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An Introduction to the Measurement of the Cerebral Oxygen Uptake Rate by Inhalation of $^{15}O_2$: Analysis of the Contribution of $^{15}O_2$ and $H_2{}^{15}O$ in Brain Radioactivity

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Abstract. This paper introduces a model for the computation of the regional cerebral metabolic rate of oxygen from the data of cerebral regional radioactivity collected after inhalation of $^{15}O_2$. A method was devised for the differential determination of the respective contribution of radiooxygen and of radiowater in the inflow and the outflow of radioactivity in the brain after or during such on inhalation. The results demonstrate the feasability of such a model and outline some of the methodological prerequisites to be taken into account in the numerical analysis of the data.

Introduction

The design of a method for the determination of the regional cerebral metabolic rate of oxygen (CMR_{O_2}) in man encounters several difficulties, mainly linked to the impossibility of conceiving a method using an analogue of oxygen, to the rapid physical decay of $^{15}O_2$ and to the high rate at which oxydoreduction processes occur in the tissues, especially in the brain.

The classical technique of *Kety and Schmidt* [1] yields only a measurement of global CMR_{O_2} and affords no idea on the spatial distribution of this parameter in physiological and physiopathological conditions.

The method using a sequential intracarotid injection of $^{15}O_2$ and of $H_2{}^{15}O$, with regional external counting of the cerebral activity [2] is presently the only technique for measuring regional values of CMR_{O_2} in man. Its application to human beings is still limited by the necessity of a carotid access.

The present research was done in view of defining the prerequisites of a model for the numerical analysis of regional cerebral activi-

ties detected after inhalation of $^{15}O_2$. The high rate of oxygen diffusion and reduction in the brain makes one of the first problems to be encountered the contribution of radiowater in the inflow and in the outflow of radioactivity in each detection area of the brain.

Methods

Experimental Setup

The time course of the $^{15}O_2$ and $H_2{}^{15}O$ activity in the internal carotid artery and in the internal jugular vein blood after a bolus inhalation of $^{15}O_2$ was determined in 3 adult baboons and in 2 rhesus monkeys, prepared by ligation of the branches of the external carotid arteries. The monkeys were paralyzed with gallamine and passively ventilated with an anesthetizing mixture N_2O(70%)–O_2(30%). End-tidal pCO_2, arterial blood pressure and rectal temperature were continuously monitored. Temperature was maintained between 37 and 39 °C with a heating pad.

Blood was sampled from one internal carotid artery and from the ipsilateral jugular vein through small catheters positioned under fluoroscopic control from the femoral artery.

After a bolus inhalation of $^{15}O_2$, carotid and jugular blood are sampled during 120 s. The radioactivity is separately measured on the total blood and on the corresponding plasma phase of each sample. Any loss of $^{15}O_2$ is avoided by handling blood under a protective oil film. Volumes of samples are measured by weighing. Countings are corrected for physical decay. A continuous withdrawal of blood through the catheters was maintained during the whole sampling procedure and the time shift between the activity in the vessels and in the samples was verified to be negligible.

Arterial pO_2, hemoglobin concentration, hematocrit, pH and pCO_2 were measured before and after each inhalation of labeled oxygen.

The cerebral blood flow (CBF) was measured by rapid injection of $H_2{}^{15}O$-labeled blood into the internal carotid artery and by detection of the radioactivity of the head with a 3 × 2 in NaI(Tl) scintillation detector, following the method of *Ter-Pogossian* et al. [2]. CMR_{O_2} was computed by multiplying CBF by the cerebral arteriovenous difference in oxygen blood content.

Determination of $^{15}O_2$ and of $H_2{}^{15}O$ Activities in the Blood

The differential determination of $^{15}O_2$ and $H_2{}^{15}O$ radioactivities in the blood are based on dual counting on the whole blood and on the plasma phase and on a data processing based on the knowledge of the distribution of oxygen and water in erythrocytes and plasma at equilibrium. Oxygen is distributed in three compartments, oxyhemoglobin, dissolved oxygen in erythrocytes and dissolved oxygen in plasma, and water is distributed in two compartments, erythrocytes and plasma.

