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Cannabinoids and Ghrelin Have Both Central and Peripheral Metabolic and Cardiac Effects via AMP-activated Protein Kinase*

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Blerina Kola‡§¶, Erika Hubina‡, Sonia A. Tucci‖, Tim C. Kirkham‖, Edwin A. Garcia‡***, Sharon E. Mitchell‡‡, Lynda M. Williams‡‡, Simon A. Hawley§§¶¶, D. Grahame Hardie§§¶¶, Ashley B. Grossman‡, and Márta Korbonits‡‖‖**

From the ‡Department of Endocrinology, William Harvey Research Institute, Barts and the London Medical School, London EC1M 6BQ, United Kingdom, the §Department of Internal Medicine, University of Ancona, 60100 Ancona, Italy, the ‖Department of Psychology, University of Liverpool, Liverpool, L69 7ZA, United Kingdom, the ‡‡Rowett Research Institute, Aberdeen, AB21 9SB, United Kingdom, and the §§Division of Molecular Physiology, University of Dundee, Dundee DD1 5EH, United Kingdom

Endocannabinoids and ghrelin are potent appetite stimulators and are known to interact at a hypothalamic level. However, both also have important peripheral actions, including beneficial effects on the ischemic heart and increasing adipose tissue deposition, while ghrelin has direct effects on carbohydrate metabolism. The AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme that functions as a fuel sensor to regulate energy balance at both cellular and whole body levels, and it may mediate the action of anti-diabetic drugs such as metformin and peroxisome proliferator-activated receptor γ agonists. Here we show that both cannabinoids and ghrelin stimulate AMPK activity in the hypothalamus and the heart, while inhibiting AMPK in liver and adipose tissue. These novel effects of cannabinoids on AMPK provide a mechanism for a number of their known actions, such as the reduction in infarct size in the myocardium, an increase in adipose tissue, and stimulation of appetite. The beneficial effects of ghrelin on heart function, including reduction of myocyte apoptosis, and its effects on lipogenesis and carbohydrate metabolism, can also be explained by its ability to activate AMPK. Our data demonstrate that AMPK not only links the orexigenic effects of endocannabinoids and ghrelin in the hypothalamus but also their effects on the metabolism of peripheral tissues.

Endocannabinoids, acting via the presynaptic cannabinoid type 1 receptor (CB_1),[1] stimulate appetite in the hypothalamus (1), but direct peripheral effects have also been observed in animal studies implicating endocannabinoids in peripheral signaling of nutritional status and lipogenesis (2). The CB_1 antagonist rimonabant (SR141716) inhibits food intake, and phase III clinical trials have shown sustained effects on weight loss in humans through effects on glucose and fat metabolism (3–5). Ghrelin is a circulating brain-gut peptide with growth hormone-releasing and appetite-inducing effects, with predominant expression in the gastric mucosa but low level widespread expression throughout the body (6–8). Intracerebroventricular (i.c.v.) ghrelin treatment increases appetite and body weight, with a *direct* stimulatory effect on adipose tissue deposition demonstrated in both *in vivo* and *in vitro* studies (9, 10). It has been suggested that ghrelin favors preservation of lipid stores and the catabolism of carbohydrate-derived fuel, thereby increasing the respiratory quotient (11, 12). Ghrelin levels are high during fasting and in subjects with low body mass index, while low ghrelin levels are observed after food intake and in patients with insulin-resistant states such as type 2 diabetes, obesity, or polycystic ovarian syndrome (8). Both cannabinoids and ghrelin have been shown to have beneficial effects on the ischemic heart (13, 14). We have previously shown that ghrelin and the endocannabinoid system interact, as subanorectic doses of rimonabant can inhibit the orexigenic effect of ghrelin (15). Here we demonstrate a previously unrecognized interaction between cannabinoids and the AMPK system.

The AMPK complex is an $\alpha\beta\gamma$ heterotrimer that acts as a sensor of cellular energy status (16, 17). It is activated by any stress that depletes cellular ATP, with a concomitant rise in AMP, causing increased phosphorylation of the α subunit on Thr-172 by the upstream kinase, the tumor suppressor serine/threonine kinase LKB1 (18, 19). Both metformin and peroxisome proliferator-activated receptor γ agonists have been shown to activate AMPK, and it is suggested that this effect is important in the anti-diabetic action of these compounds. As well as being involved in regulating energy balance at the cellular level, AMPK regulates energy intake by mediating the opposing effects of ghrelin and leptin on appetite in the hypothalamus (20, 21) and also increases energy expenditure by mediating the effects of leptin (22) and adiponectin (23) to stimulate fatty acid oxidation in the periphery. We speculated that ghrelin and the endocannabinoids might exert both their central and peripheral effects via AMPK. Here we report a stimulatory effect of both cannabinoids and ghrelin on AMPK in the hypothalamus and in heart muscle, together with a contrasting inhibitory effect in liver and adipose tissue, but no detectable effect on skeletal muscle.

