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OXYGEN-INDUCED MODIFICATIONS OF BENZODIAZEPINE RECEPTORS AND D_2 DOPAMINE RECEPTORS IN THE RAT UNDER HYPEROXIA

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Peripheral-type benzodiazepine Receptors (PBR) in the kidney and Central-type Benzodiazepine Receptors (CBR) in the cerebral cortex were not affected in rats exposed to chronic hyperoxia (85% O_2, ATA‡, 6 days). Nevertheless, cortical CBR showed a significant decrease (29%) after hyperbaric hyperoxia (100% O_2, 3.5 ATA, 2 h) in rats at a preconvulsive stage, with no concomitant alteration of kidney PBR. A similar down-regulation of striatal D_2 dopamine receptors was noticed (27%) – after hyperbaric hyperoxia – without any modification of cortical PBR. On the contrary, an up regulation of liver PBR was obtained in the same conditions (20%). It is likely that receptors implicated in neurotransmission are particularly down regulated or altered under hyperbaric hyperoxia.

KEY WORDS: hyperoxia, oxygen toxicity, benzodiazepine receptors, dopamine receptors, rat, binding parameters.

INTRODUCTION

It is widely thought that oxygen toxicity is a phenomenon caused by an increase in the tissue concentration of oxygen radicals. It is well-established that oxygen radicals can act as deleterious agents upon targets such as SH groups in enzymes, and membrane lipids.[1,2] Therefore membrane receptors represent potential targets for oxygen radicals, both because of their protein nature and of their location inside the phospholipid membrane. Alteration of the lipid environment of a receptor is likely to alter its functional properties. Several authors have studied, in different membrane receptors, the functional changes induced by submitting biological membranes to agents which modify the lipid structure. Membrane treatments, especially by phospholipase A_2, enabled description of several changes regarding Central Benzodiazepine Receptors (CBR) and Peripheral Benzodiazepine Receptors (PBR) affinity and/or density from diverse tissue sources.[3–5]

Those changes induced by submitting membranes to phospholipase A_2 have been connected with the release of arachidonic acid; the transformation of that acid into peroxides by oxygen radicals might be responsible for the impairment of receptors.

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‡ 1 ATA = 1 atm. abs. = 0.1 MPa.

On the other hand, it has already been shown that exposing membranes to ascorbic acid systematically altered numerous neurotransmitter receptors.[6-8] In this case too, close connection between ascorbate-induced lipid peroxidation and receptor-ligand impairment suggests lipid peroxidation as an essential factor.

Those studies, as well as oxygen poisoning pathology, led us to further investigations regarding the influence of hyperoxic in vivo exposures upon the behaviour of several receptors.

MATERIALS AND METHODS

Our first investigation addressed the question of what happens to cerebral cortical CBR and to kidney PBR in rats submitted to two different hyperoxic protocols compared to control situations:

– A chronic hyperoxic protocol (model I), in which animals were housed for 6 days in a plexiglass chamber containing 85% oxygen at 1 ATA (or air at 1 ATA for the control group I);

– An hyperbaric hyperoxic protocol (model II), in which animals were submitted for 2 hours to 3.5 ATA pure oxygen (or to a normoxic 3.5 ATA Heliox exposure 94% He, 6% O_2 for the control group II).

At the end of the exposures, the animals were quickly sacrificed by decapitation and the cerebral cortex and kidney were rapidly dissected. A crude synaptosome-rich fraction (P_2) from cerebral cortex was obtained by initial homogenizing in 20 volumes of 0.32 M sucrose and subsequent centrifugations described by Squires and Braestrup.[9] A crude kidney homogenate was obtained by homogenizing in 30 volumes of 50 mM Tris-HCl buffer, pH 7.4, and was filtered through gauze. P_2 cortical membrane and kidney homogenates were subsequently washed and centrifuged 3 times in Tris-HCl buffer.

In the second stage of our investigation, the same hyperbaric exposure protocol as described previously was used. Cerebral cortex, striatum and liver were removed under the same conditions, apart from the fact that mitochondrial fractions were obtained from cortex and liver by differential centrifugation.[10] The microsomal cell fraction of striatum was obtained according to the procedure of Heikkila *et al.*[6] Protein concentrations in all homogenates were determined by the 2,2′-bicinchoninic acid protein assay (BCA Pierce) using bovine serum albumin as standard.

[^{3}H] Flunitrazepam binding was measured on P_2 cortical membranes for CBR assay. The final volume of the binding assay was 500 μl and contained 300 μl membranes (0.100–0.160 mg protein) and 100 μl [^{3}H] Flunitrazepam (final concentration 0.5–15 nM) without (total binding) or with (non specific binding) 100 μl of Clonazepam (final concentration 3 μM). Incubation was at 4°C, for 30 min and the reaction was ended by rapid filtration through Whatman GF/B filters, which were then washed 3 times with 5 ml of 50 mM Tris-HCl buffer pH 7.4.

