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Retinal venous blood carbon monoxide response to bright light in male pigs: A preliminary study

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ABSTRACT

The physical mechanism by which light is absorbed in the eye and has antidepressant and energizing effects in Seasonal Affective Disorder and other forms of psychiatric major depression is of scientific interest. This study was designed to explore one specific aspect of a proposed humoral phototransduction mechanism, namely that carbon monoxide (CO) levels increase in retinal venous blood in response to bright light. Eleven mature male pigs approximately six months of age were kept for 7 days in darkness and fasted for 12 h prior to surgery. Following mild sedation, anesthesia was induced. Silastic catheters were inserted into the dorsal nasal vein through the angular vein of the eye to reach the ophthalmic sinus, from which venous blood outflowing from the eye area was collected. The animals were exposed to 5000 lx of fluorescent-generated white light. CO levels in the blood were analyzed by gas chromatography before and after 80 min of light exposure. At baseline, mean CO levels in the retinal venous blood were 0.43 ± 0.05 (SE) nmol/ml. After bright light, mean CO levels increased to 0.54 ± 0.06 nmol/ml (two-tailed *t*-test $p < 0.05$). This study provides preliminary mammalian evidence that acute bright light exposure raises carbon monoxide levels in ophthalmic venous blood.

1. Introduction

The physical mechanism by which light is absorbed and has antidepressant and energizing effects in seasonal affective disorder (SAD, winter depression) and other forms of psychiatric major depression is of scientific interest. Decades of placebo-controlled research establishing the efficacy of bright light treatment for SAD and, more recently, for non-seasonal forms of depression, invite a better understanding of the molecular mechanisms explaining such treatment effects [1–3]. Melanopsin-containing light-sensitive retinal ganglion cells have been established to play primary (but not sole) roles in regulating biological clock responses [4] that are most often associated with antidepressant responses in SAD [5].

Beyond melanopsin, one biophysical model proposed to explain the molecular and anatomic basis of light's energizing and antidepressant effect is "humoral phototransduction" [6]. In this model, based on conserved circadian and seasonal responses to light and conserved putative chromophores in plants and animals, light acting in the eye on the chromophores of humoral molecules such as hemoglobin (Hb) and bilirubin is proposed to yield carbon monoxide (CO) and nitric oxide (NO) in retinal venous blood to effectuate some of light's properties in regulating the biological clock and mediating mood. CO, NO, and H_2S gases all serve critical roles as physiological gasotransmitters, with the capacity for regulation of the eye and the brain [7–11].

Higher levels of CO and NO draining from the retinal vasculature to the cavernous sinus (CavS) following bright light exposure are then postulated to diffuse into the internal carotid artery (wrapped by the CavS) and then carried by arterial blood flow directly to the brain. In vivo studies have already demonstrated the capacity for exogenous CO administered into blood vessels draining to the CavS leading to changes in gene expression of pituitary gland GnRH and GnRH receptor mRNA levels [12].

There are two putative mechanisms by which bright light could generate CO and NO in the retinal vasculature. One potential mechanism is bright light's stimulating the dissociation of bound gases such as CO and NO from heme moieties of Hb [13]. A second relevant mechanism would be the well-described capacity of bright light to stimulate the heme oxygenase and nitric oxide synthase enzymes that, respectively, produce CO and NO. Both putative mechanisms draw on the idea that the heme moieties of Hb, heme oxygenase, and nitric oxide synthase can serve as photoreceptors that absorb the light driving these chemical reactions [6].

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In a naturalistic study of sixteen mature males of a wild boar and pig crossbreed, Koziorowski and colleagues previously demonstrated that the concentration of CO in the venous blood draining from the eye was elevated during the summer days, but not during the summer night, nor during the day or night in the winter [14]. In a control condition in that study, CO concentration in the control nasal venous blood did not differ between seasons and across the day and night, and was lower than CO levels in the ophthalmic venous blood during the summer. These results supported the humoral phototransduction model by demonstrating that CO was released from the eye into the ophthalmic venous blood in a sunlight-dependent pattern. That study did not permit assessment whether this elevation was based on an acute or chronic effect of light.

This study was designed to explore one specific aspect of the proposed humoral phototransduction model. We examined whether CO levels acutely increase in retinal venous blood in response to bright light.

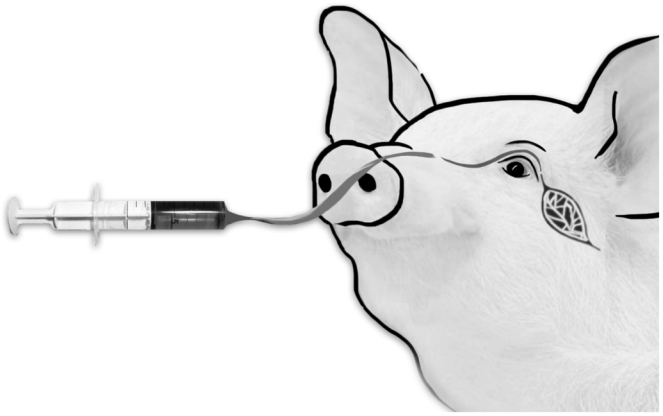

Fig. 1. Schematic of catheter insertion via dorsal nasal vein to reach the venous ophthalmic sinus.

