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β_2-Adrenoceptor agonists in the regulation of mitochondrial biogenesis ☆

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ABSTRACT

The stimulation of mitochondrial biogenesis (MB) via cell surface G-protein coupled receptors is a promising strategy for cell repair and regeneration. Here we report the specificity and chemical rationale of a panel of β_2-adrenoceptor agonists with regards to MB. Using primary cultures of renal cells, a diverse panel of β_2-adrenoceptor agonists elicited three distinct phenotypes: full MB, partial MB, and non-MB. Full MB compounds had efficacy in the low nanomolar range and represent two chemical scaffolds containing three distinct chemical clusters. Interestingly, the MB phenotype did not correlate with reported receptor affinity or chemical similarity. Chemical clusters were then subjected to pharmacophore modeling creating two models with unique and distinct features, consisting of five conserved amongst full MB compounds were identified. The two discrete pharmacophore models were coalesced into a consensus pharmacophore with four unique features elucidating the spatial and chemical characteristics required to stimulate MB.

The regulation of cellular energy demand is complex and essential for the homeostasis of cellular processes and responses to cellular stress.[1,2] While mitochondria have a number of functions, the synthesis of adenosine triphosphate (ATP) is critical to cellularactivities. When mitochondria do not properly function, ATP depletion occurs and redox imbalances result in oxidative stress that can lead to cell death. Ischemic injuries are a primary cause of mitochondrial dysfunction and include acute injuries to organs such as the kidney, liver and heart, as well as stroke.[3] Mitochondrial dysfunction is also associated with multiple chronic disease states including Alzheimer disease and diabetes.[4,5] Consequently, the discovery of compounds that stimulate mitochondrial biogenesis (MB) may have vast therapeutic potential.

MB is the continuous process to form new mitochondria within the cell. MB is necessary to maintain cellular homeostasis, and can be induced during periods of cellular stress or injury. The recent identification of a few compounds that induce MB, havehighlighted the process as an important therapeutic target. To study MB, a phenotypic assay is of particular utility. Cellular O_2 consumption rates (OCR) reflect the functional activity of the mitochondria, and are reflective of cellular health. We specifically determined MB by measuring maximal OCR after the addition of the proton ionophore carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP).[6] FCCP uncouples oxygen consumption from the production of ATP resulting in maximal activity of the mitochondrial electron transport chain. If a chemical induces MB, then the FCCP-uncoupled OCR (FCCP-OCR) increases when compared to diluent controls. We have validated this assay with compounds known to cause MB.[7,8] It should also be noted that the respiratory experiments were conducted in primary culturesof rabbit renal proximal tubule cells (RPTC) grown under improved culture conditions, which are highly dependent on aerobic respiration and similar to that found in vivo.[7,8] Although FCCP-OCR is a one dimensional parameter it is reflective of a complex process and ideal for identifying MB. Furthermore, using primary cultures of RPTC have significant scientific and clinical relevance compared to similar assays in cell lines, due to the reliance of RPTC on aerobic respiration.

The β_2-AR represents a major and well-studied receptor responsible for multiple phenotypes including smooth muscle relaxation, increased cardiac chronotropy and ionotropy, increased insulin and renin secretion, and glycogenolysis.[9–12] At the molecular level the renin secretion,and glycogenolysis$_2$-AR is a classical G-protein coupled receptor that couples to both $G_{s\alpha}$ and $G_{i\alpha}$, increasing the

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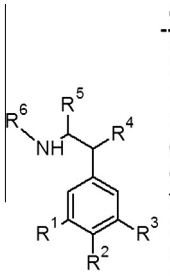

Compound	R1	R2	R3	R4	R5	R6
Epinephrine	H	OH	OH	OH	H	methyl
Norepinephrine	H	OH	OH	OH	H	H
Isoproterenol	OH	OH	H	OH	H	isopropyl
Isoetharine	OH	OH	H	OH	ethyl	isopropyl
Clenbuterol	Cl	NH2	Cl	OH	H	tert-butyl
CPB	H	OH	OH	OH	ethyl	cyclopentane
Terbutaline	OH	H	OH	OH	H	dimethylpropane
Metaproterenol	OH	H	OH	OH	H	methylpropane
Ritodrine	H	OH	H	OH	methyl	ethylphenol
Formoterol	formamide	OH	H	OH	H	methoxy4propylbenzene
Fenoterol	OH	H	OH	OH	H	4propylphenol
Procaterol	H	OH	-NH-COH-C=C-R4	OH	propane	methylpropane
Nisoxetine	H	H	H	methoxyphenol	H	methyl
Tomoxetine	H	H	H	methylphenol	H	methyl

Figure 1. Generalized chemotype of MB stimulating β_2-AR agonists and similar compounds.

