Exercise in rats does not alter hypothalamic AMP-activated protein kinase activity

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Abbreviations: Acetyl-CoA carboxylase, ACC; AMP-activated protein kinase, AMPK; peptide YY 3-36, PYY₃₋₃₆;

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Abstract

Recent studies have demonstrated that AMP-activated protein kinase (AMPK) in the hypothalamus is involved in the regulation of food intake. Because exercise is known to influence appetite and causes substrate depletion it may also influence AMPK in the hypothalamus. Male rats either rested or ran for 30 or 60 min on a treadmill (22m/min, 10% slope) ~~and~~ were sacrificed immediately after exercise or after 60 min recovery either in the fasted state or after oral gavage with glucose (3g/kg body weight~~bw~~). Exercise decreased muscle and liver glycogen substantially. Hypothalamic total or α 2-associated AMPK activity and phosphorylation state of the AMPK substrate acetyl-CoA carboxylase (ACC) were not changed significantly immediately following treadmill running or during fed or fasted recovery. Plasma ghrelin increased ($p < 0.05$) by 40% during exercise whereas the concentration of PYY was unchanged. In recovery, glucose feeding increased plasma glucose and insulin concentrations whereas ghrelin and PYY decreased to (ghrelin) or below (PPY) resting levels. It is concluded that 1 h of strenuous exercise in rats does not elicit significant changes in hypothalamic AMPK activity despite an increase in plasma ghrelin. Thus, changes in energy metabolism during or after exercise are likely not coordinated by changes in hypothalamic AMPK activity.

Introduction

AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that plays a key role in regulating energy metabolism [1-3]. AMPK is activated by an increase in the AMP:ATP ratio within the cell, and in response phosphorylates a number of targets leading to a decrease in energy utilising pathways and an increase in energy producing pathways. A number of studies have shown that AMPK activity in skeletal muscle is increased following exercise in both humans and rodents [4-8]. Furthermore, activation of AMPK in resting skeletal muscle increases both glucose uptake and fatty acid oxidation [9] as well as gene expression [10; 11], leading to speculation that AMPK could mediate the increase in these pathways in response to exercise {Winder, 1999 3011 /id [format ref](#)}. Recent studies, however, have indicated that genetic deletion of either the $\alpha 1$ or $\alpha 2$ catalytic subunits [12] or the $\gamma 3$ regulatory subunit [13] of AMPK has no effect on contraction-stimulated glucose uptake in skeletal muscle and that AMPK is not required for the increase in GLUT4 expression in response to exercise [14]. The precise physiological significance of AMPK in mediating the effects of exercise on skeletal muscle metabolism therefore remains unclear.

~~Recent studies have demonstrated that AMPK plays a role in the central regulation of energy metabolism.~~ An exciting advance in the field was the finding that AMPK activity in the hypothalamus is altered by hormones that control feeding e.g. leptin and ghrelin [15; 16]. Furthermore, inhibition of hypothalamic AMPK leads to reduced food intake [16; 17], whereas activation increases food intake [15; 16]. Exercise leads to changes in appetite such that appetite is suppressed during strenuous exercise [18; 19] and a short while after exercise whereas increases in appetite after a single bout of exercise are inconsistent (For review see[19]). The mechanism

behind this exercise-induced change in appetite is not known [19] but could involve changes in hypothalamic AMPK activity which may serve the function of coordinating energy expenditure during exercise with energy intake during or after exercise.

In this study we ~~therefore~~ have therefore investigated whether AMPK activity in the hypothalamus is affected during or after exercise and whether it is influenced by the nutritional status after exercise.

Methods

Experimental Animals

All experiments were approved by the Danish Animal Experiments Inspectorate and complied with the *Principles of Laboratory Animal Care* (National Institutes of Health publication no. 85-23, revised 1985). Fifty male Wistar rats weighing ~200 grams were subjected to three treadmill familiarisation sessions of 15 – 20 minutes duration per day leaving one rest day between familiarisation and the experimental day. The animals were divided into the following 5 groups consisting of rats that performed equally well on the treadmill. Group 1 acted as control group while groups 2 – 5 performed an exercise bout of varying duration. Group 2 ran for 30 minutes, group 3 ran for 60 minutes, group 4 ran for 60 minutes and rested for 60 minutes in the fasted condition, and group 5 ran for 60 minutes and rested for 60 minutes in the fed state. Immediately following the exercise bout, group 4 received a 1 ml oral gavage of 0.9 % saline solution while group 5 received 1 ml of saline containing 3.5 M glucose resulting in a glucose dose of 3g/kg. The night prior to the day of the experiment, rats were semi-fasted and received chow corresponding to

50% of their daily energy expenditure. On the day of the experiment, rats assigned to treadmill running ran at a speed of 22 m/min on a 10 degree incline.

Collection of tissue and plasma samples

Animals were sacrificed by decapitation. Trunk blood was collected together with dissection of the liver and soleus and the red and white part of the gastrocnemius muscle. Brains were immediately removed and the hypothalami block dissected and snap-frozen in liquid nitrogen. Plasma was separated by centrifugation, frozen and stored at -20°C until assay.

