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Concurrent tissue oxymetry and blood flowmetry to assess the effect of drugs on cerebral oxygen metabolism

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ABSTRACT
An Oxylite/LDF system (Oxford Optronix, UK) driven by a sensor made of optical fibres for the tissue oxygen tension (pO2) and for the Laser Doppler Blood Flow (BF) was implemented. This has allowed pO2 and BF real time measurements in discrete brain areas of anaesthetised rats that were then challenged with exogenous oxygen (O_2) and carbon dioxide (CO_2). The results gathered were compared with data obtained following treatment with drugs that have excitatory influence upon the brain activity such as amphetamine or with a central nervous system (CNS) depressant such as CI-966. Altogether these experiments support the methodology for in vivo investigation of pharmacological effects on cerebral oxygen metabolism and could provide new understandings on the effects of psychostimulants and anticonvulsants on selected brain regions.
Keywords: *in vivo; tissue oxymetry; blood flow; rat brain; amphetamine; CI-966.*

1. INTRODUCTION
Changes in tissue oxygen tension (pO2) reflect transient imbalance between oxygen consumption and supply, and are sensitive to local changes in oxygen metabolic rate [1, 2].

In order to monitor cerebral oxygen metabolism, we have implemented an Oxylite/LDF system (Oxford Optronix, UK) driven by a sensor (diameter 0.3mm) made of optical fibres for the pO2 and for the Laser Doppler Blood Flow (BF) real time measurements in discrete brain areas of anaesthetised rats challenged with exogenous O_2 and carbon dioxide (CO_2) for instrument calibration. In particular, mild hypercapnia has been shown to induce a haemodynamic response without significantly affecting brain metabolism [3] and has been proposed as a calibration to measure changes in oxygen metabolic rates [4, 5, 6].

Data were then compared with results obtained following treatment with drugs that have excitatory influence upon the brain activity such as amphetamine [7, 8, 9] or with a central nervous system (CNS) depressant such as CI-966. CI-966 is acting as a selective γ-aminobutyric acid (GABA) reuptake inhibitor, more specifically as a highly potent and selective blocker of the GABA transporter 1 (GAT-1) (IC50 = 0.26 μM)) [10, 11] that has been investigated as a potential anticonvulsant, anxiolytic, and neuroprotective therapeutic [12, 13].

2. MATERIALS AND METHODS
Adult rats (Wistars, 220–250 g) were supplied by Charles-River (Italy) and kept in temperature- and humidity-controlled rooms (22 °C, 50%). All animal procedures were carried out in accordance with the Italian law (Legislative Decree no. 116, 1992) which acknowledges the European Directive 86/609/EEC and were fully compliant with the internal policy on the care and use of laboratory animals and codes of practice. Furthermore, all efforts were made to minimize the number of animals and their suffering.

2.1. The Oxylite system.
This study has been performed using a combined Oxylite/LDF system (Oxford Optronix, Oxford, UK) to simultaneously measure tissue oxygen tension (pO2) and blood flow (BF) in the rat brain via a combined dual fluorescence-quenching/Laser Doppler- Flow probe [14].

Oxylite technology uses a ruthenium luminophor located at the tip of an optical fiber, which conveys light-pulses generated by a diode to the photoactive material. The lifetime of the resultant fluorescence is inversely proportional to pO2 at the probe tip. Essentially, the fluorescence quenching spectroscopy methodology is based upon:

i] Short pulses of light that via the fiber optic sensor excite a platinum based fluorophore at the sensor tip;
ii] The resulting emission of fluorescent light is quenched by O_2 then travels back up the optic fiber and detected.

The Oxylite probe is bundled with another optical fiber (200 μm diameter) for the Laser Doppler Flow measurement. This laser Doppler velocimeter emits a light beam and measures the shift in wavelengths of reflections from particles moving i.e. the red cells within the blood flow. The tips of the two probes are closely positioned to provide simultaneous measurements of the two parameters over approximately the same volume. All probes were pre-calibrated by the manufacturer, and the calibration parameters were scanned into the system before each experiment.

Successively, the Oxylite/LDF probe (approx. 0.3mm diameter) was lowered stereotactically in the medial prefrontal cortex of anaesthetised (1% isoflurane in a 30%/70% O_2:N_2 mixture) adult male rats according to the coordinates from Paxinos and Watson [15], i.e. AP +3.2 mm, ML +0.5 mm, DV -4.5.

Prior to that, each rat was tracheotomized and artificially ventilated with a mechanical respirator (Inspira, Oxford, UK). The right femoral artery was cannulated with a PE50 polyethylene

catheter for i) monitoring arterial blood pressure, ii) 'blood gas analysis' and iii) infusion of compounds i.e. D- tubocurarine (0.25 mg/kg hr, dissolved in saline heparin (25 UI/ml) to maintain muscle relaxation.

Throughout surgery the "gas" anaesthetic level was maintained at 2.5% isofluorane while during measurements this level was lowered to 1%.

