IN VIVO NITROXIDE SPIN LABEL IMAGING BY OVERHAUSER EFFECT

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Double resonance is a well known development of magnetic resonance in which one resonant transition of a system is excited while simultaneously a different transition is monitored. Since the first works of the early fifties, double resonance has been widely applied in NMR and in EPR [1]. Only recently, double resonance has been used for imaging [2] and for in vivo applications [3]. The possibility to monitor the tissular oxygen content is emphasized here because the oxygen content of tissues is an extremely important parameter for the energetic metabolism and therefore is involved in numerous physiological and pathological processes. Free radicals are commonly studied by EPR measurements performed at X or Q band (9.5 and 35 GHz respectively). At these frequencies, losses in the magnetic and electric field require the use of flat cells to achieve a good penetration of the microwave into samples of physiological conductivity. For EPR imaging, radiofrequency of 250 MHz was recently selected to obtain a good penetration into small animals [4] but the images display a very poor resolution.

Double resonance of free radicals dissolved into water combines the advantages of continous wave EPR sensitivity and pulsed NMR resolution. In order to achieve a good penetration of the RF waves, a low magnetic field of 6.8 mT was choosen. In this case the electronic resonance (ν_e) of the free radical (Fremy's salt: $ON(SO_3)_2$) is 197.4 MHz and the nuclear resonance (ν_p) of the proton of the water is 289 kHz. At equilibrium the population of each electronic and nuclear state is given by the Boltzman distribution. If, using a strong RF wave at ν_e, the electronic polarization is forced to a different value from that at equilibrium, then the steady state polarization of the protons becomes :

$$\langle P_z \rangle = \langle P \rangle_o - f\,\rho\,\frac{1}{2I+1}\,(\langle E_z \rangle - \langle E \rangle_o) \qquad (1)$$

where $\langle P \rangle_o$ and $\langle E \rangle_o$ are the equilibrium polarization of the protons and the electrons respectively, f is a leakage factor depending on relaxation mechanisms other than the coupling between P and E spins, I is the nuclear spin of the nitroxide coupled to the electron of the free radical and ρ is a dimensionless coefficient. For a scalar coupling between the P and E spins, ρ is equal to -1 and +½ for a dipole-dipole coupling [5].

A conventional NMR imaging apparatus has been used [3]. Experiments, done on rat hearts perfused with 2mM Fremy's salt in a tyrode solution, show that the NMR signal of the heart is 1.4 times more intense when oxygen is removed (fig. 1). The sensitivity of

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Overhauser effect imaging to oxygen was evaluated by imaging sample with different oxygen partial pressures (fig. 2). The great sensitivity of Overhauser imaging to oxygen content arises from the conjunction of the effect of oxygen on the leakage factor f (Eq. 1), on the linewidth of the free radical ESR line and on the NMR relaxation times [6].

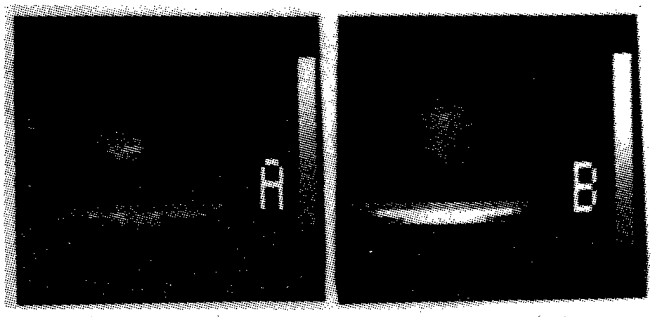

Fig. 1. Overhauser imaging of a perfused rat heart. Perfusion solution saturated with O_2 in A and with N_2 in B. (EPR irradiation 10 W, 12 min acquisition time).

The development of Overhauser imaging for monitoring the tissular oxygen content is dependent on the saturation of the electronic resonance without an exceeding deposition of RF power and on the nature of the free radical used. The saturation of the electronic resonance can be better achieved at much lower magnetic field. In this case the coupling of the electron spin and the nuclear spin of the nitrogen shifts the resonance lines of the free radical. The frequency resonances are given by the Breit-Rabi formula [7] which shows that they are very dependent on the two levels at zero field. For example the two levels at zero field of the Fremy's salt are separated by 54.7 MHz and for the tempol by 69 MHz. Therefore in a 6.8 mT field the frequency resonance is 197.4 MHz for Fremy's salt and 201.4 MHz for tempol. This is an evidence that, contrary to the high field case, at low field all the nitroxides have not the same frequency of resonance. The second problem concerns the toxicity of the nitroxides. In all our in vivo experiments the injection of the Fremy's salt has not induced any pathologic sign and the animals awaked and survived normally. Nitroxides are suspected to be toxic but, at our knowledge, no extensive toxicological studies have been performed. Nitroxides are stable free radicals and the most important problem is not their toxicity but their rapid bioreduction. These last difficulties are also encountered in EPR imaging.

However, Overhauser imaging which units the EPR sensitivity and the NMR resolution seems a promising method for in vivo animal studies of oxygen metabolism.

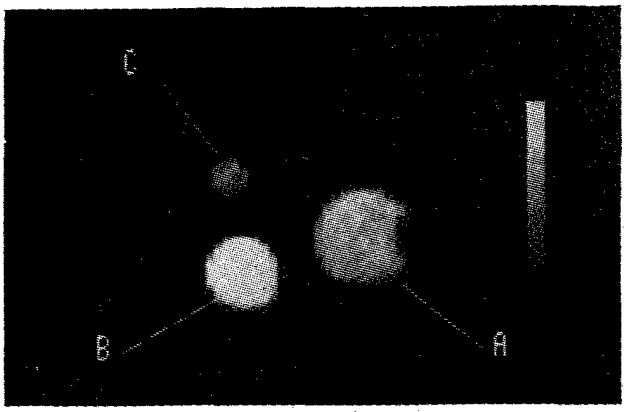

Fig. 2. Test object imaging. Cylindrical sample of 7 cm diameter filled with water in which three tubes A, B, C, with 1 mM Fremy's salt in phosphate buffered saline are set. Oxygen partial pressure A : 170 mmHg, B : 94 mmHg and C : 270 mmHg. (EPR irradiation 10 W).

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