured continuously and integrated to obtain mean arterial blood pressure (MABP). Arterial P_{O₂} (Pa_{O₂}), Pa_{CO₂}, and pH were determined periodically (IL 1306 blood gas/pH analyzer) in rats breathing air and immediately before and after HBO₂ exposure, while ventilated with 100% O₂. Rectal temperature was monitored continuously and held at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ with a heating pad. EEG was recorded continuously and assessed visually. A train of three or more EEG spikes or the first burst, consisting of several high-voltage slow waves and spikes repeated every few seconds, followed closely by generalized spiking, signaled the initiation of CNS O₂ toxicity.

Cerebral Blood Flow

Regional CBF (rCBF) was measured in HBO₂ by hydrogen clearance as described previously (13, 16). Briefly, rCBF was measured in the striatum with an insulated platinum wire electrode having a bare conical tip of 1-mm length with an apical diameter of 10–50 μm and coated with Nafion (Aldrich Chemical, Milwaukee, WI) to prevent protein fouling. To measure rCBF, 2.5% H₂ in air was introduced through the respirator for 60 s, and then H₂ washout curves were captured with WINDAQ software (D-1200 AC, DATAQ Instruments). Absolute rCBF ($\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$) was calculated by the

initial slope method with Mathematica 3.0 software (Wolfram Research) with minor modifications (14).

Total CBF (tCBF) was also measured with a modified hydrogen clearance method with a platinum disk electrode set gently on the surface of the intact dura mater over the confluence of venous sinuses. Earlier studies have shown that H₂-sensitive electrodes inserted into veins draining intact organs allow measurement of total blood flow to heart, skeletal muscle, and kidney (2, 34) or to the brain if the electrode is inserted into the confluence of sinuses beneath the torcular herophili, the concavity in the internal aspect of the occipital bone accommodating the confluence of sinuses (32). Since H₂ diffuses freely through venous walls, H₂ clearance curves can also be recorded from their outer surface without penetrating the venous wall (11). To validate this method for tCBF measurement in the rat, we compared measurements made by a needle electrode inserted into the confluence of sinuses with those made simultaneously by a disk electrode placed on the dura over the confluence of sinuses; H₂ clearance curves were calculated for both electrodes to assess H₂ diffusion through the sinus wall (Fig. 1B).

Hyperbaric Oxygen Exposure

Rats were placed in a hyperbaric chamber (Duke Center for Hyperbaric Medicine and Environmental Physiology) along with the stereotaxic frame, respirator, blood pressure transducer, heating pad, and infusion pump. Electrodes were connected through hermetic wall penetrations to amperometric amplifiers outside the chamber. After a 60-min stabilization period during which the animal breathed 30% O₂, three H₂ clearance curves were recorded to calculate control tCBF and rCBF. The respirator was then supplied with 100% O₂, and the chamber air pressure was raised to either 4 or 6 ATA at 0.6 ATA/min. Hyperbaric oxygen exposures lasted 60–75 min, and CBF was measured every 15 min. Immediately after decompression at a rate of 0.6 ATA/min (8.3 min), blood gases and pH were determined as the rats continued to breathe 100% O₂.

Preparation of PDE-5 Inhibitors for Infusion

Tablets of sildenafil (Viagra 50 mg, Pfizer) or tadalafil (Cialis 20 mg, Eli Lilly) were dissolved in 0.9 NaCl (saline), filtered, and injected intraperitoneally in amounts approximately proportional to the maximal doses administered to 70-kg human patients, 100 mg for sildenafil or 20 mg for tadalafil (1). Times of administration for these drugs were adjusted according to their onset of action (2, 5).

Experimental Design

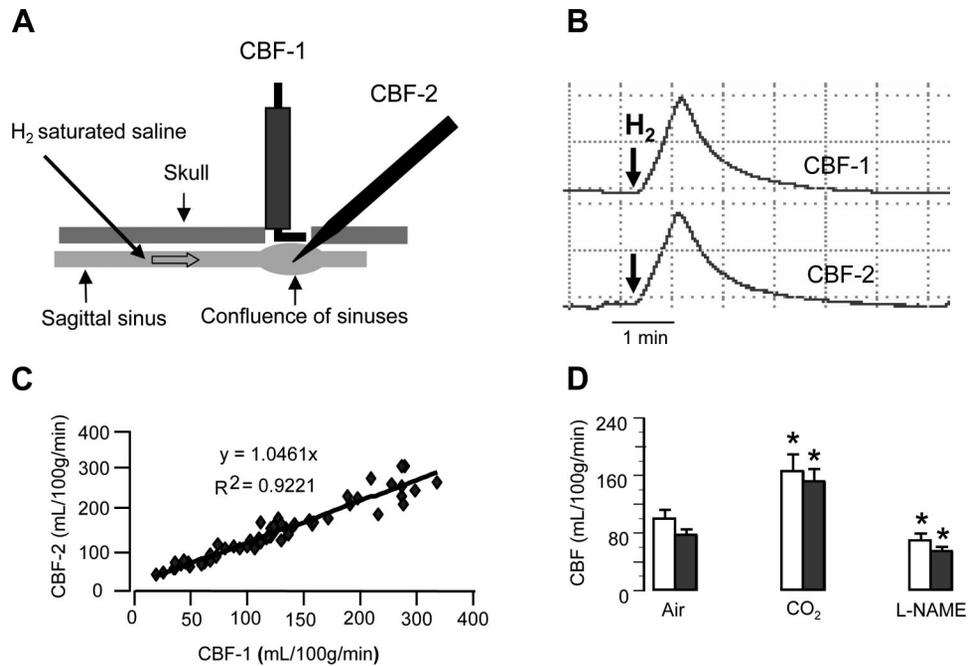
Four series of experiments were conducted, as described below. Interventions were made before the first measurement in "air" or HBO₂: saline (0.5 ml ip, 30 min), sildenafil (1.4 mg/kg ip, 30 min), tadalafil (0.3 mg/kg ip, 60 min), *N*^ω-nitro-L-arginine methyl ester (L-NAME; 30 mg/kg ip, 30 min), *S*-nitroso-*N*-acetylpenicillamine (SNAP; 0.1 mg/kg iv, 5 min).