2.2. Treatments.

The pO2/LDF/T real time measurements were started as soon as the probe was lowered in the medial prefrontal cortex and after a period of stabilisation (approx. 30-60min) rats were challenged with:

- O2 during 3min (i.e. the mixture 30%/70% O_2:N_2 was changed to 100% O_2 by stopping N_2 supply).
- 5%, 7.5% or 10% CO_2 during 1min (i.e. 0.1 L/min CO2 were added to the O_2:N_2 mixture to obtain 5% CO_2). After each CO_2 treatment time was allowed to recover control values.

Subsequently, vehicle (saline, 2ml/Kg, n=5), amphetamine (n=5) or CI-966 (n=5) were administered intravenously (i.v.) to the anaesthetized animals at doses already described to be active in brain i.e. amphetamine 1 mg/kg [7, 8] or CI-966 15mg/kg [10, 11], respectively.

2.3. Statistics.

Statistics were calculated from the raw data using repeated measures analysis of variance (two ways ANOVA) with STATISTICA software version 6.0. In the case of statistically significant differences between mean values produced by drug treatments versus controls (vehicle treatment) main factor Dunnet post-hoc test was applied. Statistical significance was set at $p < 0.05$

3. RESULTS

It appeared that both O_2 and CO_2 challenges transiently increased pO2 values, while BF transiently increased following CO_2 - induced mild hypercapnia only (figure 1).

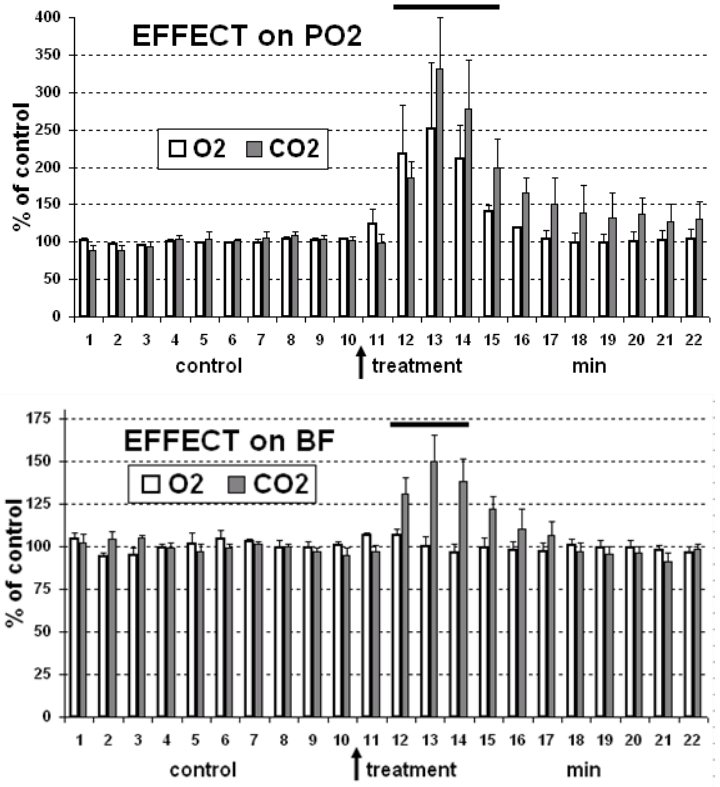

Figure 1. Evolution in time of tissue pO2 (top) or BF (bottom) in frontal cortex of five rodents following administration (arrow) of exogenous O_2 or CO_2 10%; mean ± S.D. Bar: $p<0.05$, Dunnet test.

A dose-response curve was also obtained with successive dosages of CO2 (figures 2 and 3).

The pharmacological treatments showed that amphetamine increased both pO2 and BF (figure 4). In particular, CO_2 - induced mild hypercapnia, a challenge that affects haemodynamics but not metabolism, elicited comparable changes in blood flow but substantially larger changes in tissue oxygen levels than amphetamine. A similar outcome was observed when using another compound having an excitatory influence upon the brain activity such as cocaine [16, 17]. Thus, these differences in tissue oxygen build-up suggest that increased oxidative metabolism is a significant component of the cerebral metabolic response to excitatory challenges.

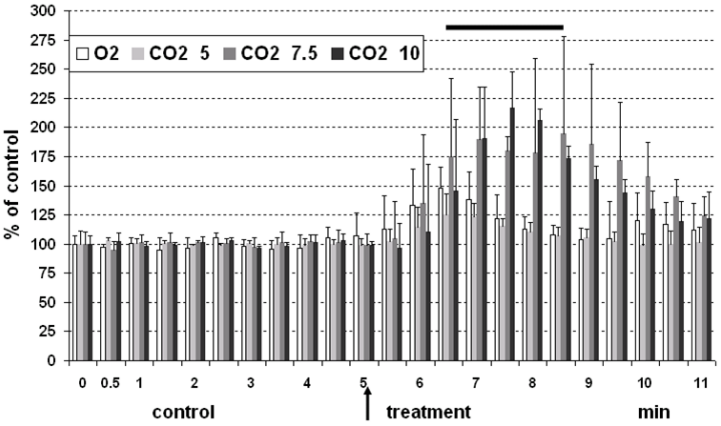

Figure 2. Evolution in time of tissue pO2 in frontal cortex of five rodents following administration (arrow) of exogenous O_2 or CO_2 at various concentrations i.e. 5%, 7.5% or 10%; mean ± S.D. Bar: $p<0.05$, Dunnet test.

