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Parallel Effect of Nicotine and MK-801 on Brain Metabolism: An *In vivo* Non Invasive Near-Infrared Spectroscopy Analysis in Rats

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Abstract

Oxygenated haemoglobin, deoxyhaemoglobin and total blood volume are measured with our recently developed Near-Infrared Spectroscopy [NIRS] apparatus allowing *in vivo* non invasive real time monitoring of brain metabolism in anaesthetized rats. These measurements are indicative of the state of vascular activity and the state of the oxygen saturation, thus of the level of metabolism in the living tissue.

Nicotine is a natural alkaloid derived from tobacco that has been implicated in various effects ranging from addiction to toxic effects and neuro-protective actions. Here we analyse the influence of nicotine as well as that of the non competitive NMDA receptor antagonist MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine maleate)] upon brain metabolism in anaesthetized rats.

In the first 30min after treatment, nicotine decreases significantly although in a transient manner oxygenated haemoglobin and total blood volume while increasing significantly deoxyhaemoglobin. In addition, MK-801 performed in another group of rats was followed by changes in these three parameters that were similar to those monitored in nicotine treated rats.

The NIRS methodology appears to be apt to analyzing non-invasively and in real time the influence of systemic pharmacological treatments upon brain metabolism. In particular the data gathered show similarity of action of the two chemicals studied on influencing metabolic brain "behaviour" proposing that their central protective action may pass via the observed similar changes in brain metabolism. These changes could be a common mechanism of adaptation and protection towards neurotoxicity: mechanism that should be also considered in the intent of developing new pharmaceutical approaches for neuro-protective treatments.

Keywords: Near-infrared spectroscopy; Haemoglobin; Deoxyhaemoglobin; Brain metabolism; Non invasive; Nicotine; MK-801; Rat

Abbreviations: MK-801: [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate)]; NIRS: Near-Infrared Spectroscopy; HbO_2: Haemoglobin; Hb: Deoxyhaemoglobin; HbT: Total Blood Volume

Introduction

The pharmacological activities of nicotine have been analyzed in animals and humans since long time. Nowadays, this natural alkaloid derived from the tobacco has been implicated in toxicity effects but not only, further to its additive effect it appeared that has nicotine had also neuroprotective actions [1]. The toxicity implies involvement of oxidative stress in various organs including the central nervous system CNS, [2] where it could also result in hypoxia and encephalopathy [3]. The central protective action of nicotine is similar to that of the non competitive NMDA receptor antagonist MK-801 [4] as it pass via the defence towards neurotoxicity induced by excitotoxic amino acids as well as beta amyloids present in the senile plaques [5]. In addition, nicotine induces the basic Fibroblast Growth Factor [FGF-2] and the Brain Derived Neurotrophic Factor [BDNF] [6] both reported to be neuroprotective for the DA cells: possible mechanism of protection within Parkinson disease.

Recently, nicotine has been used as a prototypical agent for the analysis of drug-induced changes in Functional Magnetic Resonance Imaging [fMRI] in the brain [7,8]. This non invasive methodology is using radio frequency pulses in a strong static magnetic field and is mainly based upon changes in blood flow and delivery of oxygen following neuronal activation. NIRS is another relatively new non invasive methodology that has been introduced since about three decades [9]. The technique is based on:

- The use of harmless radiations, which have wavelength in the spectral range of the near infrared [I.R., 650-1000 nanometers, nm];
- The principle that near-infrared light easily passes through biological tissues and is mainly absorbed by few tissutal chromophores such as: oxygenated haemoglobin [HbO_2] and deoxyhaemoglobin [Hb].

HbO_2 and Hb are the dominant absorbing elements between 700 nm and 1000 nm, and the transmission of light is relatively unaffected by water in this I.R. Therefore, NIRS provides a non-invasive, non-ionizing means to monitor HbO_2 and Hb.