CBF in anesthetized animals in "air." In the first series, tCBF and rCBF were measured in five groups of rats breathing 30% O₂ (balance N₂) at 1 ATA and infused either with saline (as controls) or with a drug: saline ($n = 5$), sildenafil ($n = 8$), tadalafil ($n = 8$), the NO donor SNAP ($n = 7$), or SNAP + sildenafil ($n = 7$). Because both sildenafil and tadalafil achieve maximum plasma concentrations within 1 h of intraperitoneal administration, exposures of 75 min were chosen to assess the time course of any effects of PDE-5 inhibitors on CBF.

CBF in anesthetized animals in 4 ATA O₂. In the second series, tCBF and rCBF were measured in rats infused with saline ($n = 8$) or sildenafil ($n = 8$) before exposure to 4 ATA O₂ for 75 min.

Seizures in awake animals in 4 ATA O₂. In the third series, seizure latency was assessed in freely moving rats exposed to O₂ at 4 ATA for 2 h after infusion with either saline ($n = 8$) or sildenafil ($n = 8$). Latency to the initiation of full motor seizures with loss of postural

Fig. 1. Validation of total cerebral blood flow (tCBF) measurements in rats. *A*: electrodes for recording partial pressure of H₂ on the outer wall of the confluence of sinuses (CBF-1) and in the venous blood (CBF-2). *B*: representative H₂ clearance curves recorded by CBF-1 and CBF-2 electrodes after bolus injection of H₂-saturated 0.9% NaCl into the superior sagittal sinus. *C*: correlation between tCBF measured in rats by CBF-1 and CBF-2 electrodes after 30-s inhalation of 2.5% H₂ (balance air). *D*: tCBF (open bars) and striatal CBF (filled bars) in anesthetized rats in response to inhalation of 5% CO₂ in room air or treatment with *N*^o-nitro-L-arginine methyl ester (L-NAME). **P* < 0.05 vs. air.



control (6) was determined by direct observation of animal behavior. Our previous work had shown that 2 h at this level of hyperoxia exposure time was optimal to observe seizures and other behavioral changes in rats (unpublished data) and in mice.

CBF in anesthetized animals in 6 ATA O₂. In the fourth series, tCBF and rCBF were measured in six groups of rats exposed to 6 ATA O₂, after pretreatment with saline (*n* = 8), sildenafil (*n* = 8), tadalafil (*n* = 8), L-NAME (*n* = 8), L-NAME and sildenafil (*n* = 6), or L-NAME and tadalafil (*n* = 6). Exposure to HBO₂ was limited to 60 min to avoid mortality from prolonged seizures and to allow samples to be drawn for blood gas measurements after decompression.

Data Analysis

All values from groups of animals are expressed as means ± SE. Absolute and percent changes in CBF and other physiological variables (MABP and arterial blood gas values) are compared with baseline values obtained before treatment with paired *t*-tests. Changes in CBF and MABP over time in 30% O₂ at 1 ATA or in HBO₂ are analyzed with repeated analysis of variance (ANOVA) followed by post hoc comparison with Fisher's exact test. A value of *P* < 0.05 is accepted as significant.

RESULTS

Validation of CBF Measurements Using H₂-Sensitive Electrodes

Hydrogen was found to diffuse reproducibly through the wall of the confluence of venous sinuses after bolus injection of

H₂-saturated saline into the sagittal sinus (Fig. 1*B*). Transdural detection of partial pressure of H₂ was delayed by <3 s, compared with direct measurement in venous blood. The walls of the venous sinuses are a tenuous network of endothelium and elastic and collagenous fibers within the dura (39); thus H₂ clearance curves recorded by electrodes located over the venous sinuses can be used for measuring CBF. Clearance curves obtained in this manner had two components, the first of which represents recirculation of H₂ or its diffusion between cerebral arteries and veins. H₂ is the most rapidly diffusing of all gases, and because of its evanescence there is no significant delay in its elimination from the lungs or other tissues that could alter its clearance kinetics from the cerebrovenous system. Studies in the baboon have shown that experimental artifacts are negligible if the first 40 s are discounted (37). In the rat, with a much shorter circulation time and smaller volume of distribution for H₂, we found it only necessary to discard the first 20 s of the clearance curve.

