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Direct effects of dopamine on mitochondrial thermogenesis in brown adipocytes

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Background: Brown adipose tissue (BAT) is specialized in thermogenesis, i.e. the production of heat. Uncoupling protein 1 (UCP1) mediates this effect in mitochondria of brown adipocytes [1]. Catecholamines are known to be critically involved in the regulation of BAT thermogenesis [2]. However, little is known about dopamine (DA)-mediated effects on thermogenesis. Here, we investigated direct cellular effects of DA on mitochondrial thermogenesis and mass in brown adipocytes.

Methods: Cell culture: SV-40T immortalized murine brown adipocytes were used for all experiments.
Western blot: Protein lysates were prepared and immunoblotting was performed using specific antibodies. Mature brown adipocytes were treated with dopamine (DA; 1 or 10nM) for 24h in serum free medium.
cAMP determination: Brown adipocytes were treated as stated in the figure. Cells were lysed and supernatants were analyzed using an ELISA kit (Caymen Chemical, Hamburg, Germany).
Oxygen consumption: Respiration was measured using Oxoplates (FluoSTAR OPTIMA; BMG-Labtech, Ortenberg, Germany).
JC-10 assay: Mitochondrial membrane potential ($\Delta\psi_m$) was analyzed with the JC-10 dye (Biomol, Hamburg, Germany). Fluorescence intensities of JC-10 monomers and aggregates were quantified using a microplate reader.
Statistical analysis: Paired Student's *t*-test was performed using Sigma Plot $^{**}p<0,01$;$^{*}p<0,05$.

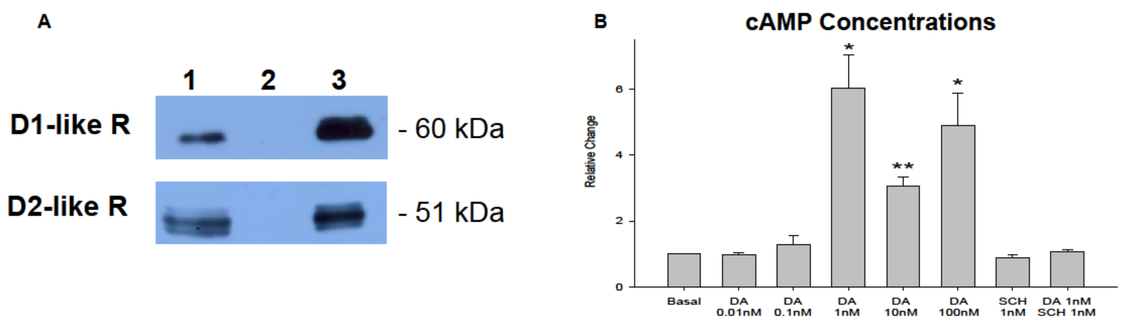

Fig.1: Western blot for D1-like and D2-like dopamine receptors expression (A) (1= BAT cell line, 2=negative control, 3=positive control). DA treatment of brown adipocytes for 2 minutes causes a dose-dependent increase of cAMP concentrations. D1-like receptor antagonist SCH 23390 (SCH) abolished the effect of DA on cAMP level, SCH alone had no effect on cAMP levels (B).