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‖‖ Supported by the UK Medical Research Council. To whom correspondence should be addressed: Dept. of Endocrinology, Rm. 114C, John Vane Science Centre, Barts and the London Medical School, Charterhouse Square, London EC1M 6BQ, UK. Tel.: 44-20-7882-6238; Fax: 44-20-7882-6197; E-mail: m.korbonits@qmul.ac.uk.

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[1] The abbreviations used are: CB_1 and CB_2, cannabinoid type 1 and type 2 receptors; i.c.v., intracerebroventricular; AMPK, AMP-activated protein kinase; THC, Δ^9-tetrahydrocannabinol; ACC, acetyl-CoA carboxylase; GHS-R, growth hormone secretagogue receptor; 2-AG, 2-arachidonoylglycerol; SAMS, substrate for AMP-activated protein kinase.

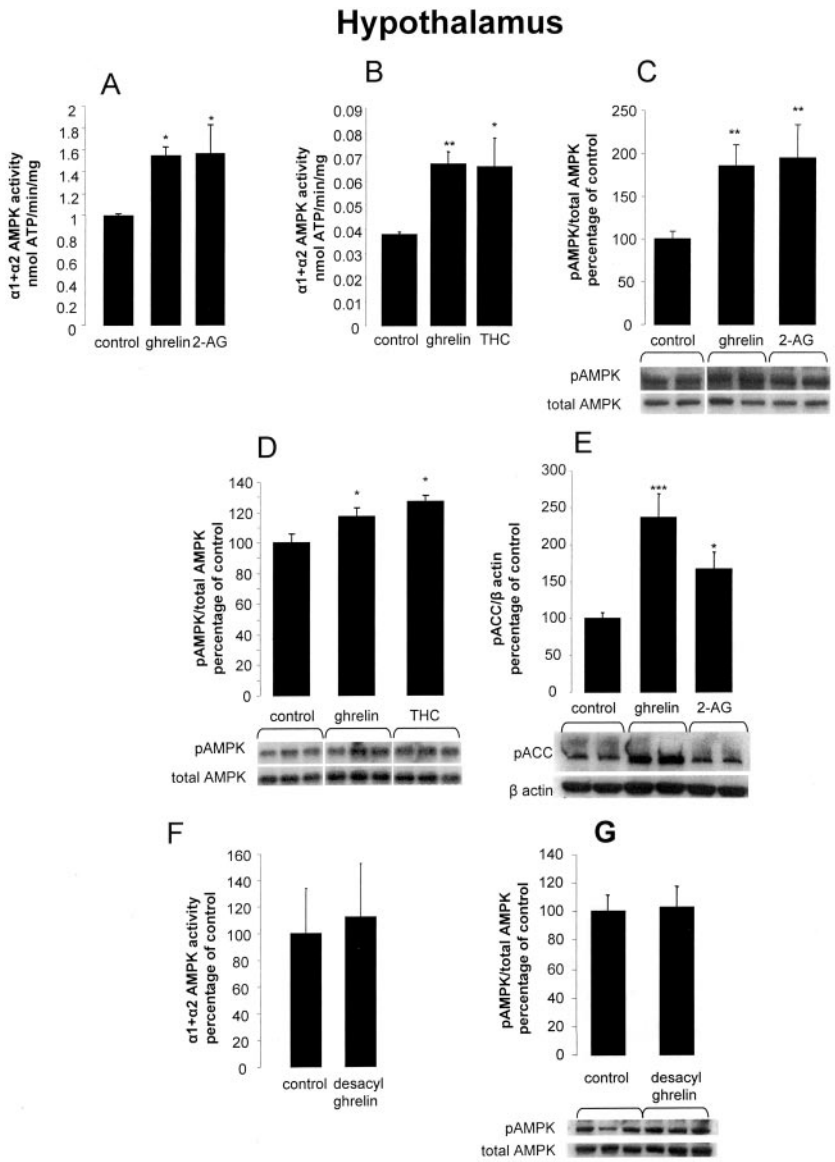

FIG. 1. **Ghrelin and cannabinoid effects on hypothalamus 1 h after i.c.v. or intraperitoneal administration.** All data are shown as mean ± S.E., $n = 6$ rats/group. *A*, i.c.v. ghrelin (1 μg) and 2-AG (50 μg) on hypothalamic AMPK activity compared with vehicle-treated animals; *, $p < 0.05$. *B*, intraperitoneal ghrelin (100 μg) and THC (2 mg/kg) on hypothalamic AMPK activity compared with vehicle-treated animals; *, $p < 0.05$; **, $p < 0.01$. *C*, i.c.v. ghrelin (1 μg) and 2-AG (50 μg) on hypothalamic Thr-172 phosphorylation of AMPK compared with vehicle-treated animals; **, $p < 0.01$. *D*, intraperitoneal ghrelin (100 μg) and THC (2 mg/kg) on hypothalamic pAMPK (mean ± S.E., $n = 6$ rats/group; *, $p < 0.05$ compared with control). *E*, i.c.v. ghrelin (1 μg) and 2-AG (50 μg) on hypothalamic phosphorylation of ACC compared with vehicle-treated animals; *, $p < 0.05$; ***, $p < 0.001$. *F*, i.c.v. desacyl ghrelin (5 μg) on hypothalamic AMPK activity compared with vehicle-treated animals. *G*, i.c.v. desacyl ghrelin (5 μg) on hypothalamic pAMPK (mean ± S.E., $n = 6$ rats/group)