Plasma assays

Samples were assayed in duplicate. Insulin in plasma was assayed using a commercial radio immuno-assay (RIA) using rat insulin as standard (Linco, St. Charles, USA). Ghrelin-like IR was measured with a specific and sensitive RIA as previously described [20]. The antibody used (SC-10368 G102 Santa Cruz, CA, USA) recognises both octanoyl and des-octanoyl ghrelin and does not cross-react with any known gastrointestinal or pancreatic peptide hormones. PYY-like IR was measured using a specific and sensitive RIA, as previously described [21]. Glucose in plasma was measured by an ABL system 615 (Radiometer, Copenhagen, Denmark). Muscle and liver glycogen content were measured as glucose residues after acid hydrolysis [22].

AMPK assay

Frozen tissues (approximately 50 mg) were homogenised in 0.2 ml ice-cold 50 mM Tris/HCl, pH 7.5, 50 mM NaF, 5 mM Na pyrophosphate, 250 mM sucrose, 1 mM EDTA, 1 mM DTT, 1 mM benzamidine, 0.1% (w/v) phenylmethylsulphonylfluoride using an UltraTurax homogeniser (3 x

30 s bursts). Insoluble material was removed by centrifugation and the resulting supernatant used for immunoprecipitation of AMPK and Western blot analysis. Total AMPK was immunoprecipitated from 100 µg protein using an anti-pan β antibody [23] bound to protein A-Sepharose and activity measured by phosphorylation of the SAMS synthetic peptide [24]. AMPK- $\alpha 2$ specific activity was determined in immunoprecipitates isolated with an antibody specific for the $\alpha 2$ subunit [25].

Western blot analysis

Tissue lysates (40 µg) were resolved by SDS-PAGE and transferred to polyvinylidene fluoride membranes. ACC phosphorylation was measured with a phospho-ACC specific antibody (Upstate) and detected with an anti-rabbit antibody linked to horse radish peroxidase (Bio-Rad). Blots were developed using enhanced chemiluminescence (Pierce) and quantified using a 16-bit charged-coupled device cooled camera and Gene Tools software (Syngene, Cambridge, UK).

Statistical analysis

All variables were analysed by ANOVA followed by post-hoc least significant difference tests. $P < 0.05$ were taken to be significant

Results

All of the exercise regimes employed in the study significantly reduced muscle and liver glycogen (Table 1). Administration of an oral glucose dose (3g/kg) following the 1h run resulted in a significant increase in tissue glycogen content in red gastrocnemius and liver relative to rats fasted during the 1h recovery period. Plasma glucose and insulin levels were not significantly changed by the exercise regime per se, although there was a trend towards decreased insulin levels following the exercise bout. In contrast, as would be predicted, there was a significant increase in both plasma glucose and insulin following the oral dose of glucose (Fig. 1A,B).

Total hypothalamic AMPK activity, measured in immune complexes isolated following immunoprecipitation with a pan- β antibody that recognises both $\beta 1$ and $\beta 2$, was not changed by any of the exercise regimes (Fig. 2A). Since it has been reported previously that the predominant change in AMPK activity in the hypothalamus is associated with the $\alpha 2$ isoform we measured AMPK activity in immune complexes isolated with an $\alpha 2$ -specific antibody (Fig. 2B). As with total AMPK activity there was no significant change in $\alpha 2$ -associated activity. Consistent with the lack of effect of exercise on hypothalamic AMPK activity, there was no significant change in the phosphorylation state of ACC (Fig. 3).

We have previously reported that AMPK activity in the hypothalamus is regulated by hormones that influence feeding: leptin inhibits, whereas ghrelin activates, AMPK [15]. The plasma level of ghrelin increased significantly following the 1h run, falling during the 1h recovery period. Administration of glucose during the recovery period resulted in the level of ghrelin returning to the control value (Fig. 4A). We were unable to accurately measure plasma leptin levels, which were very low in these animals (data not shown). Another hormone, that like leptin causes a decrease in food intake, is peptide YY 3-36 (PYY₃₋₃₆) [26]. The concentration of plasma

PYY₃₋₃₆ was not significantly altered immediately following exercise. During the 1h recovery period, however, the level of PYY₃₋₃₆ was significantly reduced and this was not prevented by the oral glucose dose (Fig. 4B).