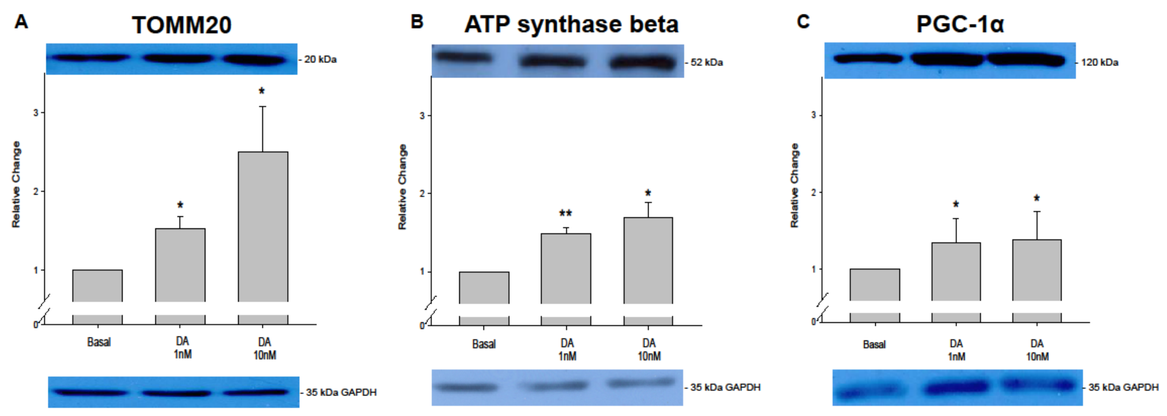

Fig.3: Dopamine (DA) increases mitochondrial mass: DA treatment of brown adipocytes for 24 hours increases expression of two mitochondrial mass marker TOMM20 (A), and ATP synthase beta (B) dose dependently also DA induces expression of PGC-1α (C) in BAT significantly to basal (PGC-1 α = peroxisome proliferator-activated receptor γ coactivator 1 α).

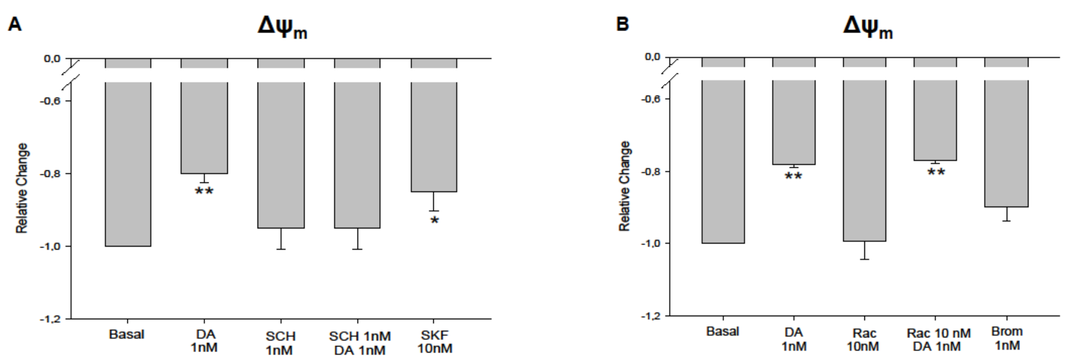

Fig.4: Dopamine (DA) effects on $\Delta\psi_m$ are mediated by dopamine 1-like receptors: Treatment of brown adipocytes with DA significantly increases $\Delta\psi_m$ within 24 hours. A D1-like receptor antagonist (SCH), but not the D2-like receptor antagonist Raclopride (Rac), abolished the DA-effect on $\Delta\psi_m$. The specific D1-like receptor agonist SKF 38393 (SKF) had a similar effect on $\Delta\psi_m$ as DA, whereas bromocriptine (Brom), a D2-like receptor agonist, had no significant effect (A,B).