MATERIALS AND METHODS

Experimental Setup—Male Lister hooded rats (Harlan, Blackthorn, UK) weighing between 220 and 250 g were used; each treatment group consisted of six rats. For i.c.v. treatment the lateral ventricle was cannulated, and the test substances or vehicle was injected in a volume of 5 μl/rat. Animals were killed 1 h later, the brain was dissected, and the hypothalamus was removed as a 2-mm thick coronal section from −2 to −4 from Bregma and from the supraoptic decussation to the dorsal end of the lower part of the third ventricle. Tissue samples were frozen in liquid nitrogen and stored at −80 °C. For the i.c.v. studies rat ghrelin (Tocris Cookson Ltd., Avonmouth, UK) was injected at a dose of 1 μg/rat, desacyl ghrelin at a dose of 5 μg/rat (a kind gift from M. Kojima, Kurume, Japan), and 2-arachidonoylglycerol (2-AG) at a dose of 5 μg/rat (Tocris), which was shown previously to increase food intake in rats (24). For intraperitoneal injection, doses of 100 μg/rat ghrelin and 2 mg/kg Δ^9-tetrahydrocannabinol (THC; Tocris) were used, in a volume of 1 ml/kg. Ghrelin and cannabinoid doses were chosen for their established effectiveness in earlier studies to promote eating (24–26). Whole heart, liver, subcutaneous and visceral adipose tissue, skeletal muscle, and whole brain or hypothalamus were immediately removed and placed in liquid nitrogen and stored at −80 °C. All injections were performed between 09.00 and 10.00. The experimental procedures carried out in this study were in compliance with the UK Animals (Scientific Procedures) Act 1986.

Immunoprecipitate Kinase Assays for AMPK and Immunoblotting—Immunoprecipitate kinase assays for AMPK, and analysis of Western blots using anti-peptide antibodies, have been described previously (19). Briefly, tissue samples were homogenized and the protein content was determined (BCA assay, Pierce). AMPK activity in the immune complex was determined by phosphorylation of SAMS, a synthetic peptide substrate of AMPK. 300 μg of protein was immunoprecipitated using protein G beads (Amersham Biosciences, Bucks, UK) and a mixture of α1 and α2 AMPK antibodies (2.5 μg/sample of each) (19). The immunoprecipitate was divided into three aliquots, and two were assayed for AMPK activity with a reaction solution containing 0.1 μCi of [γ-^{32}P]ATP, 0.1 μl of 100 mM cold ATP, 0.25 μl of 1 M $MgCl_2$, 10 μl of 1 mM AMP, and 10 μl of SAMS 0.1 mM (Upsate Biotechnology, Dundee, Scotland) and the third aliquot with the same mixture except with Hepes-Brij buffer replaced by SAMS. The reaction was performed on a shaker for 20 min at 30 °C, and samples were pipetted onto paper squares (P81; Upstate Biotechnology). The reaction was stopped by placing the paper squares into 1% phosphoric acid, and after repeated rinsing the activity was counted using a scintillation counter. For separate α1 and α2 AMPK activity determination, 600 μg of protein was used and immunoprecipitation was performed sequentially for α1 and α2 AMPK. AMPK activity was calculated using the difference of the counts between SAMS containing and SAMS negative samples and expressed as nanomoles of ATP incorporated per minute per milligram of sample peptide. Western blotting was performed running 10–20 μg of protein on a 7.5 or 10% SDS-gel (Bio-Rad, Hemel Hempstead, UK) and transferring it to a polyvinylidene difluoride membrane (Immobilon-P; pore size 0.45 μm; Millipore UK Ltd., Watford, UK). Membranes were incubated with the primary antibody anti-phosphorylated AMPK (pAMPK) antibody (which recognizes the AMPK pan-α subunit phosphorylated at Thr-172), at a concentration of 1:1000 (19) and with anti-phospho acetyl-CoA carboxylase (ACC) antibody (1:1000, Upstate