Importantly the absorption spectra of near-infrared light differ for the oxygenation–deoxygenation states of Hb [HbO_2 *vs.* Hb, respectively] so that the two compounds can be directly monitored. As calculated and reported earlier, the total haemoglobin concentration [HbO_2+Hb] is considered as equivalent to total blood volume, so called HbT [10].

Altogether, these measurements are indicative of the state of vascular activity and the state of the oxygen saturation, thus of the level of metabolism in the living tissue.

Recently we have developed a NIRS prototype allowing *in vivo* non invasive real time monitoring of brain levels of Hb, HbO_2 and then HbT,

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so that analysis of brain vascular and metabolic activities in real time can be performed in the CNS of anaesthetized rats [11]. Indeed, HbO_2 and Hb are chromophores also present in brain tissue and are markers of the degree of brain tissue oxygenation, thus providing an index of brain tissue metabolism. Using this non invasive methodology we have here investigated the influence of nicotine and MK-801on these three parameters, i.e. HbO_2, Hb and HbT *in vivo*, within the rat brain. Indeed, neuroactive drugs can be administered to elicit a physiological response [changes in local blood flow, blood volume, or blood oxygenation]. The hemodynamic changes are considered a functional readout of the underlying molecular process, the drug-receptor interaction, and can hence be used to study different neurotransmitter systems.

Methods

Experiments on animals have been performed on Male Sprague-Dawley CD rats [290 g, Charles River, Italy]. The animals were housed four per cage, fed with Purina Rat Chow by Purina Animal Nutrition U.S.A., with water available ad libitum and kept in a temperature controlled environment [22°C, 50%] with lights on from 0700 to 1900 hours.

The experimental procedures were in line with the NIH Guidelines for Small Animal Research and approved by local review committees. All procedures were carried out in accordance with the Italian law [Legislative Decree no.116, 27 January 1992], which acknowledges the European Directive 86/609/EEC, and were fully compliant with Company policy on the care and use of laboratory animal and codes of practice. Furthermore, all efforts were made to minimize the number of animals used and their suffering. Each rat was anaesthetised using urethane (1.4 g/kg ip) and placed on a stereotaxic apparatus [D. Kopf, USA]. Then the input system (four optic fibres, 200 μm diameter each) and the receiver system were both firmly placed using a stereotaxic micromanipulator onto the surface of the rat's head, close to the sagittal line without any surgery as already shown [11].

It is known that anaesthesia as well as MK-801 induces hypothermia [12]. Therefore animals were maintained normothermic at 38.5 ± 0.5˚C with a rectal thermistor connected to a heat lamp.

Calibration of the NIRS prototype was performed as reported earlier [10,11] then following a 10min control/control recording period, and in order to evaluate the sensitivity of NIRS measurements to exogenous oxygen, rats were supplied with pure O_2 [n=6, 0.5 bar, 2 min] via direct insufflation into the animal mouth. Successively, the anaesthetized adult male rats prepared for NIRS analysis in the "whole brain" have been treated with vehicle [1.2 ml/kg s.c. saline, n=6], 0.6 mg/kg nicotine s.c. [n=6] or with 0.5 mg/kg MK-801 s.c. [n=6]. NIRS measurements continued other 60 min, then the analysis was stopped and the position of the NIRS optical fibers verified versus bregma.

The model used to support the measurement executed with the NIRS instrument is based on the modified equation of Lambert-Beer:

$$\begin{bmatrix} \Delta HbO_2 \\ \Delta Hb \end{bmatrix} = \frac{1}{d \cdot DPF} \cdot \left[\alpha_{i,j}\right]^{-1} \cdot \begin{bmatrix} \Delta A(\lambda_1) \\ \vdots \\ \Delta A(\lambda_j) \end{bmatrix}$$

where ΔA is the variation of attenuation measured with the instrument at the interested wavelengths [experimental measurement]; $\alpha_{i,j}$ is the extinction coefficient matrix of the oxy- and deoxygenated haemoglobin at the same wavelengths [data obtained from *in vitro* measurements [13]; d is the source-detector distance [measurement geometry] and DPF [differential path-length-factor] that takes into account the scattering effect. The absolute value for DPF is equal to 5 in CNS as reported in the literature [13,14].

