Experimental Study 14.1 Which factors mediate exercise-induced angiogenesis in mammals?

Background

Local tissue hypoxia is one of the most potent stimuli of capillary growth (angiogenesis). However, it is not clear how a low partial pressure of oxygen is sensed. We know that there is an increase in hypoxia-inducible factor (HIF-1α)[1], which stimulates the transcription of the gene for vascular endothelial growth factor (VEGF). However, VEGF does not act alone, and attention has recently turned to the role that the transcriptional coactivator **PGC-1α**[2] may play in exercise-induced angiogenesis.

PGC-1α is a peroxisome proliferator-activated receptor (PPAR-γ) coactivator 1α. PGC-1α has been shown to induce angiogenesis in skeletal muscle without the involvement of HIF, suggesting that local hypoxia may not be an important factor in the process.

Studies were designed, therefore, to answer the questions:

- What is the role of PGC-1α in the mediation of exercise-induced angiogenesis in mammals?
- Does PGC-1α act alone or are other factors involved?

Experimental approach

A series of experiments used gene knockout technology and specific β-adrenergic receptor[3] agonists and antagonists to investigate the sequence of events in the mediation of angiogenesis following sustained exercise.

Experiment 1

This experiment was designed to answer the question:

- Is PGC-1α necessary for increased capillary growth in response to sustained exercise?

This was achieved by breeding mice lacking PGC-1α in their skeletal muscles, called knockout mice (MKO mice). MKO mice were placed with wild type controls (WT mice) in cages with running wheels for 14 days. Figure A (i) shows that both sets of mice ran spontaneously and equally as well as one another on the wheels. However, Figure A(ii) illustrates that there was no significant change in capillary density in MKO mice despite there being more than a 2-fold increase in capillary density in WT mice at the end of the 14 days.

This experiment demonstrates that PGC-1α is required for exercise-induced angiogenesis in skeletal muscle.

We know from other studies that the expression of PGC-1α increases in skeletal muscle of exercising rodents and humans, so the next set of experiments was designed to determine the mechanisms involved in the increased expression of PGC-1α.

Experiment 2

It has been demonstrated that stimulation of β-adrenergic receptors causes an increase in the expression of PGC-1α, so this experiment was designed to answer the question:

- Does stimulation of β-adrenergic receptors initiate angiogenesis in skeletal muscles?

Figure B(i) shows that stimulation of β-adrenergic receptors by a specific agonist, clenbuterol, causes increased expression of

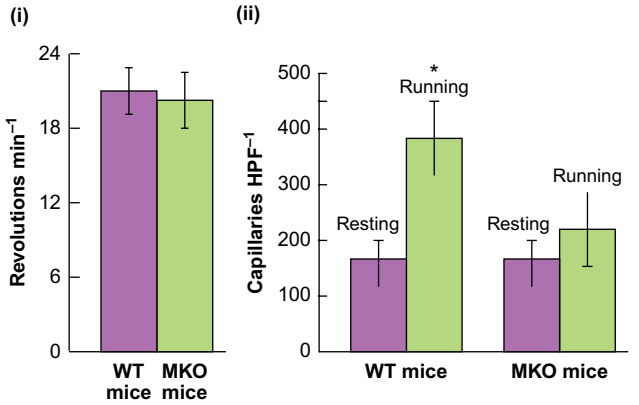

Figure A The PGC-1α gene in skeletal muscles is necessary for exercise-induced angiogenesis in mice

(i) Both wild type (WT) mice and mice with muscle-specific PGC-1α knockout (MKO mice) run spontaneously and equally well on a running wheel

(ii) There is a significant increase in capillary density (given as capillaries per high power field, HPF) in WT mice spontaneously running on a running wheel over a period of 14 days, but this is not the case in MKO mice. HPF is the area which is visible under the objective of a light microscope at maximum magnification, which is usually × 400 to × 500.

Data are mean ± SEM, n = 5. * significantly different from resting value.

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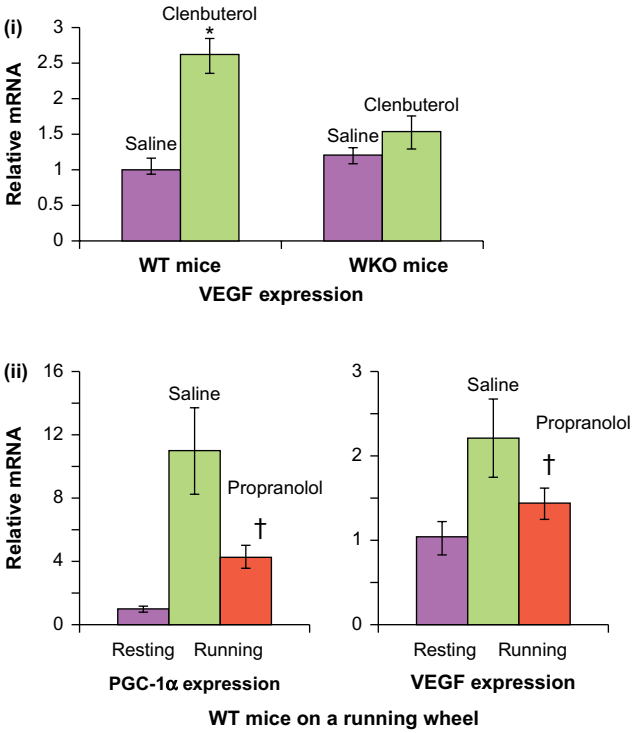