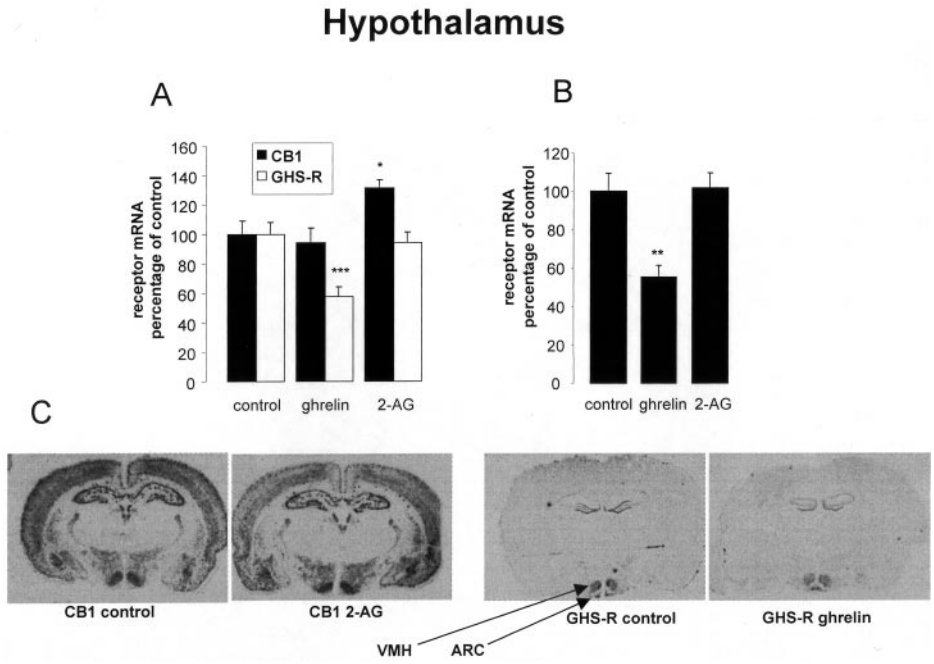

FIG. 2. *In situ* hybridization for CB_1 and GHS-R mRNA expression in the ventromedial nucleus (*A*) and in the arcuate nucleus (*B*) of rats treated with i.c.v. ghrelin (1 μg) and 2-AG (50 μg). ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. Data shown are mean ± S.E., $n = 6$ rats/group. *C*, the *left two panels* show CB_1 receptor mRNA expression with *in situ* hybridization. The ventromedial nucleus (*VMH*) shows up-regulation after i.c.v. endogenous cannabinoid 2-AG (50 μg) treatment compared with vehicle-treated controls. Ghrelin had no effect on CB_1 expression (data not shown). The *right two panels* show GHS-R mRNA expression. Both the ventromedial and arcuate nuclei (*ARC*) show down-regulation after i.c.v. ghrelin (1 μg) treatment compared with vehicle-treated controls. 2-AG had no effect on GHS-R expression (data not shown).

Biotechnology) in 5% nonfat dry milk in Tris-buffered saline-Tween overnight at 4 °C. To achieve cleaner bands, membranes for pAMPK were preincubated with unphosphorylated PT172 protein (27) for 60 min before incubation with the pAMPK antibody. Donkey anti-sheep IgG horseradish peroxidase, 1:2000 (Santa Cruz Biotechnology) was used for secondary antibody. After stripping the membrane total AMPK (mixture of primary antibody α1 and α2 AMPK at 0.2 μg/ml for total AMPK (19), secondary donkey anti-sheep IgG horseradish peroxidase antibody, 1:2000 (Santa Cruz Biotechnology)) glyceraldehyde-3-phosphate dehydrogenase (primary antibody 1:2000 (Santa Cruz Biotechnology), secondary goat anti-rabbit antibody 1:10,000 (Dako, Glostrup, Denmark)) or β-actin (primary monoclonal antibody 1:5000 (Abcam, Cambridge, UK), secondary goat anti-mouse antibody 1:10,000 (Dako)) antibodies were used to normalize for equal loading, and densitometric readings of the resulting bands were evaluated using the Scientific Imaging system (Eastman Kodak Co.).

In Situ Hybridization—Quantitative *in situ* hybridization for the growth hormone secretagogue receptor (GHS-R) was performed as described (28). A CB_1 cDNA fragment was cloned from mouse hypothalamic cDNA. The 422-bp fragment of CB_1 was amplified using primers 5′-TGGGCAGCCTGTTCCTCACG-3′ and 5′-GGGTTTTGGCCAGCCTAATGTCC-3′ (GenBank accession number NM_007726) with 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. DNA fragments were ligated into PGEM-T cloning vector (Promega, Southampten, UK), and transformed into JM 109 cells (Promega), and automated sequencing was performed to verify the sequence.

Statistical Analysis—Data were analyzed using the Kruskal-Wallis test followed by Conover-Inman comparison. Significance was taken at $p < 0.05$. The data are expressed as means ± S.E., $n = 6$ in each treatment group.

RESULTS

As both cannabinoids and ghrelin have central orexigenic effects, we first studied the hypothalamus. While leptin, which is associated with appetite suppression, inhibits α2 AMPK activity in the arcuate and paraventricular nucleus of the hypothalamus (20), ghrelin has been shown to stimulate whole hypothalamic AMPK activity after peripheral administration (21). In the current study using a functional AMPK assay we observed that in whole hypothalamus total AMPK activity increased to 153 ± 8% of control after central 2-AG injection and to 156 ± 26% after i.c.v. ghrelin injection (Fig. 1*A*). Similar responses were also seen after peripheral injection of THC (174 ± 31% of control) and ghrelin (177 ± 12%, Fig. 1*B*). This increase in AMPK activity was, as expected, associated with an increase in Thr-172 phosphorylation of AMPK (Fig. 1, *C* and *D*), while total AMPK levels did not change in either of the tissues studied. One of the best established downstream targets of AMPK (and therefore a good marker for AMPK activation) is ACC. Phosphorylation by AMPK at the equivalent sites on the two isoforms ACC1 and ACC2 causes inhibition of fatty acid synthesis and stimulation of fatty acid oxidation, respectively (17). Using an antibody that detects phosphorylation of both isoforms, we detected an increase in phosphorylation of ACC after central cannabinoid and ghrelin treatment in the hypothalamus (Fig. 1*E*). We and others have described important peripheral effects of desacyl ghrelin (see Ref. 8 and references therein), although this form cannot activate the full-length, functionally active GHS-R1a receptor. In this study no change was observed in hypothalamic AMPK activity (Fig. 1*F*) or AMPK phosphorylation (Fig. 1*G*) after i.c.v. administration of desacyl ghrelin.

