# Reply: On interaction between mitochondrial porin and anion carriers

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According to our data [1], stimulation of the controlled succinate oxidation by ADP, palmitate and DNP and sensitivity of this stimulation to carboxyatractylate are considerably less pronounced in mitoplasts than in mitochondria. Addition of porin-containing preparations (outer mitochondrial membranes or solubilized porin) to mitoplasts results in partial restorations of the oxygen consumption rate and sensitivity to carboxyatractylate. Recently, Wojtczak and Nałęcz [2] failed to reproduce some of our data, namely the lower rate of buthylmalonate-sensitive oxidation of succinate in mitoplasts than in mitochondria. The authors stressed that mitoplasts always contain contaminations of the outer membrane.

We agree that such contaminations are present in mitoplast preparations (mainly due to the contact sites of two membranes) [3–7]. The amount of these contaminations depends on the state of mitochondria, used to obtain mitoplasts. Respectively, the effect of added porin described in our paper [1] seems to be the higher the lower is the outer membrane contaminations. In particular, it was in fact absent when mitochondria have high respiratory control (above 4). The optimal control was found to be 2.5–3.5 for whole mitochondria and 1.2–1.8 for mitoplasts. Under these conditions monoamine oxidase activity in mitoplasts was $37 \pm 8\%$ of the activity in intact mitochondria. In this context, it should be mentioned that uncoupling and glycerol treatment is known to reduce the number of contact sites in mitochondria [5–7]. Maybe in experiments of Wojtczak and Nałęcz [2] the respiratory control of mitochondrial was too high resulting in a relatively high number of remaining contact sites in mitoplast preparations.

We believe that our results [1] are not due to inner membrane damage because reduced sensitivity of mitoplast respiration to the studied activators and inhibitors could be partially restored upon addition of porin-containing preparations. This restoration was observed within the narrow protein concentration range only (1.0–2.0 μg/ml and 0.5–1.5 ng/ml of protein for outer membranes and solubilized porin, respectively) and was not observed upon incubation of mitoplasts in the glycerol-containing medium. The respiratory parameters of intact mitochondria were not affected by addition of either outer membranes or solubilized porin.

In the literature there are some data to support our interpretation of the obtained results [1] namely (i) different surface charges of the outer and inner membranes [4]; (ii) dependence of activity and inhibitor sensitivity of ADP/ATP antiport and respiratory substrate transports on membrane surface charge [8–11]; (iii) decrease in ADP/ATP exchange upon a stepwise removal of outer membrane from mitoplasts [12]; (iv) activation of ADP/ATP exchange by anionic and cationic effectors which was observed in mitoplasts and reconstituted systems, but was not seen in mitochondria [13, 14]; (v) existence of a large positive charge at the porin channel surface [15]. These data may be the arguments for suggesting porin to be a potential activator of the porters in contact sites.

In papers concerning isolation and reconstitution of anion carriers the procedure of porin removal has not been discussed. However, the reproduction of isolation procedures for some anion carriers [16–18] and uncoupling protein [19] enabled us to obtain preparations forming porin-like channels in planar bilayers ([20] and unpublished data). Therefore it seems that the possibility of porin contamination in carriers preparations cannot be excluded.

We do not think that our observations [1] adequately prove the direct functional link between porin and mitochondrial carriers, but a number of available data might be well explained by this hypothesis.

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