BDNF, BUT NOT GDNF, INCREASES MOUSE BRAIN MITOCHONDRIAL RESPIRATORY COUPLING AT COMPLEX I, BUT NOT COMPLEX II

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Brain derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) are neurotrophic factors responsible for growth and development of neurons. Previous studies confirmed the ability of BDNF to modify rat brain mitochondrial ATP synthesis (Markham et al., 2004). The present study investigates the effects of neurotrophic factors in mouse brain mitochondria.

Forebrains from female Swiss Webster CD-1 mice (30-35g) were removed and placed in isolation buffer containing 220mM mannitol, 60mM sucrose, 5mM Tris-HCl, 5mM Tris-base, 0.5mM EGTA and 1mg ml⁻¹ bovine serum albumin (BSA; fatty acid free) pH 7.4. Homogenates were centrifuged at 2,000 rpm for 6 min at 0-4°C, the resulting supernatant decanted off and spun for 8 min at 10,000 rpm. The pellet produced was resuspended in 9 ml of buffer and aliquots layered onto 10 ml ice-cold Percoll solution containing 250mM sucrose, 5mM Tris-HCl, 0.1mM EGTA and 18% (v/v) Percoll, pH 7.4. The resulting density gradient was centrifuged at 10,000 rpm for 45 min. A loose mitochondrial pellet, free from most contamination, was formed at the bottom of the tube with a synaptosomal fraction forming a separate layer at the top of the tube. The two layers were then isolated and centrifuged at 10,000 rpm in isolation buffer minus EGTA (incubation buffer) to remove the Percoll.

Oxygen consumption was measured polarographically (Sweetman & Weetman, 1972) using a Clark-type oxygen electrode (Rank Bros, Bottisham, UK). Respiratory studies were performed at 20°C in incubation buffer plus 5 mM KH₂PO₄, saturated with oxygen (463.3 ng atoms O ml⁻¹). Experiments were initiated by the addition of brain mitochondria in the presence or absence of synaptosomes, in a final volume of 0.3 ml. The respiratory substrates 5 mM Glutamate plus 5 mM Malate and 5 mM succinate were added after 1 min and 167 µM ADP added after a further min. Calculating the RCI (Respiratory Control Index; a measure of the efficiency of respiratory coupling) assessed mitochondrial integrity. Data were analysed using a one-way ANOVA followed by Dunnett’s post-test.

BDNF (66.6 – 666 ng ml⁻¹; Alomone laboratories) produced no significant effect on mitochondria in the absence of synaptosomes. In the presence of synaptosomes BDNF (133 – 666 ng ml⁻¹) produced an increase in RCI. The RCI was significantly increased from a control of 3.98 ± 0.42 to 5.9 ± 0.26 (sem, n = 6; P<0.01) at the highest concentration, using 5 mM glutamate plus 5 mM malate as a substrate. 10µM PD98059, 1µM U0126 (inhibitors of MEK; Tocris) and 1.33µg ml⁻¹ Ab-1 (an anti-BDNF antibody, Calbiochem) abolished this effect. BDNF (66.6 – 666 ng ml⁻¹) produced no effect on mitochondrial respiration when 5 mM succinate was used as a substrate. GDNF (66.6 – 666 ng ml⁻¹) produced no effect on mitochondrial function regardless of substrate or the presence / absence of synaptosomes.

BDNF produced an increase in RCI in mouse brain mitochondria at concentrations similar to rat brain (Markham et al., 2004). The different effects of the neurotrophic factors on mouse brain mitochondria may be explained by the finding that BDNF is known to act via tyrosine kinase B (TrkB) linked receptors, whereas GDNF acts via RET tyrosine kinase linked receptors (Ibanez, 1998).