Intracerebroventricular 2-AG treatment stimulated CB_1 mRNA expression in the ventromedial nucleus to 132 ± 5% of control, while CB_1 was not detected in the arcuate nucleus. Ghrelin inhibited GHS-R expression to 55 ± 6% and 58 ± 6% of the control in the ventromedial and arcuate nuclei (Fig. 2, *A–C*). These nuclei were included in the whole hypothalamic tissue samples assayed for AMPK activity and pAMPK content.

Cannabinoids and ghrelin markedly stimulated AMPK in the heart: total AMPK activity was increased to 388 ± 94% and 273 ± 17% of control, respectively (Fig. 3*A*). Specific assays for the α1 and α2 isoforms of AMPK showed similar activities at base line, although the increase after THC and ghrelin treatment was greater for α2 (Fig. 3*B*), which could be explained by the greater sensitivity of α2 AMPK to AMP (29). Immunoblotting showed an increase in phosphorylation of the α subunit to 372 ± 25% of control in response to THC and to 373 ± 40% after ghrelin treatment (Fig. 3*C*). In contrast to the hypothalamus and myocardium, in liver tissue we found strong inhibitory effects of THC (62 ± 11% of control) and ghrelin (38 ± 7%) on AMPK activity (Fig. 4*A*) and on phosphorylation of Thr-172 of AMPK (74 ± 12% and 55 ± 16%, respectively; Fig. 4*B*). We also observed an inhibitory effect of endocannabinoids (75 ± 6% of control) and ghrelin (72 ± 6%) on combined subcutaneous and visceral adipose tissue AMPK activity ($p < 0.05$). While the AMPK activity response was similar in the two adipose tissue

types (Fig. 5*A*), more marked decrease was observed in visceral adipose tissue pAMPK content compared with subcutaneous adipose tissue: phosphorylation of AMPK at Thr-172 was 72 ± 10% of control in response to THC and 70 ± 8% in response to ghrelin in visceral adipose tissue and 108 ± 7% and 123 ± 23% of control in subcutaneous adipose tissue, respectively (Fig. 5*B*). In visceral adipose tissue the relative contribution of the α1 isoenzyme was more pronounced than that of the α2 isoenzyme (α1 THC 89 ± 11% of control, ghrelin 79 ± 9% $p < 0.05$; α2 THC 91 ± 11% of control, ghrelin 102 ± 9%, not significant) in accordance with previous data (30). No effects of cannabinoids or ghrelin were observed on skeletal muscle AMPK, either on activity or phosphorylation of Thr-172 (Fig. 6, *A* and *B*).

DISCUSSION

Cannabinoids were found to stimulate AMPK activity in the hypothalamus after both central and peripheral administration. We propose that this effect of cannabinoids on AMPK represents an important pathway which mediates their orexigenic effect. As both ghrelin and endocannabinoids are synthesized in peripheral tissues (7, 31), we suggest that the effects we have observed after peripheral administration are physiologically important. The role of AMPK in appetite regulation has also been demonstrated using the AMPK activator 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside, the fatty acid synthase inhibitor C75 (32), and most recently, glucose (33). We predict that other appetite-modulating agents will also have effects on hypothalamic AMPK.