Therefore, tCBF values obtained from H₂ clearance curves recorded directly in the blood in the confluence of sinuses correlated closely with those measured extravascularly on the overlying dura mater (Fig. 1*C*); close correlation between intra- and extravascular measurements also indicates a lack of significant disruption of the blood-brain barrier by the intravascular microelectrode. Validation of these tCBF measurements was also confirmed by determining cerebrovascular

Table 1. Blood gases and mean arterial blood pressure in rats breathing 30% O₂ (balance N₂) before and after PDE-5 inhibition

Group (F _{IO₂})	Before Treatment				75 min After Treatment			
	MABP	PaO ₂	Paco ₂	pH	MABP	PaO ₂	Paco ₂	pH
0.3 ATA (Control)	119±6	118±6	36±3	7.40±0.03	115±6	107±6	37±3	7.38±0.04
0.3 ATA (sildenafil)	117±5	121±6	36±3	7.38±0.03	113±7	111±6	36±2	7.38±0.04
0.3 ATA (tadalafil)	120±5	116±5	37±3	7.39±0.02	111±6	110±5	39±3	7.37±0.03

Values are means ± SE. PDE-5, phosphodiesterase-5; F_{IO₂}, inspired PO₂ [atm absolute (ATA)]; MABP, mean arterial blood pressure (mmHg); PaO₂ and Paco₂, arterial PO₂ and PCO₂ (Torr).

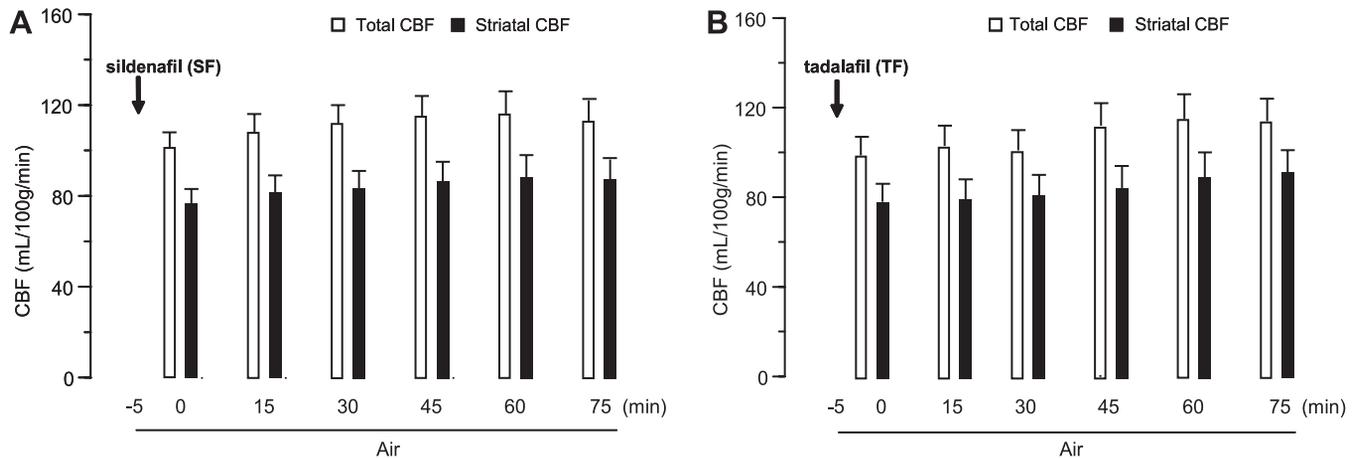


Fig. 2. Temporal profiles of tCBF and striatal CBF after phosphodiesterase (PDE)-5 inhibition. *A*: treatment with sildenafil (SF, 1.4 mg/kg ip; at arrow). *B*: treatment with tadalafil (TF, 0.3 mg/kg ip; at arrow). No statistically significant changes were observed.

reactivity to CO₂ or L-NAME (Fig. 1D). In the rat, the confluence of sinuses drains multiple brain regions, including the cerebral cortex, the white matter, and some subcortical structures (3, 44), and points of juncture between large veins and sinuses in rats can be located reproducibly (3). Therefore, H₂ curves recorded over the confluence of sinuses represent venous drainage from about two-thirds of the rat brain and can be reproducibly used to calculate tCBF.

Effects of PDE-5 Inhibitor on MABP

The average value of MABP in anesthetized rats at steady state was 119 ± 6 mmHg and did not differ significantly among experimental groups. Although intravenous administration of sildenafil decreased MABP transiently by 15–25%, 5–10 min later it did not differ from control levels. By contrast, the effect of intraperitoneal sildenafil or tadalafil on MABP was milder and slower; MABP decreased transiently by ~10–15% and returned nearly to control levels after 20–30 min. In anesthetized rats breathing “air,” MABP did not change significantly during the 75-min experiment (Table 1).

In untreated rats exposed to 4 ATA O₂, MABP rose during compression and increased continuously during HBO₂ exposure, remaining elevated until decompression (data not shown). MABP profiles in rats pretreated with sildenafil and exposed to 4 ATA were no different from those of untreated animals. At 6 ATA, MABP was higher during compression and a sharp elevation in blood pressure occurred immediately before EEG spikes were observed, whether or not PDE-5 was inhibited. MABP in rats pretreated with L-NAME and exposed to 6 ATA was higher than in untreated animals. MABP profiles in animals pretreated with both L-NAME and a PDE-5 inhibitor were no different from those treated solely with L-NAME.