Equations 1, 2 and 3 can be written, with the following symbols: C values are quantities per unit volume of whole blood, erythrocytes or plasma, as indicated by the subscripts b, e and p; diss O_2, HbO_2 and H_2O indicate dissolved oxygen, hemoglobin-linked oxygen and water, respectively; α_eO_2 is the Bunsen coefficient of solubility of oxygen in erythrocytes at 37 °C; α_pO_2 is the corresponding coefficient for plasma; pO_2 is the partial pressure of oxygen in blood in Torr; H is the hematocrit value; CO_2 is the oxygen content of the blood, as oxyhemoglobin, expressed as [ml O_2(NTP)] × [ml whole blood]$^{-1}$; λ was experimentally measured by the measurement of the distribution of $H_2{}^{15}O$ between red cells and plasma.

$$k \triangleq \frac{C_{e\,diss\,O_2}}{C_{eHbO_2}} = \frac{\alpha_eO_2 \times pO_2 \times H}{CO_2}, \quad (1)$$

$$\gamma \triangleq \frac{C_{p\,diss\,O_2}}{C_{e\,diss\,O_2}} = \frac{\alpha_pO_2}{\alpha_eO_2} = 0.81, \quad (2)$$

$$\lambda \triangleq \frac{C_{eH_2O}}{C_{pH_2O}} = 0.81. \quad (3)$$

The value of α_eO_2 is 3.39×10^{-5} [ml O_2(NPT)] × [ml erythrocyte]$^{-1}$ × [Torr.]$^{-1}$; the value of α_pO_2 is 2.75×10^{-5} [ml O_2(NPT)] × [ml plasma]$^{-1}$ × [Torr]$^{-1}$ [3]; pO_2 and pH are measured on blood sample; C_{O_2} is determined from pO_2, pH and the concentration in hemoglobin of the blood.

The equilibrium distribution of $^{15}O_2$ and $H_2{}^{15}O$ may be assumed to be the same as that of unlabeled oxygen and water in the blood, and the above-mentioned equations be applied, with C values indicating activites per unit volume.

The activities of O_2 and H_2O in each of the five compartments can be calculated from the measurement of C_b, the activity of whole blood in cps × [ml whole blood]$^{-1}$ and C_p, the activity of the corresponding plasma phase, in cps × [ml plasma]$^{-1}$.

$$C_{eHbO_2} = \frac{C_b - (1 - H + \lambda H)\, C_p}{H\,(1 + k - \lambda kv)}, \quad (4)$$

$$C_{e\,diss\,O_2} = kC_{eHbO_2}, \quad (5)$$

$$C_{p\,diss\,O_2} = kvC_{eHbO_2}, \quad (6)$$

$$C_{pH_2O} = C_p - C_{p\,diss\,O_2}, \quad (7)$$

$$C_{eH_2O} = \lambda C_{pH_2O}. \quad (8)$$

The values of interest, radio oxygen and radio water concentrations in the blood, are finally computed as follows, expressed as cps × [ml whole blood]$^{-1}$.

$$C_{bO_2} = HC_{eHbO_2} + HC_{e\,diss\,O_2} + (1-H)\, C_{p\,diss\,O_2} \quad (9)$$

$$C_{bH_2O} = HC_{eH_2O} + (1-H)\, C_{pH_2O} \quad (10)$$

The errors resulting from the aleatory character of radioactive decay are also estimated.

The application of these equations to blood samples labeled in vitro only with $^{15}O_2$ or $H_2{}^{15}O$ yielded values for C_{bH_2O} and C_{bO_2} which were, respectively, not significantly different from zero, thus validating the method.

Results and Discussion

Rationale of the Study

The measurement of regional uptake rate of oxygen in the brain has been carried out from the externally detected activity on the head after rapid and sequential injection of $^{15}O_2$ and of $H_2{}^{15}O$ in the internal carotid artery [2]. This method has been thoroughly validated [4–6] and applied to physiological and physiopathological studies [7, 8]. The application of this reference method is still limited by the ethical impediment of performing and, especially, repeating a carotid artery puncture in most patients.