There is mounting evidence that endocannabinoids may play important roles in limiting damage to the heart and circulation in shock and in myocardial infarction (34, 35). Cannabinoids reduce infarct size associated with ischemia/reperfusion in rat isolated hearts, and the endogenous release of cannabinoids has been implicated in survival after coronary artery occlusion in rats via the CB_1 receptor (14, 36). AMPK is activated by ischemia in the heart, leading to increased glucose uptake and phosphorylation of the heart-specific 6-phosphofructo-2-kinase, which activates production of ATP by glycolysis under anaerobic conditions. Activation of AMPK during ischemia (37) also lowers malonyl-CoA and thus increases ATP generation via fatty acid oxidation during reperfusion (38). Recent results using mice expressing a dominant negative AMPK mutant in the heart suggest that the presence of AMPK protects cardiac ATP levels and reduces infarct size and damage to myocytes during ischemia (34). The lack of fat tissue cytokine adiponectin (known to stimulate AMPK activity) results in pressure overload and cardiac hypertrophy in "knock-out" animals, and this could be reversed by the reintroduction of adiponectin (39). Recent data suggest that long term cannabinoid treatment improves atherosclerosis via a CB_2-mediated effect on immune cells (40). Our present data suggest that the beneficial effects of cannabinoids could be mediated via activation of AMPK, although excessive activation of AMPK may be deleterious to the heart (38, 41). We also found a large increase in the phosphorylation and activity of AMPK activity in response to ghrelin. There have been several previous studies describing the beneficial effects of ghrelin and its synthetic analogues on cardiovascular function (8, 13, 42). These seem to be direct effects that are independent of growth hormone release, as positive results were obtained both in hypophysectomized rats and in *in vitro* studies on embryonic (H9c2) and adult (HL-1) heart muscle cell lines (43, 44). Ghrelin and GHS-R mRNA are present in human myocardium (7), and protein expression has also been detected both in primary tissue and in the HL-1 cell line, where ghrelin has anti-apoptotic effects (44). Diastolic dysfunction associated with myocardial stunning is improved with ghrelin analogue treatment (45). Human studies have shown that ghrelin increases stroke volume both in healthy volunteers and in chronic heart failure, while chronic administration of ghrelin improves left ventricular dysfunction and attenuates the development of cardiac cachexia in rats with heart failure (46). As elevated ghrelin levels have been reported in patients with cardiac cachexia, this could be part of a compensatory mechanism in response to catabolic-anabolic imbalance (44). In patients with obesity (or other insulin-resistant states that are associated with low ghrelin levels, such as type 2 diabetes and polycystic ovarian syndrome) the low levels of ghrelin could contribute to heart failure, where cardiomyocyte apoptosis is known to play a role (47). In contrast, the beneficial effects of weight loss on cardiac function may, at least in part, be the result of the beneficial effects of increased ghrelin levels.

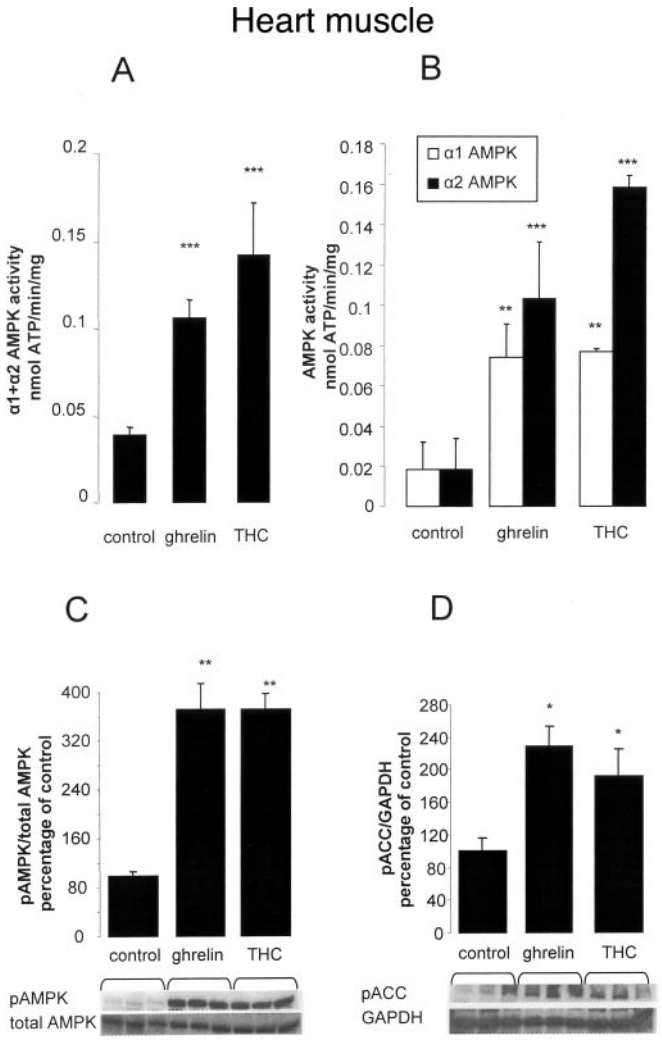

FIG. 3. **Ghrelin (100 μg, intraperitoneal) and THC (2 mg/kg, intraperitoneal) stimulates AMPK activity and AMPK phosphorylation in heart muscle (mean ± S.E., n = 6 rats/group).** *A*, ghrelin and THC activates α1 + α2 AMPK 1 h after injection; ***, $p < 0.001$. *B*, ghrelin and THC activates α1 and α2 AMPK 1 h after intraperitoneal injection; ***, $p < 0.001$; **, $p < 0.01$. *C*, myocardial Thr-172 phosphorylation of AMPK compared with vehicle-treated animals; **, $p < 0.01$. *D*, ghrelin and THC increases the phosphorylation of ACC in the heart compared with vehicle-treated animals; *, $p < 0.05$.