Effects of PDE-5 Inhibitors on CBF in Air

In anesthetized rats breathing 30% O₂ at 1 ATA, MABP as well as PaCO₂ and pH were maintained in the physiological range throughout the 75-min experiment (Table 1). The average baseline tCBF was 94 ± 5 ml·100 g⁻¹·min⁻¹ ($n = 43$), and striatal rCBF was 71 ± 4 ml·100 g⁻¹·min⁻¹ ($n = 52$). After administration of sildenafil or tadalafil, no significant

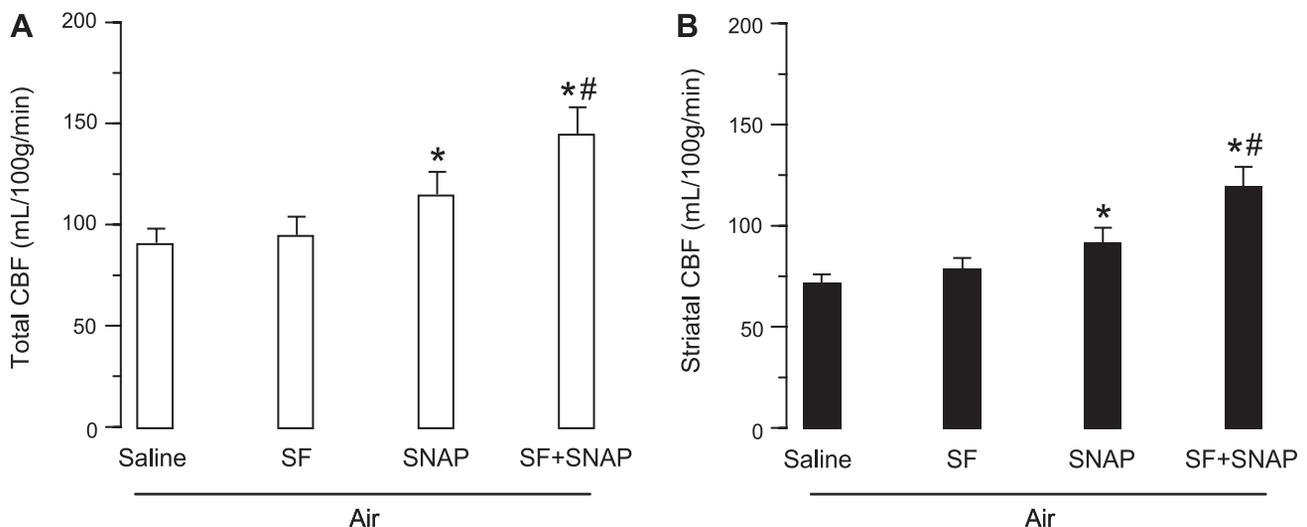


Fig. 3. PDE-5 inhibition with and without a nitric oxide (NO) donor. SF does not significantly change tCBF (A) or striatal CBF (B). S-nitroso-N-acetylpenicillamine (SNAP) increases CBF in rats breathing air, but in rats pretreated with SF both tCBF and striatal CBF are significantly higher than after SNAP treatment alone. * $P < 0.05$ vs. saline, # $P < 0.05$ vs. SNAP alone.

Table 2. Blood gases and MABP in rats pretreated with PDE-5 inhibitors before and after HBO₂

Group (F _i O ₂)	Before HBO ₂ (30% O ₂ at 1 ATA)				After HBO ₂ (100% O ₂ at 1 ATA)			
	MABP	PaO ₂	PaCO ₂	pH	MABP	PaO ₂	PaO ₂	pH
4 ATA	120±5	119±5	36±2	7.39±0.03	122±6	428±22*	39±2	7.39±0.03
4 ATA (SF)	117±5	119±6	35±3	7.40±0.02	119±5	409±23*	40±3	7.38±0.03
6 ATA	113±6	112±5	36±2	7.41±0.04	118±6	426±21*	39±2	7.39±0.04
6 ATA (SF)	116±4	116±5	35±2	7.39±0.04	123±6	418±22*	41±3	7.38±0.05
6 ATA (TF)	118±5	114±6	36±3	7.42±0.03	118±5	432±23*	42±3	7.38±0.04
6 ATA (L-NAME)	133±6	117±7	36±3	7.41±0.04	138±7	416±25*	39±2	7.39±0.04
6 ATA (L-NAME+SF)	131±6	122±7	35±3	7.42±0.03	137±6	401±32*	41±3	7.39±0.05
6 ATA (L-NAME+TF)	130±5	121±7	35±2	7.39±0.04	124±6	387±29*	38±2	7.39±0.04

Values are means ± SE MABP (Torr), PaO₂ (Torr), PaCO₂ (Torr), and pH. HBO₂, hyperbaric oxygen; SF, sildenafil; TF, tadalafil; L-NAME, N^ω-nitro-L-arginine methyl ester. *P < 0.05 vs. before HBO₂ exposure.

changes occurred in tCBF and rCBF over 60 min of observation (Fig. 2). No EEG changes were observed in rats breathing "air" that were pretreated with a PDE-5 blocker or a NO donor.

