017P BDNF INCREASES MITOCHONDRIAL RESPIRATORY COUPLING, AND GLUTAMATE METABOLISM, AT COMPLEX I, BUT NOT COMPLEX II

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Brain-derived neurotrophic factor (BDNF) governs both the selective survival of neurons during development and the experience-based regulation of synaptic strength throughout life (Black, 1998). Neuronal plasticity, coupled to neuronal activity, will require considerable energy expenditure, but the links between plasticity and mitochondrial function are unknown. We show that BDNF, concentration-dependently, increases the respiratory control index (RCI, a measure of the efficiency of respiratory coupling and organelle integrity) of brain mitochondria for 5 mM glutamate plus 5 mM malate oxidation (complex I) by a MAP kinase pathway: oxidation of 5 mM succinate (complex II) is not affected.

Mitochondrial respiratory coupling is adversely affected if extensive preparatory procedures are used and the signal transduction systems for neurotrophin activity must be intact. We therefore used a rat forebrain preparation from female Wistar rats (250-300 g). Brains were removed and placed in buffer containing 220 mM mannitol, 60 mM sucrose, 5 mM Tris-HCl, 5 mM Tris-base, 0.5 mM EGTA and 1 mg ml⁻¹ bovine serum albumin (BSA; fatty acid free) pH 7.4. A homogenate was centrifuged at 2,000 rpm for 6 min at 0-4 °C, the resulting supernatant was decanted off and spun for 8 min at 10,000 rpm, the pellet produced by this spin was gently dislodged and the process was repeated a further 5 times. For the last two spins pellets were resuspended in the buffer minus 0.5 mM EGTA.

Oxygen consumption was measured polarographically using a Clark-type oxygen electrode (Sweetman & Weetman, 1973).

Mitochondrial respiration studies were performed at 20 °C in an incubation medium containing 5 mM KH₂PO₄ and the above buffer minus EGTA at pH 7.4. Incubation medium was saturated with oxygen (463.3 ng atoms O ml⁻¹) and the experiment was initiated by the addition of brain mitochondrial protein (0.5–1 mg ml⁻¹) in a final volume of 0.75 ml. Calculating the RCI for each preparation assessed mitochondrial integrity. Statistical analysis were performed via one way ANOVA (post hoc test).

Mitochondrial respiration, in response to ADP (160 µM), in the presence of 5 mM glutamate plus 5mM malate (complex I) or 5mM succinate (complex II) substrates, was tightly coupled. BDNF did not modify endogenous respiration, state 4 or state 3 respiration when 5 mM glutamate plus 5 mM malate was used as a substrate, but there was a reduction of the return to state 4 respiratory rate. Furthermore, BDNF caused a significant concentration-dependent increase (66 ng ml⁻¹- 666 ng ml⁻¹) in the RCI. The RCI was increased from 3.12 ± 0.08 (s.e.m., n = 5) to 5.12 ± 0.33 (s.e.m., n = 5; P<0.01) at the highest concentration, a 64% increase in the efficiency of coupling was observed. RCI for 5 mM succinate was unchanged by BDNF and the effects of BDNF on complex I were abolished by co-treatment with a neutralising anti-BDNF antibody (1.3µg ml⁻¹, Calbiochem). The effects of BDNF were completely abolished by pre-treatment with PD 98059, at a concentration (10 µM) which is specific for inhibition of MEK kinase. This is the first report of an effect of neurotrophins on mitochondrial metabolism.