Results

It appeared that pure O_2 supply is increasing significantly HbO_2 levels from steady state baseline [considered as zero] up to approx. 12-14 [μmoles/L] and significantly decreasing Hb to a similar (negative) extent. This effect is reversible as soon as the influx of O_2 is stopped (Figure 1).

Systemic vehicle treatment was followed by non significant fluctuation of the NIRS parameters similar to those monitored in control/control experiments [no treatment performed; (Figure 2)]. In contrast, nicotine treatment [performed at time zero in Figure 2] was followed by significant changes of these parameters: as it appeared that in the first 20-30min after treatment, nicotine decreases significantly although in a transient manner steady state levels of HbO_2 and HbT [blood volume] down to 4.8 or 2.7 μmoles/L, respectively [repeated ANOVA: effect on time $F(6,60)=4.73$, $p=0.0005$ HbO_2; $F(6,60)=4.82$, $p=0.0005$ HbT; effect on group $F(1,10)=9.79$, $p=0.01$ and group by time interaction $F(6,60)=4.60$, $p=0.0006$ HbO_2)]. In addition, significant increase of Hb up to 2.4 μmoles/L was monitored: effect on group $F(1,10)=8.71$, $p=0.014$ and group by time interaction $F(6,60)=3.04$, $p=0.011$. Similarly, systemic MK-801 performed in another group of rats decreased steady state levels of HbO_2 down to approximately 2 μmoles/L.significantly [repeated ANOVA: effect on time $F(6,60)=4.22$, $p=0.0005$); effect on group.

$F(1,10)=8.98$, $p=0.01$ and group by time interaction $F(6,60)=4.34$, $p=0.0004$]. It also increased levels of Hb up to approximately 2.6 micromoles /l from baseline (considered as 0 micromoles /l) within 30 min significantly: effect on group $F(1,10)=8.55$ $p=0.012$ and group by time interaction $F(6,60)=3.66$, $p=0.013$.

Discussion

Data monitored following pure O_2 supply are in accord with other similar experiments performed with the association NIRS - BOLD fMRI [15] with pharmacological experiments using fMRI to study central vascular activities [16] and with the precedent NIRS works indicating changes in tissue oxygen saturation [StO_2] from approx. 70% in the normal state (with 21% of O_2 in the inspired air) to approx 85-90% when giving pure oxygen [17]. Indeed our data show a similar increase of approximately 13 μmoles/L from steady state baseline when pure O_2 is supplied. These results strength the applicability

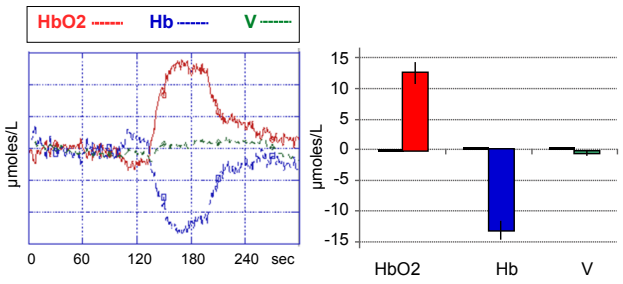

Figure 1: Typical response of NIRS parameters to exogenous supply of pure oxygen presented as changes from basal levels considered as zero (μmoles/L in ordinates).
Left n=1; right n=6, data are mean ± SD. All changes are statistically significant versus control; repeated ANOVA indicated that exogenous O2 a has significant effect of group: HbO2: $F(2,21)=434.72$, $p=0.001$. Post Hoc analysis (Dunnett) indicated significant differences between control and treated groups ($p<0.001$).