Methods proceeding by inhalation instead of arterial injection of oxygen were consequently investigated, either with bolus inhalation of $^{15}O_2$ and detection by probes [9] or with sequential continuous breathing of $^{15}O_2$ and C $^{15}O_2$ and detection by positron emission tomography (PET) [10, 11].

As the intracarotid method [2] measures the extraction fraction of $^{15}O_2$ by the brain from the detection of the first capillary transit of the tracer in the tissue, the data which are taken into account are not altered by any interference of $H_2{}^{15}O$ activity, however rapid the reduction of $^{15}O_2$ may be.

On the contrary, if the administration of $^{15}O_2$ is spread more over time and if the detection data are collected during a longer period of time, the contribution of metabolically produced $H_2{}^{15}O$ is to be taken into account in the analysis of the results.

Inflow and Outflow of Labeled Oxygen and Water in the Brain

Figure 1 illustrates that $H_2{}^{15}O$ activity rises in the arterial and jugular blood very early after the oxygen inhalation, in connection with the high rate at which oxygen is metabolically reduced in the tissues and with the relatively small tissular oxygen pool. Thus the arterial activity, the inflow of which is to be considered in the analysis of externally detected cerebral activity, is built up with a mixture of radiooxygen and of radiowater from the earliest tens of seconds after the inhalation of $^{15}O_2$. The integral – from 0 up to 120 s – arterial radiowater activity ranged from 14.0 to 29.6% of the total blood activity in our observations. The jugular vein activity also shows the outflow of radiowater from the encephalus, the difference of $H_2{}^{15}O$ activities between the arterial and the venous sides depending on the cerebral production and back diffusion of labeled metabolic water.

Simulation of a Modeled Determination of CMR_{O_2}

The determination of the time course of the arterial and venous $^{15}O_2$ and $H_2{}^{15}O$ activities after a bolus inhalation of $^{15}O_2$ provides orientation data in order to specify the prerequisites of a model for the quantitative analysis of the externally detected brain activity after such an inhalation.

The calculation of CMR_{O_2} is conceivably accessible from the data of a sufficiently rapid PET [12] after a bolus inhalation of $^{15}O_2$, with the aid of a model describing oxygen tissular kinetics and of a numerical analysis technique derived from the published method for the measurement of regional substrate utilization rates by emission tomography [13]. This general model deals with tracers chemically identical to the studied substrates, with metabolic processes in which the tracee is in a steady state during the time of data collection and, finally, with systems in which no back diffusion of labeled metabolites occurs during the period under concern. The equation yielding, quite generally Φ, the estimated metabolic rate of the substrate, expressed as [mass substrate] $\times$ [unit time]$^{-1}$ $\times$ [unit mass tissue]$^{-1}$, is as follows

$$\Phi = \frac{q(t) - q_b(t)}{\int_0^t a_b(u)du + \frac{a_i(t)}{k(\alpha - 1)}} \qquad (11)$$