The effects of cannabinoids on liver metabolism have been little studied in the past. In one study, anandamide (but not THC) caused inhibition of ACC and fatty acid synthesis, similar to the effects of AMPK activation (48). However, in that case the authors concluded that this inhibition was not mediated via the CB_1 receptor but via arachidonic acid, a product of anandamide breakdown. However, $CB1^{-/-}$ animals show a lean phe-

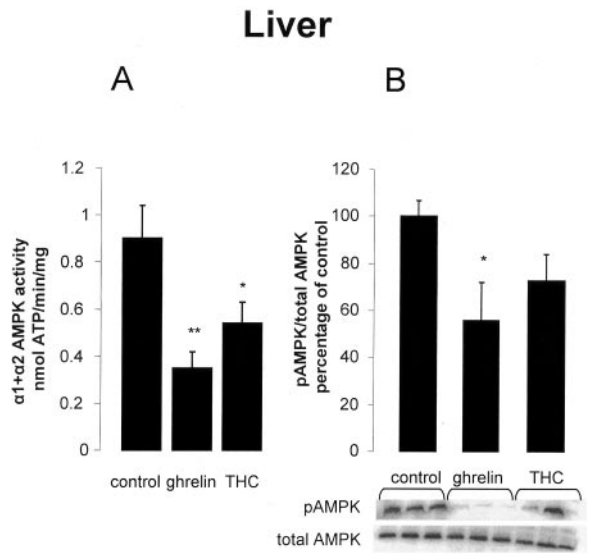

FIG. 4. **Ghrelin (100 μg, intraperitoneal) and THC (2 mg/kg, intraperitoneal) inhibited liver AMPK activity and Thr-172 phosphorylation of AMPK (mean ± S.E., *n* = 6 rats/group).** *A*, α1 + α2 AMPK activity 1 h after intraperitoneal injection; *, $p < 0.05$; **, $p < 0.01$. *B*, liver Thr-172 phosphorylation of AMPK compared with vehicle-treated animals; *, $p < 0.05$.

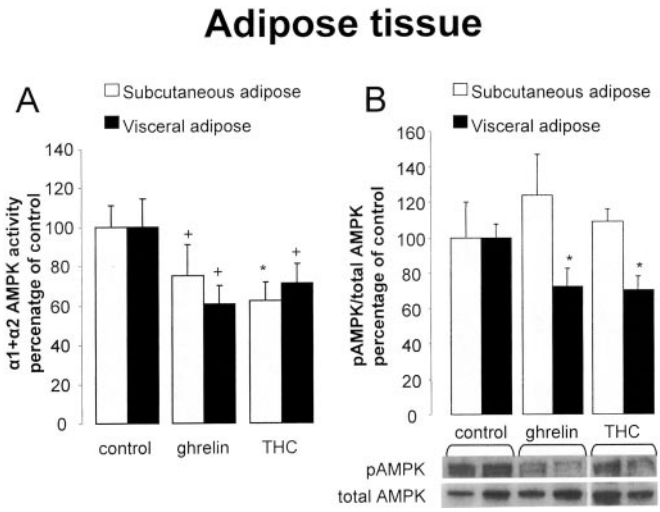

FIG. 5. **Ghrelin (100 μg, intraperitoneal) and THC (2 mg/kg, intraperitoneal) inhibited adipose tissue AMPK activity and Thr-172 phosphorylation of AMPK (mean ± S.E., *n* = 6 rats/group).** *A*, α1 + α2 AMPK activity 1 h after injection on subcutaneous and visceral adipose tissue; *, $p < 0.05$; +, trend [$p < 0.1$]. *B*, adipose tissue Thr-172 phosphorylation of AMPK compared with vehicle-treated animals; *, $p < 0.05$. The *lower panel* shows immunoblotting data from visceral adipose tissue.

notype that is not entirely due to decreased appetite and reduced food-intake, as $CB1^{-/-}$ animals still have a lower body weight than pair-fed wild-type littermates (2). An unknown peripheral mechanism was suggested to account for this effect (2). We now propose that the inhibitory effect of cannabinoids on AMPK activity in the liver could constitute this postulated mechanism. Our proposal is supported indirectly by a recent paper suggesting that ACC is a target of CB_1 activation in the liver (49). Ghrelin opposes the effect of insulin on the expression of the rate-limiting enzyme of gluconeogenesis, phosphoenolpyruvate carboxykinase, therefore up-regulating gluconeogenesis in a human hepatoma cell line (50), while AMPK activation stimulates fatty acid oxidation in rat hepatocytes, down-regulates phosphoenolpyruvate carboxykinase expression in human hepatoma cells, and inhibits glucose production in mouse liver *in vivo*. We therefore propose that the effect of ghrelin on gluconeogenesis could occur via the inhibition of AMPK activity. Indeed, 4-day ghrelin treatment has recently been reported to reduce the signal obtained by a phospho-specific antibody against Thr-172 on AMPK in rat liver, although the effects on kinase activity were not assessed (51).