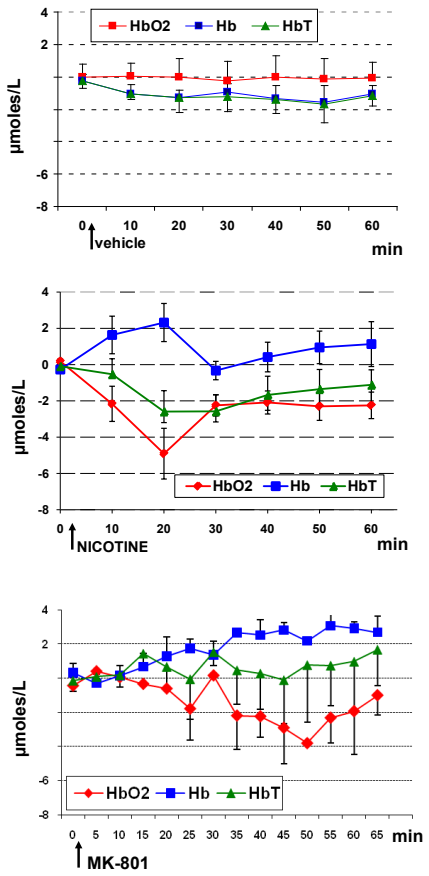

Figure 2: Top: effect of systemic vehicle (saline, 1.2 ml/kg s.c., n=6), nicotine (0.6 mg/kg s.c. n=6) or MK-801 on NIRS parameters recorded in anaesthetized rats. Mean ± S.D. See Results for statistical analysis.

of NIRS for measurement of turnover of endogenous oxygen that is directly related to neuronal functions coupled with blood flow. In particular they confirm NIRS capabilities on the direct non invasive detection of HbO_2 and Hb in rat brain as proposed earlier [11]. The ligand-receptor interaction triggers neuronal activity which is linked to an increase in metabolism (metabolic coupling). This in turns leads to an increase in the local concentration of deoxygenated blood and cerebral blood volume (neurovascular coupling). What is measured are the changes in hemodynamic (i.e. concentration of oxygenated and deoxygenated blood), that are directly related to the metabolism itself. Moreover, the present "pharmacological findings" could be indicative of the central processes involving nicotine within its toxic influences on CNS functions and in particular possibly within the hypoxia and encephalopathy effects of such drug as reported earlier [2,3] as well as its effect on blood pressure and heart rate [18]. Therefore, the NIRS methodology could be an interesting tool in analyzing non-invasively and in real time the efficacy of pharmacological treatments against such nicotine toxicity.

Many works have shown that MK-801 blocks the development of sensitization or tolerance to several psychoactive substances; i.e. amphetamine, cocaine [19] morphine [20] so that these findings suggest that diverse drugs can trigger a common mechanism of adaptation that is sensitive to MK-801. It has been also shown that MK-801 can attenuate the locomotor stimulation seen with chronic nicotine treatment in rats [21]. Thus, nicotine may be included amongst the other psychoactive drugs that are sensitive to MK-801: a similarity in the mechanism of action of these psychoactive drugs suggests a mechanism for the effect of MK-801. In particular, the dopaminergic [22-24] the nicotinergic [25] and/or the glutamatergic [21] systems have been involved. However, another possible interpretation of the present data may consist in the hypothesis that MK-801 could act in a way similar to that of nicotine upon the metabolic brain activity therefore mimicking the influence of such drug. This similarity between these two compounds on influencing metabolic brain "behaviour" may also be a component of the resulting sensitivity of nicotine towards MK-801 [21].

Conclusion

Altogether these data may propose that changes in brain metabolism could be a common mechanism of adaptation and protection towards neurotoxicity of these two chemicals and this should be also considered in the intent of developing new pharmaceutical approaches for neuroprotective treatments.

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