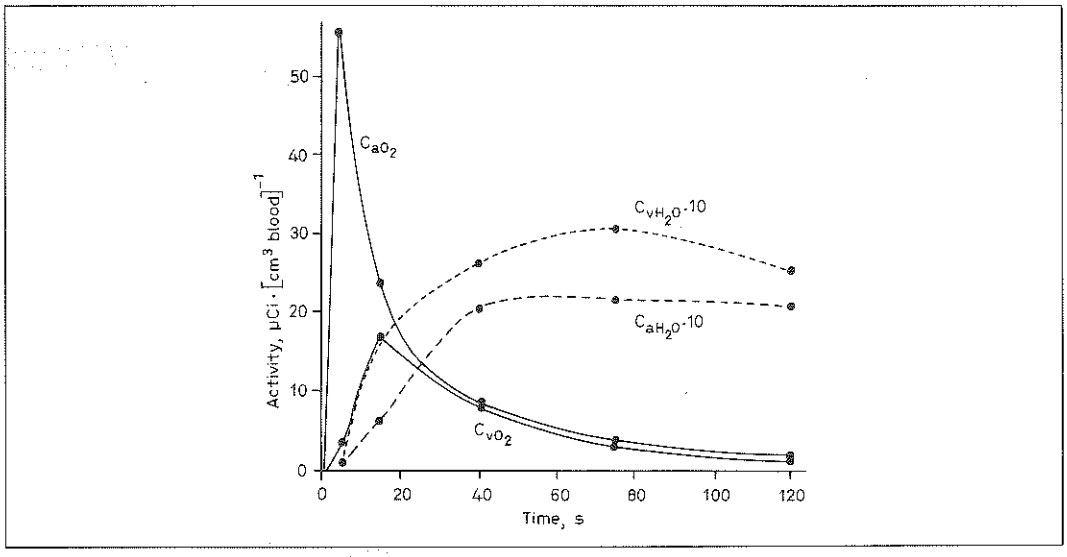
where q(t) is the radioactivity in 1 PET element at time t, calibrated in [activity] $\times$ [unit mass tissue]$^{-1}$; $q_b(t)$ is the intravascular contribution in this activity; $a_b(u)$ is the specific activity of the tracer in the capillary blood of the studied region, expressed as [activity] $\times$ [unit mass tracee]$^{-1}$; $a_i(t)$ is the corresponding specific activity of the unmetabolized tracer in the extravascular space; α is the ratio between forward and reverse fluxes of the tracee across the capillary wall, and k is the ratio between the above-mentioned forward flux and the quantity of free tracee in the extravascular space, it is expressed as [unit time]$^{-1}$.

The application and the possible adaptation of this model to the measurement of CMR_{O_2} can be approached, using data derived from the observation in the monkey: the knowledge of the time course of the arterial and venous activities of $^{15}O_2$ and of $H_2{}^{15}O$, together with the cerebral blood flow, allow to compute simulated PET data to be fed in equation 11 or in a modified version of it and so to test the possibility of accurately computing CMR_{O_2} by this method.

The following points can be successively considered.

(1) As far as oxygen kinetics in the brain is concerned, the second term in the denominator of equation 11 has a negligible value, because α and k are high and because $a_i(t)$ is low and vanishes with time. In the present observation, α computed value is 14.75 and k estimation ranges between 8.1 and 41.1 s^{-1} when the apparent extravascular diffusion space allotted to oxygen varies from 76 to 15% of the tissular mass.

(2) The back diffusion and the circulatory washout of radiowater from the brain, the early occurrence of which is demonstrated in the present study, leads to an underestimation of PET data, q(t), in the numerator of equation 11. The value of the numerator is to be increased with an additional variable, $q_w(t)$ which contributes the net loss of radioactivity from the detected region at time t, i.e. the brain produced radiowater having left the region at time t (equation 12). Values for $q_w(t)$ can be computed from the cerebral blood flow and from the activity of $H_2{}^{15}O$ in the arterial and venous blood (fig. 2).



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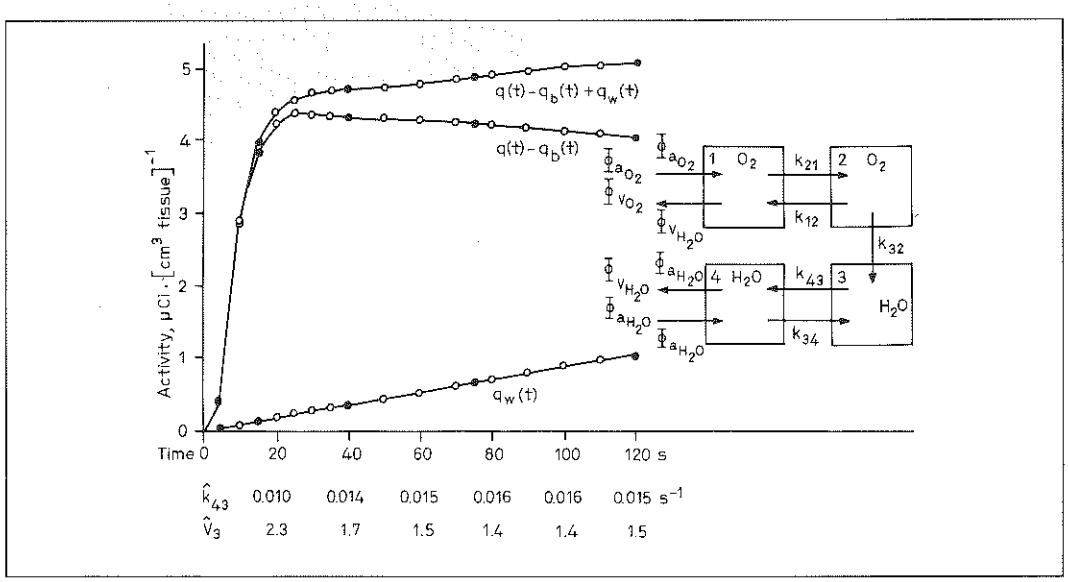

