CHANGE IN FLUX CONTROL COEFFICIENT OF CYTOCHROME *C* OXIDASE IN COPPER DEFICIENT MOTTLED BRINDLED MICE

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INTRODUCTION

The brindled mouse is a variant of mottled mutants (Mo^{br}) with severe copper deficiency, and is considered to be an animal model of Menkes syndrome [1]. The copper homeostasis disorders in mottled mice are associated with distinct mitochondrial alterations. Among these mitochondrial abnormalities is the decreased activity of cytochrome *c* oxidase a well documented phenomenon [2,3]. However, in cases where the defect is not a rate-limiting step of oxidative phosphorylation, the determination of maximal rates of respiration will not reveal the hidden mitochondrial defect. Recently, Mazat and coworkers reported that control analysis can be a successful approach for the investigation of the pathogenesis involved in certain mitochondrial defects [4,5]. However, direct evidence is still absent and acceptable pathological models were still not used for flux control analysis. To elucidate if the brindled mouse is a good model for mitochondrial myopathies with cytochrome *c* oxidase deficiency and if this defect can be further characterized by the application of control analysis, we determined the flux control coefficient of cytochrome *c* oxidase by inhibition of mitochondrial respiration with increasing concentrations of sodium azide.

METHODS

Animals: Five mottled brindled mutant male mice (Mo^{br}) obtained from Genetic Division MRC Radiobiology Unit, (Chilton, DIDCOT Oxon OX11 ORD, England) and five normal controls were used to study mitochondrial and enzymatic properties of cardiac and skeletal (*M. quadriceps*) muscles. Animals were euthanized by cervical dislocation, hearts and *M. quadriceps* were rapidly removed, washed and placed in ice cold Krebs solution. 50-100 mg of each tissue were frozen in liquid nitrogen for enzyme assays and the remaining tissue placed in relaxing solution for respiration experiments.

Isolation of skinned fibers: The bundles of muscle fibers were isolated from heart (left ventricle) or skeletal muscle (*M. quadriceps*) of control and Mo^{br} mice. Saponin-skinned fibers were prepared by incubation of bundles of intact fibers in relaxing solution (composition see below) containing 50 µg/ml saponin [6]. Glycerol-skinned fibers were prepared with the same relaxing solution containing 20% glycerol and 10 mg/ml fatty acid free BSA without saponin and kept in liquid nitrogen until analysis.

Solutions: The relaxing solution contained 10 mM Ca-EGTA buffer, free concentration of calcium 0.1 µM, 20 mM imidazole, 20 mM taurine, 100 mM K-MES, 0.5 mM DTT, 5 mM $MgCl_2$, 5 mM ATP, 15 mM phosphocreatine, pH 7.2.

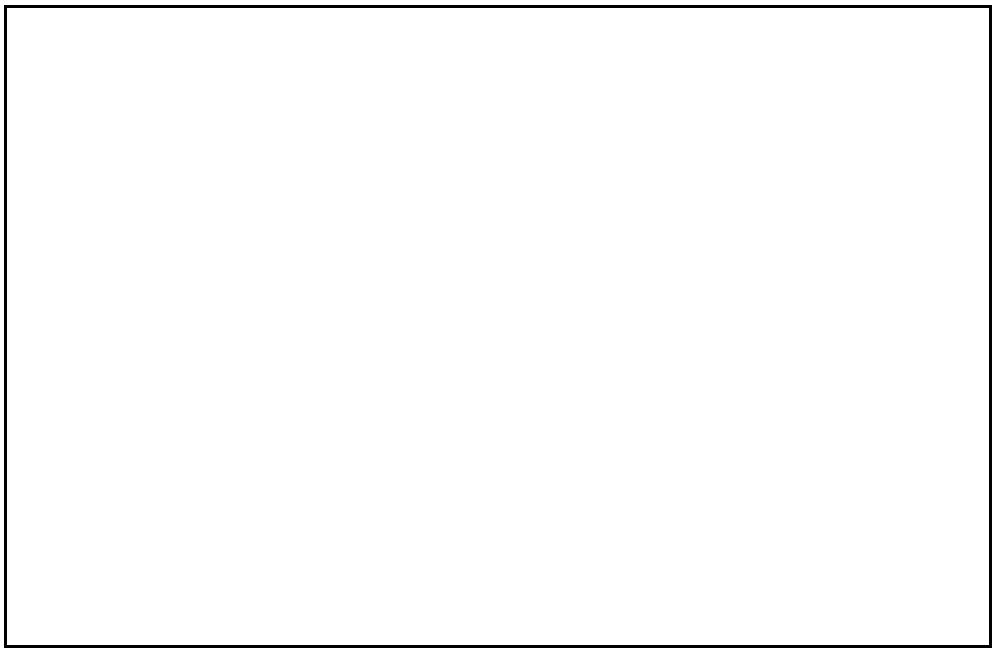

Fig. 1. Inhibition of mitochondrial respiration of skinned cardiac fibers by Na-azide. Skinned fibers (1.5-2.0 mg dry weight) were isolated from heart (left ventricle) of control (○) and brindled (●) mice. Respiration was measured in the presence of 10 mM glutamate, 5 mM malate, 10 mM succinate and 1 mM ADP (see Methods).