Figure B Stimulation of β-adrenergic receptors induces angiogenic response via PGC-1α

(i) Stimulation of β-adrenergic receptors by the specific agonist, clenbuterol, induces expression of VEGF in wildtype (WT) mice but not in mice lacking expression of PGC-1α (MKO mice)

(ii) The β-adrenergic receptor antagonist, propranolol, prevents or reduces expression of both VEGF and PGC-1α in WT mice running on an exercise wheel for 16 h

Data are means ± SEM, n = 3. * significantly different from control (saline injection) in (i), † significantly different from mice injected with saline in (ii).

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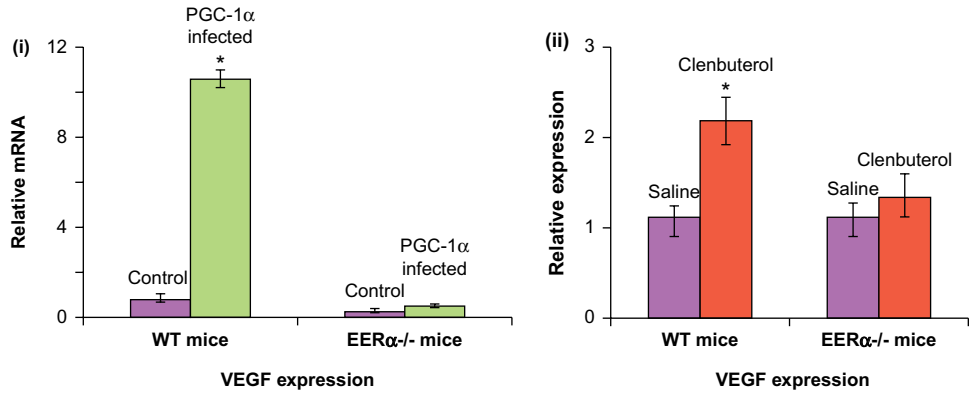

Figure C The angiogenic response to endurance exercise is dependent on EERα

(i) Infecting muscle cells with PGC-1α induces expression of VEGF in cells from WT mice but not in those from EERα-/- mice

(ii) Treatment with the β-adrenergic receptor agonist, clenbuterol, causes an increase in expression of VEGF in WT mice but not in EERα-/- mice

Data are means ± SEM, n = 3 (i) and 5 (ii). * significantly different from control (i) or saline injection (ii).

Reproduced from Chinsomboon J et al (2009). The transcriptional coactivator PGC-1α mediates exercise-induced angiogenesis in skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America 106, 21401–21406.

VEGF in wild-type (WT) mice. In mice lacking the PGC-1α gene (MKO mice), clenbuterol did not increase expression of VEGF. Figure B(ii) illustrates that WT mice treated with a β-adrenergic receptor antagonist, propranolol, show reduced expression of VEGF and PGC-1α following 16 h on the running wheels compared with WT mice injected with saline.

These data suggest that both exercise and stimulation of β-adrenergic receptors increase expression of VEGF via PGC-1α.

Experiment 3

Previous experiments have demonstrated that PGC-1α regulates VEGF via actions on the nuclear receptor EERα (oestrogen-related receptor α). Thus, experiments tested whether (and to what extent) EERα is involved in the process of exercise-induced angiogenesis. Skeletal muscle cells were isolated from EERα knockout mice (EERα-/-) and from WT mice. Figure C(i) shows that infection of muscle cells from WT mice with PGC-1α virus induced an over 10-fold increase in the expression of VEGF. By contrast, PGC-1α had no effect on expression of VEGF in cells from the EERα-/-mice. Thus, PGC-1α induces VEGF in skeletal muscle cells via ERRα.

Similarly, injection of the β-adrenergic receptor agonist, clenbuterol, causes an increase in VEGF expression in WT mice but not in EERα-/-mice, as shown in Figure C(ii). Also, after 14 days in a cage with a running wheel, there was marked angiogenesis in WT mice, but almost no change in capillary density in EERα-/- mice.

The data from these experiments demonstrate that angiogenesis induced by endurance exercise depends on the presence of EERα (for the expression of VEGF).

Overall findings

The results of the three sets of experiments indicate that exercise-induced angiogenesis in mice involves the following pathway:

$$\text{Exercise} \rightarrow \text{stimulation of } \beta\text{-adrenergic receptors} \xrightarrow{\text{EER}\alpha} \text{PGC-1}\alpha \rightarrow \text{VEGF (+ other factors)} \rightarrow \text{angiogenesis}$$

Find out more

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[1] HIF-1α is discussed in section 22.2.3

[2] PGC-1α interacts with a broad range of transcription factors that are involved in a number of physiological responses in animals, such as thermogenesis, glucose/fatty acid metabolism and conversion of type II skeletal muscle fibres to type I oxidative fibres

[3] Adrenergic receptors, their agonists and antagonists, are discussed in section 16.1.3