[^{3}H] Ro 5-4864 binding was measured as described by Marangos *et al.*[11] on kidney homogenates and on mitochondrial-rich membrane fractions from cerebral cortex and liver for PBR assay. The final volume of the binding assay was 500 μl (kidney) or 300 μl (cortex, liver) and contained 100 μl [^{3}H] Ro 5-4864 (final concentration 0.5–30 nM for kidney/0.25–15 nM for cortex and liver) and 400 μl membranes (0.08–0.11 mg protein for kidney) or 200 μl membranes (0.5–0.7 mg protein for cortex/0.15–0.30 mg protein for liver).

These membrane preparations were preincubated with 5 μM Ro 5-4864 (non specific binding) or Tris-HCl buffer (total binding). Incubation was conducted at 4°C for 90 min, and the reaction ended as described above.

[^{3}H] Spiperone binding was measured for D_2 dopamine receptor assay. Binding activity was tested in a final volume of 500 μl containing 300 μl membranes (0.02–0.03 mg protein), 50 μl [^{3}H] spiperone (final concentration 0.05–1 nM) in the presence of 1 μM(−)-butaclamol (total binding) or of 1 μM (+)-butaclamol (non specific binding). The incubation was conducted at 25°C for 30 min and ended as described above.

In all binding assays, after the washing step, filters were dried and placed into scintillation vials with 10 ml Picofluor 15 (Packard) as scintillant. The radioactivity retained by filters was measured in a Tricarb 4430 liquid scintillation spectrometer (Packard).

The binding parameters (Bmax, Kd) were analysed for each structure individually by a non linear regression analysis using a software package (MxN-FIT, Bureco AG, CH-4310 Rheinfelden). A Wilcoxon two-sample test was used for intergroup comparisons.

RESULTS

In the first part of our study, cortical CBR and renal PBR were analysed. Results are shown in Table I. We did not observe any significant change in the affinity of any receptor under any of the protocols used. Regarding the chronic hyperoxic exposure (model I), none of the binding parameters for either cortical CBR or renal PBR were modified. Concerning the hyperbaric hyperoxic exposure (model II), renal PBR was not altered compared either to the hyperbaric control group II or to the air control group I. On the other hand, there was a significant decrease in the cortical CBR density of the hyperbaric oxygen group compared either to the hyperbaric control group II (29%, $p < 0.05$) or to the air control group I (31%, $p < 0.01$). No

TABLE I

Binding parameters of [^{3}H] flunitrazepam to cerebral cortical membranes (central benzodiazepine receptor, CBR) and of [^{3}H] Ro 5-4864 to kidney membranes (peripheral benzodiazepine receptor, PBR) from rats exposed to different hyperoxic/normoxic conditions

Receptor	Exposure conditions	B_{max} ± S.E.M. (pmol/mg prot.)	Kd ± S.E.M. (nM)	n
Cerebral cortical CBR	He, O_2 3.5 ATA, 2 h			
	normoxic (control group II)	2.25 ± 0.30	1.73 ± 0.31	6
	O_2 100% 3.5 ATA 2 h (model II)	1.62 ± 0.71$^{\bullet\circ}$	1.56 ± 0.04	7
	Air 1 ATA 6 days (control group I)	2.36 ± 0.17	1.57 ± 0.06	6
	O_2 85% 1 ATA 6 days (model I)	2.02 ± 0.18	1.51 ± 0.06	5
Renal PBR	He, O_2 3.5 ATA, 2 h			
	normoxic (control group II)	11.11 ± 0.34	5.42 ± 0.80	7
	O_2 100% 3.5 ATA 2 h (model II)	11.54 ± 0.39	4.60 ± 0.37	7
	Air 1 ATA 6 days (control group I)	11.06 ± 0.34	5.41 ± 0.39	6
	O_2 85% 1 ATA 6 days (model I)	11.62 ± 0.42	5.01 ± 0.36	5

B_{max}: Maximal binding capacity given as pmol/mg protein

Kd: Dissociation constant given as nM.

Significance using a Wilcoxon two-sample test: ● $p < 0.05$ O_2 100% 3.5 ATA group compared to He, O_2 3.5 ATA group. ○ $p < 0.01$ O_2 100% 3.5 ATA group compared to Air 1 ATA group.