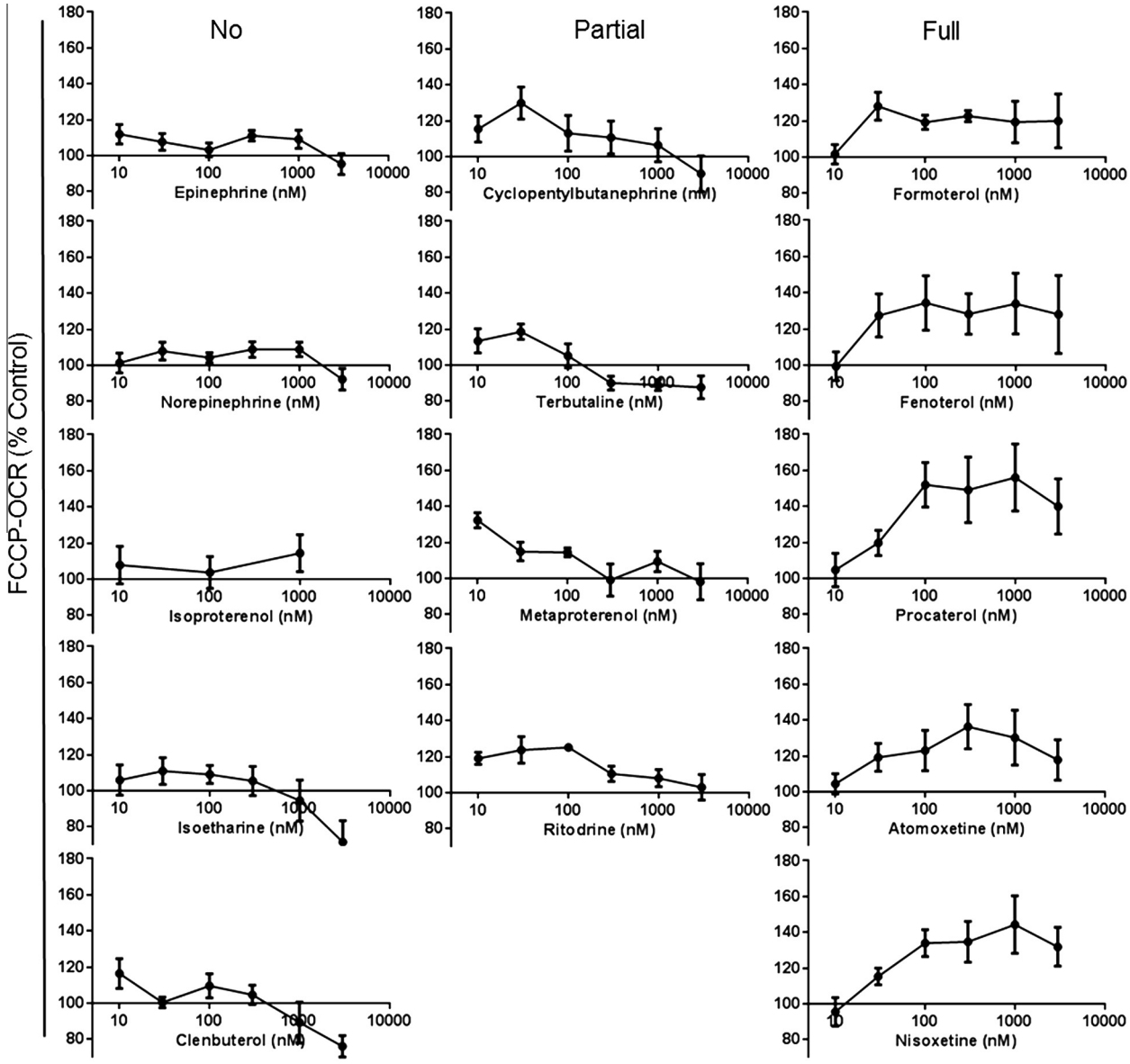

Figure 2. Representative β_2-AR agonists and similar compounds induce concentration-responsive increases in FCCP-uncoupled OCR after 24 h. Values indicate a percent of fold change relative to DMSO controls. Data is represented a mean ± SEM, $N = 4$.

scope of potential effects.[13,14] The induction of MB though the β_2-AR has been demonstrated but the effect of ligand chemotype is still a major question.[15–19] Using RPTC respirometry we showed that the selective β_2 agonist formoterol was a potent stimulator of MB while the non-selective β_2-AR agonist, isoproterenol, was not. Using formoterol as a template we used Tanimoto coefficient