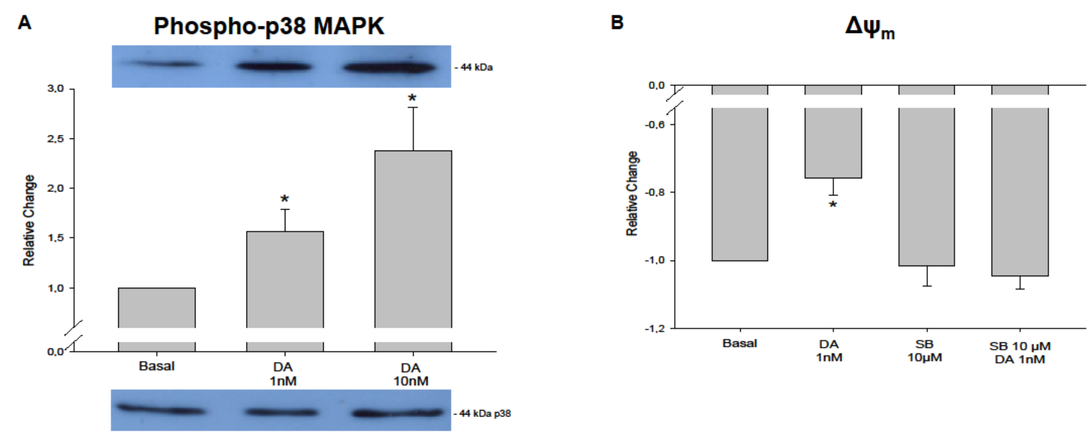

Fig.5: Dopamine (DA) effects on $\Delta\psi_m$ are p38 MAPK-dependent: Treatment of brown adipocytes with DA significantly increases p38 MAPK phosphorylation (A) and pharmacological inhibition of p38 MAPK with specific inhibitor SB 202190 (SB) abolished the DA-mediated effect on $\Delta\psi_m$ (B).

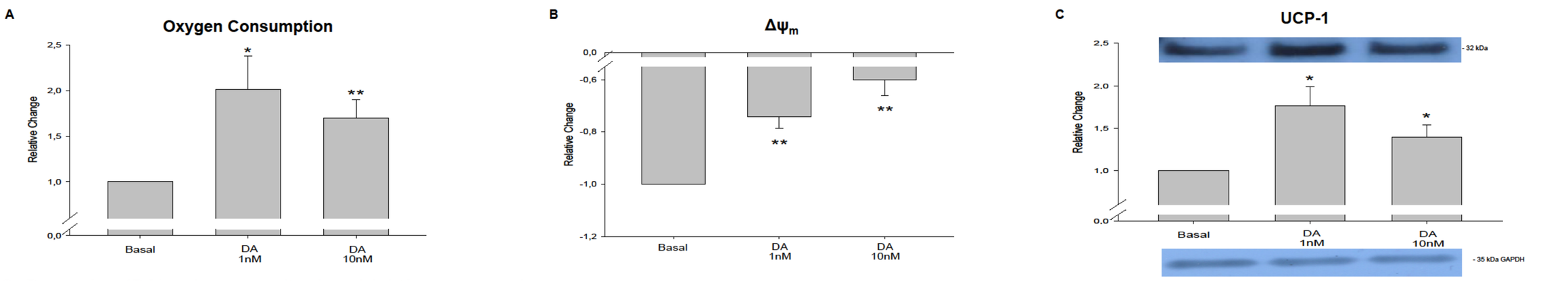

Fig.2: Dopamine (DA) enhances mitochondrial thermogenesis: DA treatment of brown adipocytes for 24 hours increases oxygen consumption rates (A) and mitochondrial membrane potential ($\Delta\psi_m$)(B). Protein levels of UCP-1 increase significantly upon treatment with DA (C).

Summary:

D1- like and D2- like dopamine receptors were detectable in brown adipocytes. Treatment of brown adipocytes with dopamine:

- increased cAMP concentrations
- increased oxygen consumption, $\Delta\psi_m$ and UCP-1 levels
- increased expression of mitochondrial mass markers

These direct effects of dopamine on mitochondrial thermogenesis were mediated by D1-like receptors and p-38 MAP kinase.

Conclusion:

Dopamine directly increased thermogenesis in brown adipocytes. Targeting D1-like receptors on brown adipocytes may help to induce thermogenesis, pointing towards novel therapeutic approaches to treat obesity.

References:
[1] Kozak LP, Koza RA, Anunciado-Koza R (2010) Int J Obes. 34 Suppl 1:S23-7.
[2] Collins S, Yehuda-Shnaidman E, Wang H (2010) Int J Obes. 34 Suppl 1:S28-33.

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