To determine whether PDE-5 activity varies with NO production, we investigated the effects of the NO donor SNAP on CBF in rats pretreated with sildenafil. Both tCBF and rCBF increased in rats treated with SNAP alone, and both were significantly augmented in animals also pretreated with sildenafil (Fig. 3).

Effect of PDE-5 Inhibitors on CBF in HBO₂

PaCO₂ and pH values remained within the expected range throughout the exposures at 4 and 6 ATA O₂, as indicated by blood gas measurements made immediately before and after HBO₂ exposures (Table 2). High PaO₂ values persisting after decompression indicated that normal pulmonary gas exchange was preserved throughout the experiments and that very high PaO₂ values were attained during exposure to HBO₂, when blood gases could not be measured (Table 2).

PDE-5 inhibitors modulated the CBF profile in HBO₂ in a pressure- and time-dependent manner. However, in animals not

treated with PDE-5 inhibitors but exposed to HBO₂ at 4 ATA, tCBF and striatal CBF decreased progressively for the first 45 min, after which there was no significant change in CBF until decompression (Fig. 4). Rats pretreated with sildenafil and exposed to 4 ATA O₂ showed significant decreases in CBF over the first 30 min, after which the blood flow gradually rose, approaching preexposure levels over the next 45 min (Fig. 4). In these two groups no EEG spikes were observed, indicating that major CNS O₂ toxicity did not occur.

HBO₂ at 6 ATA produced marked increases in both CBF and EEG activity, and no initial cerebral vasoconstriction was observed in either the control groups or the drug-treated groups. In untreated rats, tCBF and rCBF increased significantly (by 31 ± 4% and 67 ± 5%, respectively) above preexposure levels 30 min after the onset of 6 ATA O₂, whereas pretreatment with sildenafil more than doubled those increases (67 ± 5% and 149 ± 11%, respectively) and hastened their onset (Fig. 5, A and B). Thus in groups of eight rats, EEG discharges, consisting of single or multiple paroxysmal spikes with amplitudes of >100 μV, were observed in six of eight control animals at 47 ± 5 min after the onset of hyper-

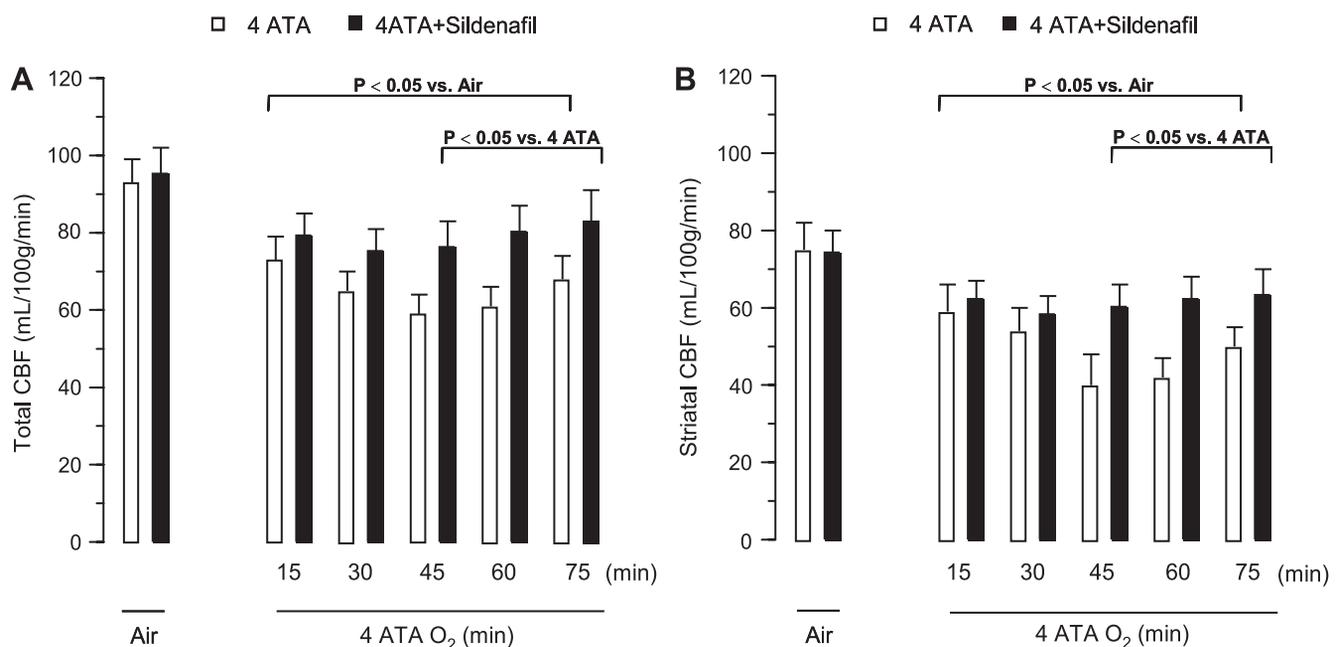


Fig. 4. Hyperoxic vasoconstriction. In untreated rats, 4 atm absolute (ATA) O₂ significantly decreases tCBF (A) and striatal CBF (B) within 75 min, while hyperoxic vasoconstriction is attenuated in rats pretreated with SF 30 min before HBO₂ exposure.

baric hyperoxia and in all eight sildenafil-treated animals at 36 ± 5 min ($P < 0.05$).

