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Adrenergic Receptor β3 Up-regulates Uncoupling Protein 1 and Cyclooxygenase 2 Expressions in The Brown Adipocyte

Rongxin Zhu,Pengyu Fu,Yang Hu,Lijing Gong
Beijing Sport University

Objective Brown adipose tissues (BAT) activation is important for losing weight as its high energy expenditure in Mammalian. Recent studies showed that exercise may also be essential for BAT activation. Uncoupling protein 1 (UCP1), specifically expressed in BAT's mitochondria, uncouples oxidative phosphorylation and dissipates energy from Free Fatty Acids into heat. Activating the Adrenergic Receptor β3 (Adrβ3) provides fuel for mitochondrial heat production and up-regulates Cyclooxygenase 2 (COX2), which is a key factor of UCP1 synthesis. Sympathetic nerve excitement stimulated by exercise can release norepinephrine as a neurotransmitter, which can affect Adrβ3. Brown adipocyte (BAC) is a kind of adipocyte *in vitro* as a model to study heat production. Isoprenaline Hydrochloride (ISO) is a widely used as an Adrβ agonist. In this research, we tried to figure out the response of BAC to Adrβ3 activations with different time points and whether ISO can be used as a BAC activator.
Methods $C_3H_{10}T_{1/2}$ cells were maintained in a humidified, 37°C, 5% CO_2 incubator in DMEM/F12 medium with 10% fetal bovine serum (FBS). For brown adipogenesis, cells were first split into differentiation medium (DMEM/F12 containing 10% FBS, 20nM insulin, 1nM 3,3'5-Triiodo-L-thyronine(T_3)) for 4 days, the medium was changed every other day. Confluent cells were treated for 2 days with brown adipose adipogenesis cocktails (differentiation medium containing 2μg/mL dexamethasone, 0.5mM isobutylmethylxanthine (IBMX), 0.125mM indomethacin and 1μM rosiglitazone) on day 4. Then the medium was replaced by differentiation medium and changed every other day. At day 10, the full differentiation adipocytes were treated with 10μM ISO for 0 (as control), 1, 3, 6, 12 and 24 hours. For the lipid droplets staining, the cells were fixed by 4% paraformaldehyde solution then stained with Oil Red O. The cells were harvested and the total cell lysates were extracted for protein analysis after each time point. The UCP1, COX2, and Adrβ3 expression levels were detected by western blot, using Actin as the internal protein. The results were expressed as the mean ± standard error of the mean (SEM). Group comparisons were performed using two-way ANOVA and LSD's post-hoc tests.
Results After differentiation, the cell shapes converted from fibroblastic to a spherical shape. Dispersed small lipid droplets were observed in the cells. After ISO treatment, the red color after Oil Red Staining became lighter and the size of the lipid droplets turned to smaller. The Adrβ3 protein expressions were 1.00±0.00, 1.34±0.32, 1.07±0.50, 4.65±1.84*, 2.44±0.73, and 3.43±1.09 at 0h, 1h, 3h, 6h, 12h, and 24h after ISO treatment, respectively. After introduced to ISO, the UCP1 expression levels were 1.00±0.00, 1.95±0.39, 2.72±0.57, 5.68±1.82*, 3.49±0.92, and 2.79±1.05 at 0h, 1h, 3h, 6h, 12h, and 24h, respectively. And for COX2, the protein expressions were 1.00±0.00, 2.13±0.67, 1.82±0.33, 4.67±1.82*, 2.88±0.44, and 2.65±0.54, respectively. The * means $p < 0.05$, compared with oh controls. The proteins expressions were reached to peak after 6 hours ISO treatment from the above results.
Conclusions UCP1 and COX2 protein expressions were increased in BAC according to Adrβ3's expression in different time points, indicating that Adrβ3 may induce adipolysis in BAC and help to burn fat and produce heat.