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Fig. 1. An example of the time course of $^{15}O_2$ and $H_2{}^{15}O$ activities in carotid artery and in jugular vein of a baboon, after bolus inhalation of $^{15}O_2$. $H_2{}^{15}O$ metabolism appears very rapidly in the arterial and the cerebral venous blood.

Fig. 2. Simulated PET data (differential form) after inhalation of a bolus of $^{15}O_2$. q(t) is the total activity per unit volume of tissue at time t; $q_b(t)$ is the corresponding intravascular activity of $^{15}O_2$; $q_w(t)$ is the net loss of activity of brain-produced radiowater at time t. Inset: Tentative model for the description of the washout of radiowater from the brain with compartment 1, the intravascular oxygen; compartment 2, the extravascular oxygen; compartment 3, the extravascular metabolically produced water; compartment 4, the intravascular water. Estimations of k_{43} and of V_3 as a function of time after the inhalation of $^{15}O_2$.

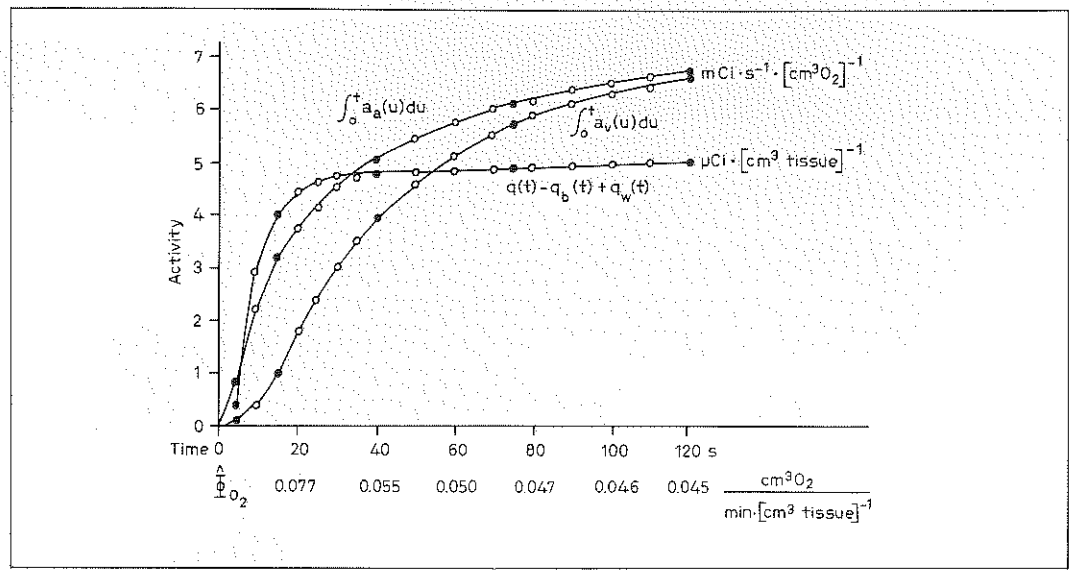

Fig. 3. The time course of the radioactivity in the tissue and of the integral vascular specific activity of oxygen after a bolus inhalation of $^{15}O_2$. q(t) is the total radioactivity per unit weight of brain; $q_b(t)$ is the net loss of radiowater produced in the brain; a_a and a_v are the specific activities of oxygen in carotid and jugular blood.

