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RESPONSE TO LETTER

# Response to the Letter to the Editor: "Mitochondria isolated from the striatum of the brain exhibit a higher degree of oxidative phosphorylation coupling, which shows that they are not subject to energetic dysfunction upon acute paraquat administration"

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In his Letter to the Editor, Professor Rendon states that our published work entitled "Impairment of striatal mitochondrial function by acute paraquat poisoning", published in *J Bioenerg Biomembr* 2015; 47(5): 395–408, a part of the methodology used and the interpretation of the results exhibit serious fallacies and contradictions that do not support the stated conclusions with respect to the negative alterations of the bioenergetics observed in the striatum mitochondria after paraquat treatment.

First of all, we want to thank Professor Rendon, for the attention he gave to our work. However, we would like to respond to Professor Rendon concerns as follows:

1. Regarding the oxygen uptake data exposed in Fig. 2A, Professor Rendon expresses that we do not include what is taking place in the case of the paraquat redox cycling. However, we think that this is not the case. In fact, we describe in Materials and Methods (page 397) and Results (pages 400–401) both the methodology and the results obtained for oxygen consumption rates measured in the absence and presence of 1.3 mM KCN. This strategy allowed us to discriminate between KCN-sensitive and -insensitive oxygen consumption. We also explained that we subtracted the rates of KCN insensitive oxygen consumption (with KCN preincubation) to the original data (without KCN preincubation). As shown in Fig. 2A, paraquat inhibited striatal state 4 and state 3 KCN-sensitive respiration by 80 % and 62 % respectively, indicating a direct effect on the respiratory chain. Also, respiratory rates were measured after incubation of mitochondrial samples with 1 μM DPI in order to evaluate the contribution of paraquat redox cycling to the total oxygen consumption. Increases of 2.2 and 2.3 fold were observed in state 4 and state 3 KCN-insensitive respiration respectively in striatal mitochondria from paraquat-treated animals, suggesting that paraquat redox cycling also consumed oxygen in a respiratory chain-independent manner.
2. Concerning why we do not calculate the RC in the case of KCN-sensitive respiration using data presented in Fig. 2A, we consider that the RC in the presence of KCN does not represent an indicator of mitochondrial viability or a physiological parameter of mitochondrial function. Therefore, it would be more accurate to analyze the mitochondrial metabolic states, instead of presenting the RC parameter. Our results show that the analysis of oxygen uptake after acute paraquat treatment presented a clear alteration of the respiratory rate in the mitochondrial metabolic states 4 and 3.
3. Professor Rendon states that during the preparation of the mitochondrial membranes through freezing and thawing procedures, a fraction of the fragments of the inner mitochondrial membranes formed vesicles with the catalytic part of mitochondrial

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**Table 1** Effect of FCCP on mitochondrial electron transfer activities from striatal submitochondrial membranes from control and paraquat-treated animals

| | NADH-cytochrome c reductase (nmol/min.mg protein) | | Succinate-cytochrome c reductase (nmol/min.mg protein) | | Cytochrome oxidase (k'/mg protein) | |
|---|---|---|---|---|---|---|
| | (−) FCCP | (+)FCCP | (−)FCCP | (+)FCCP | (−)FCCP | (+)FCCP |
| Control | 18.9 ± 0.5 | 16.7 ± 0.1 | 14.3 ± 0.9 | 16.0 ± 0.6 | 62 ± 2 | 56 ± 1 |
| Paraquat | 13.7 ± 0.3* | 12.1 ± 0.9* | 14 ± 2 | 15.2 ± 0.3 | 50 ± 2# | 46 ± 1# |

* $p < 0.001$ and # $p < 0.05$, as compared with its respective control value

complexes facing their interior. As reported previously in our work (Czerniczyniec et al. 2011), after two sessions of freezing and thawing, the mitochondrial preparation was homogenized by passage through a tuberculin syringe with a 29-gauge hypodermic needle (Boveris et al. 2002). As a result, the preparation obtained mostly consisted of a fraction of outer and inner membranes, which do not present restriction to substrate access. However, we tested the hypothesis of Professor Rendon and we measured mitochondrial respiratory complexes activity in the absence and presence of FCCP (4 μM). As shown in the enclosed Table 1, incubation of submitochondrial membranes with FCCP did not increase the values of neither of the complexes activities, probably indicating a low content of vesicles in our preparations. Also, the inhibitory effect of paraquat on Complex I-III and IV (27 % and 19 % respectively) did not change in the presence of FCCP (28 % and 18 %), as compared with control samples, indicating that the activity of the respiratory complexes reported in our original results does not seem to be underestimated.

4. The author of the letter also states that the mitochondrial enzymatic activity reported in Figure 1A is not exclusively from complex I-III enzymatic activity. He suggested that two different enzymatic systems: 1) the NADH–cytochrome *c* reductase present in the fragments of the outer mitochondrial membrane and 2) the mitochondrial complex I-III of the inner mitochondrial membrane fragments, were capable of oxidizing and reducing both exogenous NADH and cytochrome *c*, respectively. Taking into account that the external enzymatic system is insensitive to rotenone treatment (Ernster et al. 1963), we repeated the measurements of complex I-III activity in the absence and presence of 3 μM rotenone in order to evaluate the contribution of the external NADH cytochrome c reductase system to the total NADH oxidation. In addition, determinations were carried out in the presence of FCCP. As shown in the enclosed Table 2, we observed that in the presence of rotenone, a significant inhibition of approximately 80 % was found in control samples. These results were in agreement with studies using liver, kidney and brain submitochondrial membranes (Navarro et al. 2010; Puntel et al. 2013). A similar effect of rotenone was also found in striatal samples from paraquat-treated animals (77 % inhibition). Using this approach, we calculated the rotenone-sensitive complex I-III activity from control (13.3 ± 0.2 nmol/min. mg protein) and paraquat samples (9.3 ± 0.9 nmol/min. mg protein). The results indicated that the herbicide treatment inhibited by 32 % the rotenone-sensitive complex I-III activity, which was similar to the data reported in our published work, indicating that the external NADH cytochrome *c* reductase system does not significantly contribute to NADH oxidation in our assays. It is important to note that the inhibition of complex I and the increase in free radicals production by paraquat has been extensively described in different models of toxicity (Cocheme and Murphy 2008; Drechsel and Patel 2009; Fukushima et al. 1993; Tawara et al. 1996) and our results were in accordance with those studies.

**Table 2** Effect or rotenone on complex I-III activity from striatal submitochondrial membranes from control and paraquat-treated animals

| NADH-cytochrome c reductase (nmol/min.mg protein) | | |
|---|---|---|
| | (−)Rotenone | (+)Rotenone |
| Control | 16.7 ± 0.1 | 3.4 ± 0.2* |
| Paraquat | 12.1 ± 0.9 | 0.85 ± 0.08# |

* $p < 0.001$, as compared with (−) rotenone value. # $p < 0.001$, as compared with control value

Finally, according to the experimental evidences presented here and the exposed arguments, we can conclude that in our work published in *J Bioenerg Biomembr* 2015; 47(5): 395–408, the employed methodology supports the results of the

effects of acute paraquat treatment on striatal mitochondria bioenergetics.

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