2. Methods

2.1. Regulatory

All procedures were carried out in compliance with Polish legal regulations (Agreement 6/2014 of January 24, 2014) specifying terms and conditions for performing experiments on animals, and were in conducted under the supervision of an experienced veterinary surgeon (Dr. Koziorowski) in accordance with the protocol of the Local Ethics Commission for Animal Experiments in Lublin, Poland.

2.2. Subjects

We studied eleven mature male pigs (*Sus domesticus*), approximately six months of age, born and raised in the pig farm of the University of Rzeszów Institute of Applied Biotechnology and Basic Sciences farm in Werynia, Poland. The pigs were kept in darkness (red light < 5 lx) for one week and fasted for 12 h prior to surgery.

2.3. Experimental Methods

2.3.1. Surgical Procedure

The animals were pre-medicated in the dim light with 0.05 mg/kg atropine (Biowet, Gorzów Wielkopolski, Poland) injected intramuscularly (IM) followed 10 min later by 2 mg/kg Stresnil (azaperone) IM (Janssen Pharmaceutica, Beerse, Belgium). Once sedation was achieved, anesthesia was then induced with 10 mg/kg thiopental sodium (Thiopental, Sandoz GmbH, Austria). A specially-constructed soft-cushioned eye mask to prevent passage of external light was then used to cover the pigs' eyes. A silastic catheter (o.d., 2.4 mm, i.d. 1.8 mm) was then inserted into the dorsal nasal vein in a cephalic direction through the angular vein of the eye to reach the venous ophthalmic sinus, from which venous blood was collected (Fig. 1). The catheter was then entirely covered to protect its contents from light exposure.

2.3.2. Blood Collection

Immediately prior to light exposure and after 80 min of bright light exposure, 10 ml of blood was collected into a light-shielded heparin-containing test tube. The tube was kept in darkness (<5 lx) and transferred to a dark laboratory (<5 lx). Two aliquots of 2 ml blood were then transferred under vacuum to two helium-rinsed vials. 0.2 ml of CO (at 1000 hPa ambient air pressure) was then added to one of the vials to serve as a standard for the CO analysis (described below). The vials were incubated in a water bath at 37 °C for 30 min. The vials were then transferred to the gas chromatograph for analysis.

2.3.3. Light Exposure

After a post-surgery period of 40 min in darkness, the animals were then exposed for 80 min to 5000 lx of fluorescent-generated white light (Lumie "Brazil" light box, Cambridge, United Kingdom) through a plastic UV-protective diffuser. Spectral irradiance is shown in Fig. 2.

2.3.4. Carbon Monoxide Analysis

CO levels in the blood were analyzed using a procedure adapted from previously published standardized addition techniques [14] using a ThermoFisher Scientific Trace GC Ultra gas chromatograph. The analytical procedure combined the Headspace (HS) analytical technique (extraction of CO from the blood sample via its gaseous phase) with the column gas chromatography technique using a packed column for the separation of the gaseous sample components (molecular sieve 5 Å, 80–100 mesh, 15 m × 0.53 mm i.d., activated at 280 °C). Trace concentrations of CO were determined as concentrations of methane (CH_4) using a flame ionization detector (FID) after stoichiometric catalytic conversion of CO to CH_4 in a Raney nickel-packed microreactor located between the outlet of the column and the inlet of the FID detector. The application of the 5 Å molecular sieve column ensured the separation of CO and oxygen (O_2), eliminating the possible influence of O_2 on the determination of trace amounts of CO. Other specifications were: carrier: helium: 5 cm^3/min; oven temperature: 80 °C; injection port: splitless; 100 °C-injection using gas-tight syringe; sample volume: 0.5 cm^3; limit of detection (LOD): 0.5 ppm (v/v) CO.

The concentration of CO (nmol/ml) in the blood samples was calculated using the formula: $C_X = (C_W V_W * (A_W - A_2) * A_1) \div (V_L * A_W * (A_2 - A_1))$ where C_X is the concentration of CO in the blood sample, C_W is the concentration of CO in the standard mixture added to the HS vial containing the sample, V_L is the volume of the liquid sample (blood) put into the HS vial, V_W is the volume of the standard gas mixture introduced into the HS vial, A_1 is the CO peak area for the blood sample, A_2 is the CO peak area for the blood sample with the standard addition and A_W is the CO peak area for the standard mixture.

2.4. Statistics

The group means for the pre-light and post-light CO levels for each individual animal were compared with a paired *t*-test (GraphPad.com/Quickcalcs).

3. Results

At baseline, mean CO levels in the retinal venous blood were 0.43 ± 0.05 (SE) nmol/ml. After bright light, mean CO levels increased to 0.54 ± 0.06 nmol/ml. The mean increase (0.10 nmol/ml ± 0.05 SE)