As the quantities of labeled water metabolically produced in the tissue are known from the stoichiometrical conversion of radioxygen to radiowater, it is possible to tentatively describe the kinetics of water in the brain tissue from these available data by testing in a first step a two-compartment model including only intravascular and extravascular water spaces separated by the blood brain barrier.

If the permeability constant of the brain blood barrier is kept as 0.023 ml s^{-1} g^{-1} [5], estimations can be made for k_{34} and for the apparent values k_{43} and V_3 Figure 2 shows that the apparent extravascular volume of radiowater exceeds 1 ml × [ml tissues]$^{-1}$ and is not stable, especially in the beginning of the production of labeled water. This study therefore gives an approximation of the apparent value of k_{43} and shows that the washout of radiowater should be better described by a three compartmental model (to be published).

(3) The method further allows the definition of criteria for finding the appropriate experimental estimation for the integral in the denominator of equation 12. Figure 3 shows, in a monkey whose CMR_{O_2} is 0.044 ml O_2 min^{-1} [ml tissue]$^{-1}$, that the value obtained for Φ_{O_2} satisfactorily converges toward the independently determined CMR_{O_2}, when the value kept as an estimator of the integral oxygen specific activity in the capillaries is the corresponding arterial integral at its point of convergence with the same integral on the venous side.

These results conclusively show that the arterial specific activity is not a good estimator of the capillary specific activity during the whole time when $^{15}O_2$ exchanges with $^{16}O_2$ in

the tissue and that restrictive conditions are to be applied, should the arterial activity be introduced in equation 12 instead of the capillary, inaccessible values.

Beyond this condition of convergence, the results demonstrate that only the arterial specific activity in oxygen is to be considered as the interference of an additional $H_2{}^{15}O$ activity linearly introduced an underestimation of Φ_{O_2}.

The good convergence of the results argues for the feasibility of the measurement of CMR_{O_2} by bolus inhalation of $^{15}O_2$ with a rapid PET detection, using an equation generally formulated as

$$\Phi_{O_2} = \frac{q(t) - q_b(t) + q_w(t)}{\int_0^t a(u)_{O_2}\, du} \qquad (12)$$

where q(t) is the total regional activity in the tissue at time t; $q_b(t)$ is the corresponding activity in the vascular compartment; $q_w(t)$ is the net loss of brain-produced radiowater at time t; a_{O_2} is the specific activity of $^{15}O_2$ – excluding the contribution of $H_2{}^{15}O$ – in the arterial blood the time kept for integration fulfills the convergence condition between integral arterial and venous activities in $^{15}O_2$.

The equation is to be suitably integrated to take into account the duration of collection of data for PET.

The separate determination of $^{15}O_2$ and $H_2{}^{15}O$ activities in the blood also leads to the possibility of actualizing the measurement of the cerebral regional extraction fraction of oxygen from the PET data collected during a continuous inhalation of $^{15}O_2$. In point of fact for the equation deriving this parameter involves the values of $^{15}O_2$ and $H_2{}^{15}O$ activities in the arterial blood during inhalation of $^{15}O_2$ and of $C^{15}O_2$ [11]. Further, the present method offers the opportunity of making sure that the state of equilibrium is effectively reached at the time PET is performed.

Conclusion

The disposal of a method yielding separate determinations of the $^{15}O_2$ and $H_2{}^{15}O$ respective contributions in the blood activity appears to be of importance in the approach to the numerical analysis of the external detection data of the brain after inhalation of $^{15}O_2$.

The application of this technique to animal observations demonstrates the feasibility of a method of measurement of CMR_{O_2} based on a compartmental distribution of oxygen in the brain but also shows some limits of validity of the formula derived from such a model: the main points to be considered in this case are: the imperative of measuring the $^{15}O_2$ activity in arterial blood by withdrawing the rapidly raising contribution of $H_2{}^{15}O$; the necessity of integrating the arterial oxygen specific activity during a sufficiently long time to meet the convergence condition (equalization of the time-integrated arterial and venous oxygen specific activities in the region of interest), and the importance of introducing an estimation of the brain-produced radiowater activity which is washed out the region of interest.

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