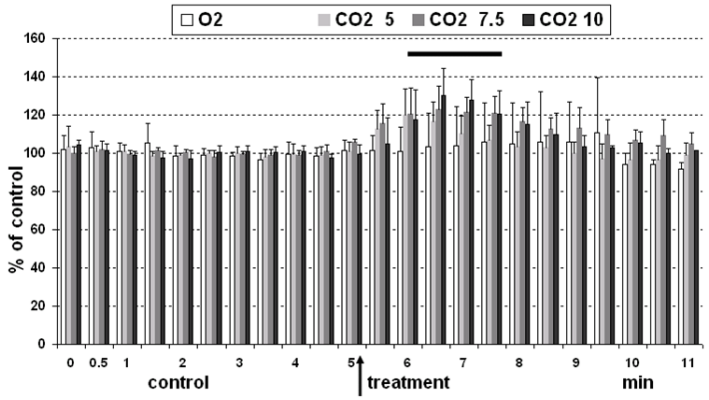

Figure 3. Evolution in time of tissue BF in frontal cortex of five rodents following administration (arrow) of exogenous O_2 or CO_2 at various concentrations i.e. 5%, 7.5% or 10%; mean ± S.D. Bar: $p<0.05$, Dunnet test.

In contrast, the anticonvulsant CI-966 resulted in reduced BF while increasing pO2. In particular, these CI-966 -induced changes in pO2 and BF showed a negative temporal correlation, with a very similar time-profile i.e. Increased tissue oxygen tension was parallel to reduced tissue perfusion. These data are in accord with previous observations obtained with another anticonvulsant such as tiagabine [18, 19] and support the

possibility that during cerebral (cortical) GABAergic deactivation [i.e. by CI-966 or tiagabine] tissue oxygen consumption decreases more than O_2 supply.

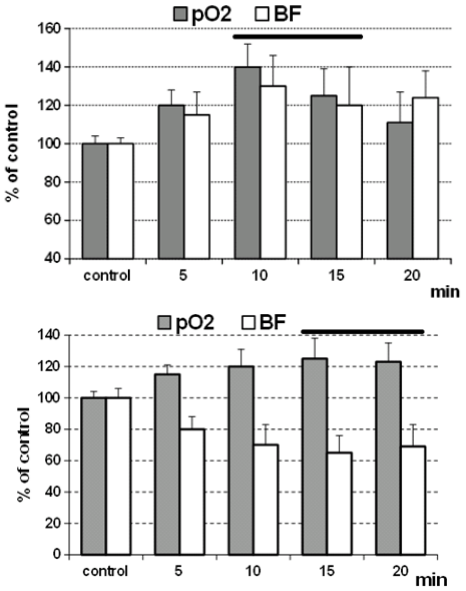

Figure 4. Evolution in time of tissue pO2 and BF monitored in the frontal cortex of five rodents following administration (arrow) of amphetamine (TOP 0.5mg/Kg i.v.) or CI-966 (BOTTOM. 10mg/Kg i.v.) Data are presented as % of control values, mean ± S.D., Bar: $p<0.05$, Dunnet test.

Taken together with the CNS excitatory pharmacologic challenge i.e. due to amphetamine as well as cocaine treatment resulting in increased tissue oxygen levels and positive BF changes, these measurements add further indicators of the excitatory or inhibitory effects of drugs corroborating the data relative to the measurement of oxygen levels only.

Oxylite presents several advantages over polarographic measurements of pO2 for the scope of this experiment. Firstly, Oxylite does not consume oxygen during the measurement, and allows continuous monitoring of oxygen levels for extended periods of time [20].

Moreover, the Oxylite probe measures an average pO2 over a sensing volume that is substantially larger than that probed by microelectrodes [21]. Thus, Oxylite is less sensitive to heterogeneity of oxygen concentration on the length-scale of capillaries.

4. CONCLUSIONS

The present data support the methodology as a useful tool to monitor oxygen tension and blood flow in discrete brain areas such as prefrontal cortex. In particular they indicate the methodology as able to distinguish changes in pO2 – BF parameters induced either via application of exogenous oxygen or via CO_2 - induced mild hypercapnia. In addition, the progressive CO_2 concentrations tested proposes the feasibility of dose –effect studies with the flow – oxymetry methodology.

Furthermore, the selective data obtained with drugs having excitatory influence upon brain activity such as amphetamine and cocaine or with sedative drugs such as ci-966 and tiagabine support the methodology for in vivo investigation of pharmacological effects on cerebral oxygen metabolism and could provide new understandings on effects of psychostimulants and anticonvulsants on selected brain regions.

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