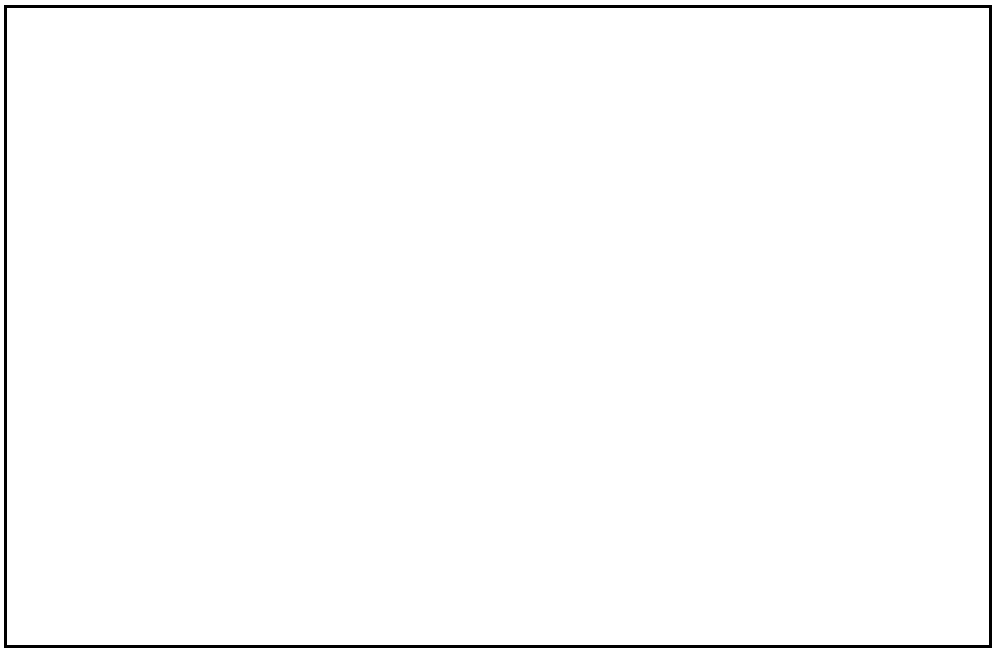

Fig. 2. Inhibition of mitochondrial respiration of skinned fibers from skeletal muscle by Na-azide. Skinned fibers (3.0-3.5 mg dry weight) were isolated from *M. quadriceps* of control (○) and brindled (●) mice. Respiration was measured as described in Fig.1.

Respiration: Respiration of skinned fibers was measured at 25 °C using an OROBOROS® *Oxygraph* (Anton Paar, Graz) in a medium containing: 5 mM $MgCl_2$, 100 mM KCl, 75 mM mannitol, 25 mM sucrose, 10 mM KH_2PO_4, 20 mM Tris-HCl (pH 7.4), 10 mM glutamate and 5 mM malate as mitochondrial substrates or with 10 mM succinate and 0.8 mM cytochrome *c* in addition to the above. The concentration of oxygen at 25 °C air saturation was assumed to be 230 nmol O_2/ml [7].

RESULTS

Determination of activities of cytochrome *c* oxidase in *M. quadriceps* and heart revealed that in muscle tissues of brindled mice only about one half of the control activity is present (*M. quadriceps*: 5.5 ± 0.5 U/g W_w versus 10.1 ± 0.6 U/g W_w; heart: 19.7 ± 2.1 U/g W_w versus 37.5 ± 2.4 U/g W_w). However, no difference was found in maximal rates of respiration of skinned quadriceps fibers isolated from normal and brindled mice. Although skinned fibers isolated from the cardiac muscle of copper deficient animals had slightly lower respiratory parameters than control, no significant difference between control and brindled groups of mice was found. To determine the flux control coefficients for cytochrome *c* oxidase the maximal rate of respiration reached in the presence of 1 mM ADP was inhibited with increasing amounts of sodium azide. The analysis of the inhibition curves revealed a much sharper slope for cardiac muscle fibers of the brindled mice than of the control mice (Fig. 1; control coefficients: C_i = 0.58 and 0.33, respectively). The same result was also obtained for skeletal muscle (Fig. 2) (C_i = 0.87 for Mo^{br} mice and C_i = 0.30 for the control).

CONCLUSION

Our data demonstrate that determination of flux control coefficients can be a valuable approach to study defects of oxidative phosphorylation which are not observable with simple measurement of maximal respiratory activities. Furthermore, this technique is a helpful method for analyzing mitochondrial dysfunction in mitochondrial myopathies and encephalomyopathies.

REFERENCES

1 Hunt DM (1977) *Comp Biochem Physiol* **57**: 79-83
2 Maehara M, Ogasawara N, Mizutani N, Watanabe K, Suzuki S (1983) *Brain Dev* (Tokyo) **5**: 533-540
3 Rezek DL, Moore CL (1986) *Exp Neurol* **91**: 640-645
4 Mazat JP, Letellier T, Malgat M (1993) In *Modern Trends in Biothermokinetics* (Schuster S, Rigoulet M, Ouhabi R, Mazat J-P, eds) Plenum Press, New York: 343-346
5 Letellier T, Malgat M, Mazat JP (1993) *Biochim Biophys Acta* **1141**: 58-64
6 Kunz WS, Kuznetsov AV, Gellerich FN et al (1993) *Biochim Biophys Acta* **1144**: 46-53
7 Kunz WS, Kuznetsov AV, Gellerich FN (1993) *FEBS Lett* **323**: 188-190