Tadalafil also promoted significant increases in both tCBF and rCBF compared with untreated animals (Fig. 5, C and D). CBF peaked ~ 30 min into the exposure, and EEG spikes in tadalafil-treated animals occurred after an average latency of 37 ± 4 min.

Awake, freely moving rats pretreated with sildenafil and exposed to O₂ at 4 ATA manifested convulsions earlier than untreated control animals. By the end of the 2-h exposure, seven of eight pretreated animals had convulsions compared with five of eight control animals. The mean convulsion latency in sildenafil-treated rats (79 ± 6.6 min) was signifi-

cantly less ($P < 0.05$) than in control animals (105 ± 5.8 min). Three animals treated with tadalafil exhibited early signs of CNS O₂ toxicity very similar to those treated with sildenafil (data not shown).

Combined Inhibition of PDE-5 and NOS

CBF fell dramatically in rats pretreated with L-NAME before exposure to 6 ATA O₂ (Fig. 6). tCBF and rCBF fell significantly in the first 15 min compared with values in rats without L-NAME treatment. Minimum values were reached after 30–45 min and persisted for the 60-min exposure. In

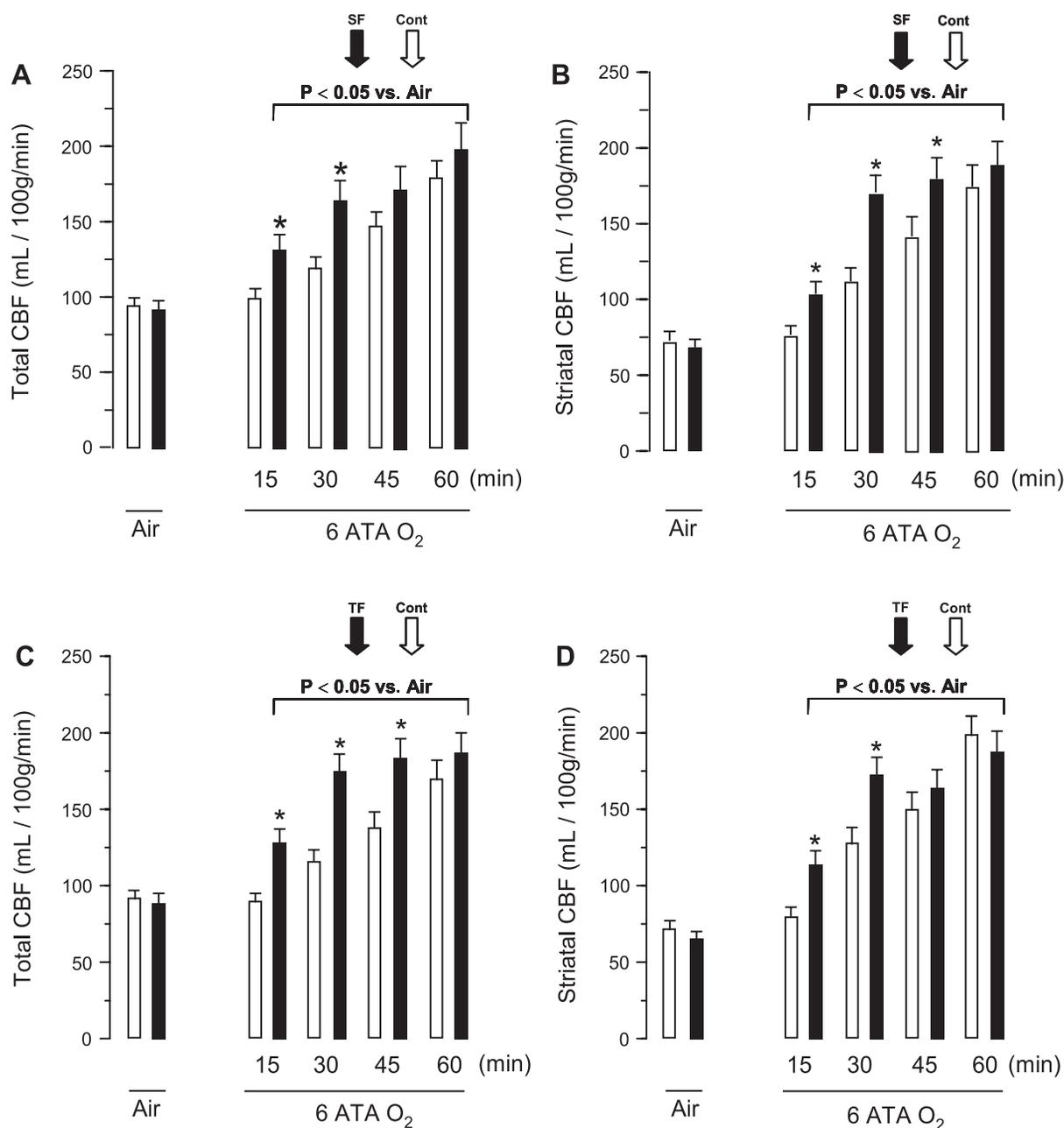


Fig. 5. Loss of cerebral vasoconstriction at 6 ATA O₂. At this pressure, CBF increases progressively in both treated and untreated animals. In untreated rats, EEG spikes are observed at a mean time of 47 min (open arrows). Pretreatment with SF (filled bars in A and B) or with TF (filled bars in C and D) accelerates the increases in both total and striatal CBF and significantly decreases mean seizure latency. Open arrows indicate onset of seizures in control rats; filled arrows mark onset of seizures in rats treated with SF or TF. * $P < 0.01$ vs. untreated rats.