AMPK activation has been shown to inhibit lipogenesis and stimulate lipid oxidation and to up-regulate adiponectin expression and down-regulate tumor necrosis factor α and interleukin 6 expression in adipose tissue (30). Thus, the inhibition of AMPK would lead to increased lipid stores, a phenomenon that has been described previously for both ghrelin and cannabinoids. Cannabinoid CB_1 receptors are expressed in adipose tissue and the CB_1 antagonist SR141716 (rimonabant) stimulates adiponectin synthesis, which in turn stimulates AMPK activity (52), so this is concordant with our results of inhibition of AMPK activity by a CB_1 agonist. Furthermore, adipose tissue is able to store lipophylic cannabinoid substances, and monoacylglycerol lipase, one of the main endocannabinoid-degrading enzymes, is expressed in fat tissue (2). A recent study showed that administration of rimonabant led to a marked and sustained reduction of adiposity in diet-induced obese mice, which could not be explained fully by the transient reduction in food intake caused by this drug (3). Ghrelin selectively increases adipose tissue *in vivo* (11), while isoproterenol-stimulated lipolysis was shown to be significantly reduced by simultaneous ghrelin treatment in a dose-dependent manner *in vitro*. Ghrelin (as well as desacyl ghrelin) infused in the bone marrow specifically stimulates bone marrow fat cell proliferation (10). These data suggest that ghrelin may play an important role in the process of adipogenesis and storage of energy. This is interesting because, while low levels of circulating ghrelin are typical in obesity and other insulin-resistant states, visceral adipose tissue is more sensitive to even these low levels compared with subcutaneous adipose tissue. Thus, circulating ghrelin would still be expected to promote lipid deposition preferentially in the visceral fat depots.

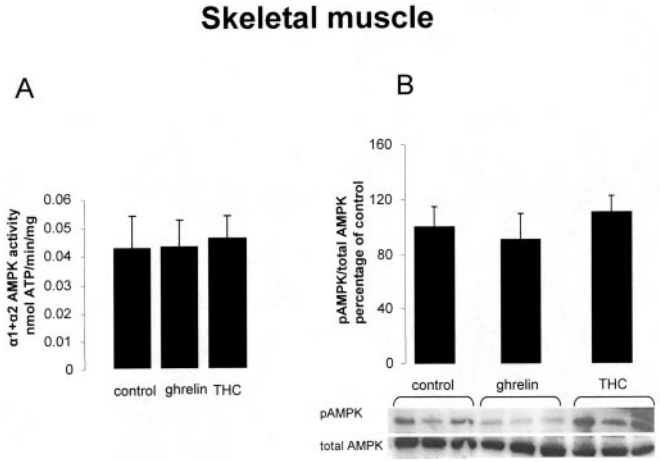

FIG. 6. Intraperitoneal **ghrelin (100 μg) and THC (2 mg/kg) on skeletal muscle AMPK activity (*A*) and Thr-172 phosphorylation of AMPK (*B*) compared with vehicle-treated animals.** Data are shown as mean ± S.E.; no significance is shown with Kruskal-Wallis test, $n = 6$ rats/group.

CB_1, CB_2, and GHS-R1a receptors are expressed in the hypothalamus, heart, and adipose tissue, so in these tissues the classical receptors may mediate the effects of their cognate ligands, although some of the reported cardiovascular effects of cannabinoids are mediated by non-CB_1, non-CB_2 receptors (53). Previous data found no change in CB_1 binding in the hypothalamus after acute or chronic stimulation, while other

brain areas show variable down- and up-regulation after acute and chronic CB_1 agonist THC administration (54). The down-regulation of GHS-R1a is in accordance with earlier desensitization studies and also corresponds with *in vivo* data following repeated GHS-R agonist administration (55–57); however, up-regulation of hypothalamic GHS-R has also been described after ghrelin administration (28). Desacyl ghrelin, which does not activate GHS-R1a, has similar adipogenic effects to ghrelin on adipose cells, suggesting the involvement of an alternative receptor (10). Very recently, CB_1 has been detected on hepatocytes and suggested to be involved in the metabolic effects of cannabinoids (49), while the presence of GHS-R1a on hepatocytes is questionable (7, 58). Thus, both classical and alternative receptor(s) for cannabinoids and ghrelin might be involved in some of the effects we describe.

We have shown that both cannabinoids and ghrelin stimulate AMPK activity in the hypothalamus and the heart and inhibit AMPK activity in the liver and adipose tissue, while we found no effect on skeletal muscle. Given the proposed role of AMPK in energy sensing and metabolism, the present findings provide important evidence of interactions between this enzyme and the orexigenic actions of cannabinoids and ghrelin. Either class of agent could potentially increase appetite by central AMPK stimulation or by facilitating the restorative actions of AMPK as the hypothalamus senses fuel deprivation. By contrast, peripheral inhibition of AMPK by cannabinoids and ghrelin may lead to fuel, particularly fat, storage. The combined effect of both central and peripheral signals would therefore be increased food intake and lipid storage, leading to lipid deposition. The cardiac and metabolic effects of cannabinoids we report may have important implications for the anticipated widespread clinical use of rimonabant and other CB_1 antagonists in the treatment of obesity. We suggest that AMPK-mediated actions may be important for the metabolic influences of cannabinoid and ghrelin agonists and antagonists and provide a possible explanation for some of the previously unexplained effects of these compounds.

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