TABLE II

Binding parameters of [^{3}H] Ro 5-4864 to cerebral cortical/liver mitochondrial membranes (peripheral benzodiazepine receptor, PBR) and of [^{3}H] spiperone to striatal membranes (D_2 dopamine receptor) from rats exposed to different hyperoxic/normoxic conditions

Receptor	Exposure conditions	B_{max} ± S.E.M. (fmol/mg prot.)	Kd ± S.E.M. (nM)	n
Cerebral cortical PBR	He, O_2 3.5 ATA, 2 h normoxic (control group II)	316 ± 28	8.20 ± 1.09	7
	O_2 100% 3.5 ATA 2 h (model II)	304 ± 11	6.84 ± 0.49	7
Liver PBR	He, O_2 3.5 ATA, 2 h normoxic (control group II)	2,315 ± 140	4.01 ± 0.25	8
	O_2 100% 3.5 ATA 2 h (model II)	2,780 ± 134$^{\bullet}$	4.60 ± 0.37	8
Striatal D_2 receptor	He, O_2 3.5 ATA, 2 h normoxic (control group II)	693 ± 36	0.22 ± 0.04	5
	O_2 100% 3.5 ATA 2 h (model II)	503 ± 36$^{\bullet\circ}$	0.18 ± 0.02	5
	Air 1 ATA (control group I)	665 ± 15	0.22 ± 0.03	4

B_{max}: Maximal binding capacity given as Fmol/mg protein

Kd: Dissociation constant given as nM.

Significance using a Wilcoxon two-sample test: ● $p < 0.05$ O_2 100% 3.5 ATA group compared to He, O_2 3.5 ATA group. ○ $p < 0.05$ O_2 100% 3.5 ATA group compared to Air 1 ATA group.

significant difference was observed between those last two groups, which suggests that the cortical CBR density decrease in the hyperbaric oxygen group is due to hyperoxia and not to the effect of pressure per se.

In the second part of our study, cortical PBR, liver PBR and striatal D_2 Dopamine receptors were analysed in rats submitted to an hyperbaric hyperoxic protocol. Table II shows the results obtained. For PBR, we investigated a mitochondrial membrane fraction, since this receptor is located in the mitochondrial outer membrane.[12] Again, no significant change in the affinity of any receptor could be observed. As regards cortical PBR, no significant alteration was observed in the hyperbaric hyperoxic group whereas a significant enhancement of this receptor density was measured in the liver of the same animals (20%, $p < 0.05$). On the other hand, a significant decrease in striatal D_2 receptor density was observed out this same group compared to the hyperbaric control group II (27%, $p < 0.05$) and to the air control group I (24%, $p < 0.05$).

DISCUSSION

Our results lead us to conclude that hyperbaric oxygen causes a decrease in the density of striatal D_2 receptors similar to that observed for cortical CBR in rats. By contrast, hyperoxia does not alter PBR either in cerebral cortex or in kidney. The enhanced PBR density observed in liver may be specific to that organ. Such differences between PBR and CBR have already been observed by treating membranes with phospholipase A_2[3], or by submitting animals to a pharmacological treatment like chronic exposure to phenobarbital.[13]

PBR may not be involved in neurotransmission, but might play a role in the regulation of mitochondrial oxidative phosphorylation as well as in the regulation of steroid hormones synthesis.[14,15] An increase in the density of liver receptors might be

related to both hypertrophy and swelling of mitochondria observed under hyperbaric hyperoxia.[16] Indeed, such membrane disruptions could secondarily unmask PBR receptors.

On the other hand, we observed that the two CNS receptors we studied (CBR and D_2 Dopamine receptors) were both decreased under hyperbaric hyperoxia. Several explanations for this phenomenon can be put forward. First, the receptors may be directly damaged by oxygen. More likely, peroxidation of membrane lipids could damage them.[6–8] Third, a decrease in the density of receptors involved in neurotransmission may be a down-regulation due to an increasing release of neurotransmitters under hyperoxia. Such processes of down-regulation are commonly observed during long-term pharmacological treatment.[13]

Some investigators have described a decrease in cerebral GABA or dopamine concentrations in animals exposed to hyperbaric oxygen during the preconvulsive stage.[17,18] Other workers report an increase in dopamine and related metabolite concentrations in the striatum of rats sacrificed after a first hyperoxic seizure.[19] However, we must stress the fact that those investigations dealt with global concentrations of neurotransmitters and not with receptors.

However, it is interesting to note that an up-regulation of GABA/benzodiazepine receptor was recently discovered in the rat exposed to a relatively short-term hypoxia (10% O_2, 6 h).[20] The authors concluded that this phenomenon was related to a decrease in GABA synthesis due to the inhibition of glutamic acid decarboxylase, the presynaptic key enzyme of GABAergic neurons.

Our results show that further investigation of the different neurotransmission pathways under hyperoxia is warranted.

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