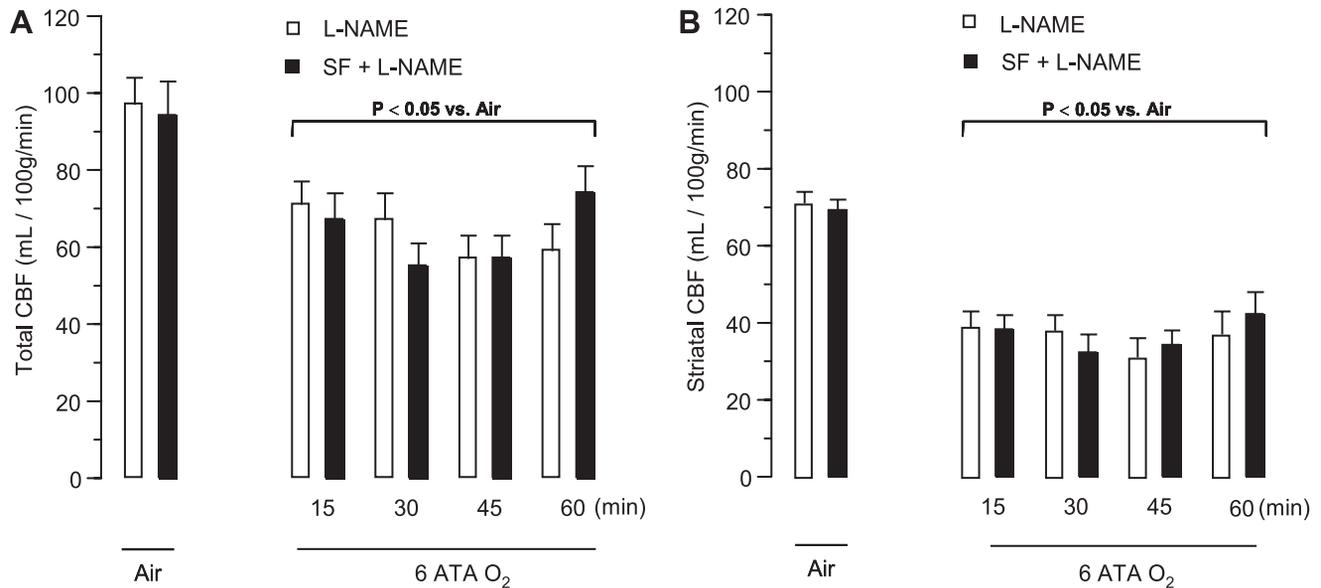


Fig. 6. Inhibition of NO production before 6 ATA O₂, with or without PDE-5 inhibition with SF, prevents the increases in both tCBF (A) and striatal CBF (B); EEG spikes are not observed.

animals pretreated with both L-NAME and sildenafil, tCBF and striatal CBF decreased over time (Fig. 6), but no significant differences were found between animals pretreated with L-NAME plus sildenafil and those treated with L-NAME alone. None of these animals exhibited EEG spikes.

DISCUSSION

The effects of PDE-5 inhibitors on CBF responses to HBO₂ are reported here for the first time. In rats breathing 30% O₂ at 1 ATA, sildenafil and tadalafil do not significantly alter basal CBF, unless cGMP production is stimulated with an NO donor. However, rats pretreated with either drug and exposed to HBO₂ show significantly faster and greater increases in CBF and are more susceptible to CNS O₂ toxicity than rats exposed to HBO₂ alone. Also, the effects of PDE-5 inhibitors on CBF and the development of oxygen seizures in HBO₂ are NO dependent.

Although sildenafil and tadalafil, as selective PDE-5 inhibitors, share the same mechanism of action, both were tested because their half-lives and onsets of action differ. Theoretically, both inhibitors should dilate cerebral blood vessels, because PDE-5 is found in cerebral arteries of animals (26, 42); PDE-5 inhibitors dilate isolated cerebral arteries, with a potency that varies with species and vessel (25); and the vascular relaxation elicited by PDE-5 inhibitors is endothelium dependent (26, 36). However, our *in vivo* data indicate that sildenafil and tadalafil alone, without HBO₂, have very weak effects on tCBF or striatal CBF. These findings are consistent with other normoxic studies of the effects of PDE-5 inhibitors on CBF (28), cerebral vessel diameter (26), and blood velocity (1).

The lack of significant CBF responses to PDE-5 inhibitors is compatible either with an absence of basal cGMP accumulation in smooth muscle cells in the cerebral vasculature or with low PDE-5 activity under resting conditions. However, the first premise is inconsistent with numerous studies in which NOS inhibition decreases CBF and that demonstrate a significant NO/cGMP contribution to basal vasodilator tone (15, 22).