Discussion

A number of recent studies have shown that AMPK plays a key role in regulating both energy intake and expenditure [1; 3]. In peripheral tissues, such as skeletal muscle, activation of AMPK switches on energy producing pathways and switches off energy consuming pathways. In the hypothalamus, activation of AMPK leads to increased feeding, thereby increasing energy intake. Conversely, inhibition of AMPK in the hypothalamus reduces food intake. These dual functions of AMPK suggest that it may act to coordinate energy expenditure with energy intake. There is already some evidence that this may be the case in one situation. Leptin activates AMPK in skeletal muscle leading to increased fatty acid oxidation [27], whilst inhibiting AMPK in the hypothalamus leads to decreased food intake [16; 17]. We were interested to investigate whether exercise could affect AMPK activity in the hypothalamus, thereby providing a potential mechanism for the coordination of energy expenditure and energy intake during or following exercise. For instance, exercise leads to changes in appetite such that appetite is suppressed during strenuous exercise and a short while after exercise [18; 19] whereas increases in appetite after a single bout of exercise are not always found [19]). These changes in appetite could therefore be expected to stem from changes in hypothalamic AMPK activity. However, in this study we did not detect any change in hypothalamic AMPK activity in rats subjected to either 30 minutes or 1h treadmill running. Similarly, there was no change in hypothalamic AMPK activity after a 1h run followed by a 1h recovery period in which the rats were either fasted or given an oral glucose dose. Glycogen levels in muscle and liver were markedly reduced following exercise, indicating that the exercise regime was strenuous and created substrate depletion.

ACC lies downstream of AMPK and we previously reported that ACC phosphorylation correlated with AMPK activity in the hypothalamus in response to treatment with either leptin or

ghrelin [15]. In the current study, however, we did not observe a change in the phosphorylation state of ACC in the hypothalamus, consistent with the lack of effect of exercise on AMPK activity in this tissue. However, it is possible that different intensities or durations of exercise, or different periods of recovery, could result in changes in hypothalamic AMPK activity. Future studies will have to determine this.

Plasma ghrelin concentrations increased with exercise reaching significance after 60 min of running. In humans, exercise has been reported not to influence plasma ghrelin levels even at high exercise intensities [28-30]. We are unaware of other published data in which the plasma ghrelin response to exercise has been studied in rats. The reason for the increase of plasma ghrelin with exercise in the present study is not known but may indicate that the present exercise regime caused a larger depletion in substrate depots than in the human studies. In agreement with this assumption, plasma ghrelin concentrations only reached basal levels when recovery was accompanied by glucose feeding and partial restoration of liver and muscle glycogen stores. PYY is a gastrointestinal tract-derived hormone that is released post-prandially in proportion to the amount of calories ingested and leads to a reduction in food intake in rodents and man [26]. In contrast to the effect of exercise on plasma ghrelin concentration, the plasma concentration of PYY did not change during exercise but surprisingly decreased in recovery regardless of the feeding status of the rats. In humans the plasma concentration was also found not to change with exercise [31]. We have previously shown that injection of PYY, despite its actions on food intake, does not change AMPK activity in the rat hypothalamus [15].

We reported previously that intraperitoneal injection of ghrelin to rats activates AMPK in the hypothalamus [15]. Plasma ghrelin concentration increased during 1h treadmill running, and this may have been expected to increase hypothalamic AMPK activity. However, although significant, the increase in ghrelin following exercise was small and presumably not sufficient to activate AMPK.

In conclusion, although prolonged strenuous exercise in rats leads to increased plasma ghrelin concentrations it does not lead to changes in AMPK activity in the brain during or 1 hour after exercise regardless of the feeding status of the rats after exercise. Changes in appetite induced by exercise are thus unlikely to be due to alterations in hypothalamic AMPK activity and changes in energy metabolism during or after exercise are likely not coordinated by changes in hypothalamic AMPK activity.

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Figure Legends

Figure 1.

Plasma glucose (A) and insulin (B) concentrations during and after exercise. Results shown are the mean $\pm$ S.E.M of 10 animals per group. Statistically significant changes compared to control groups are denoted * ($p < 0.05$).

Figure 2. Hypothalamic AMPK activity in response to exercise.

Total AMPK activity (A) and AMPK α 2-specific activity (B) were determined in immune complexes isolated from 100 μ g lysate. Results are plotted as the percentage activity relative to the control group (non-exercised) and are the mean $\pm$ S.E.M of 10 animals per group

Figure 3. Acetyl-CoA carboxylase (ACC) phosphorylation was determined by Western blotting using a phospho-specific antibody. Blots were quantified and the results plotted as a percentage of the control group (A). Results shown are the mean $\pm$ S.E.M of 10 animals per group. A representative blot for each of the groups is shown in (B). Migration of the α and β isoforms of ACC is indicated.

Figure 4. Plasma levels of ghrelin (A) and PYY₃₋₃₆ (B) during and after exercise.

Results shown are the mean $\pm$ S.E.M of 10 animals per group. Statistically significant changes compared to control groups are denoted * ($p < 0.05$) and between 1h run and 1h run followed by 1h oral glucose administration by † ($p < 0.05$).

Tables.

	Control	0.5h run	1h run	1h run+ 1h fast	1h run+ 1h glucose
Gastroc. muscle (white)	38±3	22±2*	22±2*	22±3*	26±3*
Gastroc. muscle (red)	36±2	19±2*†	15±2*†	19±1*†	26±3*
Liver	91±13	53±9*	36±11*	25±4*†	60±6*

Table 1. Muscle and liver glycogen content ($\mu\text{mol/g}$ wet wt). Results are given as mean±S.E.M. of 10 animals per group. *Significantly from control ($p<0.05$). †Significantly different from 1h run+1h glucose.

Figure 1

Figure 2

Figure 3

Figure 4