Moreover, we found a decrease in basal tCBF and rCBF of ~30% in rats pretreated with L-NAME (Fig. 6). Because basal cGMP production is thus involved in maintaining constitutive vasodilation, low PDE-5 activity is a more plausible explanation for the lack of CBF responses to sildenafil or tadalafil under resting conditions. This does not rule out the possibility that basal vascular tone in the brain may also be influenced by other PDEs, perhaps PDE10A, which degrades cGMP in the brain (18). Moreover, PDE-5 in cerebrovascular smooth muscle in the rat could be a splice variant (30) that neither sildenafil nor tadalafil inhibits completely.

In hyperbaric hyperoxia, PDE-5 inhibition opposes the initial, protective cerebral vasoconstrictor response and accelerates secondary increases in CBF, which promote the development of oxygen seizures. Although sildenafil did not completely abolish hyperoxic vasoconstriction, it did enhance CBF after 30 min at 4 ATA O₂, compared with untreated control rats. Control rats exposed to 6 ATA O₂ showed consistent elevation of tCBF and striatal CBF only after 30 min of HBO₂, but pretreatment with sildenafil or tadalafil increased CBF even within the first 15 min of hyperbaric exposure, by which time CBF had risen 50% more than in untreated animals at the same point in the experiment (Fig. 5).

Although brain Po₂ was not measured here, one of our earlier studies (16) clearly demonstrates that Po₂ follows blood flow in the same brain region in HBO₂. For example, in rats breathing air, the relationship of CBF to Po₂ is nonlinear, but at 6 ATA the plot of these two parameters approaches linearity. Thus at 1 ATA O₂ a doubling of rCBF effects a change in Po₂ of 17 mmHg, whereas at 6 ATA O₂ the same increase in CBF elevates brain Po₂ by 770 Torr (16). In the present study, animals pretreated with tadalafil showed a 72% increase in tCBF and an 86% increase in rCBF after 30 min of HBO₂ compared with untreated rats. According to our earlier study (16), these increases in CBF could elevate Po₂ in the brain to toxic levels within 15 min of reaching 6 ATA O₂, which could

easily account for the significantly shorter latency to EEG spikes in rats pretreated with PDE-5 inhibitors.

The difference in CBF dynamics between rats pretreated with sildenafil or tadalafil and exposed to 4 or 6 ATA O₂ and similarly exposed control rats implies that the effect of PDE-5 inhibition is NO dependent. As expected, rats pretreated with L-NAME showed a pronounced decrease in CBF in HBO₂, confirming that the hyperemia of extreme hyperoxia is NO dependent. Thus rats treated with L-NAME plus sildenafil and exposed to 6 ATA showed the same decreases in CBF as rats treated with L-NAME alone. Furthermore, the importance of NO in the regulation of rCBF responses to extreme hyperoxia and in the development of CNS O₂ toxicity is well demonstrated in rodents (12, 13, 15, 35). For example, as shown by H₂ clearance together with in vivo microdialysis, HBO₂ alters CBF and NO levels in the striatum in a manner that depends on both time and pressure. Thus oxygen at 4 ATA for 75 min induces vasoconstriction and decreases NO and its metabolites (NO_x), while oxygen at 6 ATA consistently elevates striatal CBF and NO_x levels (15). These data provide clear evidence that CNS O₂ toxicity in the rat brain is initiated by an increase in NO production resulting in excessive cerebral O₂ delivery. Because L-NAME dramatically reduces rCBF and inhibits EEG discharges at 6 ATA, even when PDE-5 is inhibited, the NO/sGC/cGMP signaling pathway is involved in the amplification mechanism.

The vasodilator effects of PDE-5 inhibitors are most pronounced when PDE-5 activity increases during upregulation of the NO/sGC/cGMP pathway, for example, in the myocardium (23). When cultured aortic smooth muscle cells are stimulated by NO, a sharp increase in cGMP is followed by its rapid degradation; sildenafil attenuates the decay of the cGMP peak, resulting in a sustained plateau after NO stimulation (5). This finding agrees with other studies demonstrating that PDE-5 is activated immediately upon cGMP increase and that PDE-5 is the major PDE involved in cGMP hydrolysis in smooth muscle (33).

The interaction between cGMP and PDE-5 comprises a feedback loop, since cGMP induces PDE-5 activity, which in turn regulates cGMP levels. Thus PDE-5 is activated when cGMP binds the GAF A domain of the cGMP-binding sequence, inducing a conformational change (41). Another positive feedback mechanism involves PDE-5 phosphorylation by PKG that induces conformational changes that increase cGMP binding affinity in the regulatory GAF domain and enhances cGMP catalytic activity by 50–70% (9). These mechanisms moderate vascular relaxation induced by increased NO production but have little impact on basal vascular tone.

In summary, because CNS O₂ toxicity is a significant risk in HBO₂ therapy and in several diving modes as well as in the use of high-pressure O₂ breathing to accelerate decompression and because alterations in CBF modulate the toxic effect of HBO₂, the use of cerebral vasodilator agents also increases the threat of CNS O₂ toxicity. PDE-5 inhibitors increase the risk of CNS O₂ toxicity by enhancing CBF under conditions of increased NO production that can occur in diving or during HBO₂ administration. Because PDE-5 inhibitors have biological half-lives ranging from 5 h for sildenafil to 3 days for tadalafil, these risks should be evaluated before these agents are used by divers or those undergoing HBO₂ therapy.

GRANTS

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