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Evidence that α-Calcitonin Gene-Related Peptide Is a Neurohormone that Controls Systemic Lipid Availability and Utilization

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α-Calcitonin gene-related peptide (αCGRP) is released mainly from sensory and motor nerves in response to physiological stimuli. Despite well-documented pharmacological effects, its primary physiological role has thus far remained obscure. Increased lipid content, particularly in skeletal muscle and liver, is strongly implicated in the pathogenesis of insulin resistance, but the physiological regulation of organ lipid is imperfectly understood. Here we report our systematic investigations of the effects of αCGRP on *in vitro* and *in vivo* indices of lipid metabolism. In rodents, levels of αCGRP similar to those in the blood markedly stimulated fatty acid β-oxidation and evoked concomitant mobilization of muscle lipid via receptor-mediated activation of muscle lipolysis. αCGRP exerted potent *in vivo* effects on lipid metabolism in muscle, liver, and the blood via receptor-mediated pathways. Studies with receptor antagonists were consistent with tonic regulation of lipid metabolism by an endogenous CGRP agonist. These data reveal that αCGRP is a newly recognized regulator of lipid availability and utilization in key tissues and that it may elevate the availability of intramyocellular free fatty acids to meet muscle energy requirements generated by contraction by evoking their release from endogenous triglyceride. (*Endocrinology* 149: 154–160, 2008)

THE WORLDWIDE PREVALENCE of type 2 diabetes is projected to rise to 300 million by 2025, with the majority of this increase likely to occur in the developing world (1). Central to the pathophysiology of the disease are insulin resistance in the liver, skeletal muscle, and adipose tissue and impaired insulin secretion from the β-cell. Insulin resistance is the strongest predictor of type 2 diabetes, and historically its development has been attributed to altered regulation of carbohydrate metabolism. There is growing evidence that disordered lipid metabolism is fundamental to the pathology of this condition (2). Skeletal muscle plays a critical role in the maintenance of whole-body energy balance. It can normally use both lipid and carbohydrate as fuels and switches readily between the two; however, this switching becomes impaired in insulin-resistant states, apparently in relation to pathological accumulation of intramyocellular triglyceride (TG) (3, 4). There is, therefore, significant interest in biological processes that stimulate intramyocellular TG breakdown and fatty acid oxidation.

The two calcitonin gene-related peptides, α-calcitonin gene-related peptide (CGRP) and βCGRP, and amylin are closely related 37-amino acid peptides. αCGRP, the more widely distributed of the two CGRPs, has pleiotropic biological activities in processes that mediate vasodilatation, nociception, and cardioprotection (5–7) and various metabolic effects such as modulation of insulin sensitivity in skeletal muscle and liver (8). It has thus been postulated as a regulator of the circulation and a neurotransmitter. Pharmacological αCGRP doses evoke insulin resistance in rodents, mainly through effects in muscle and liver (8–11). However, mice with specific deletion of αCGRP displayed no evident phenotypic abnormalities (12), and the primary physiological role of CGRP is still uncertain.

αCGRP is coreleased into the neuromuscular junction with the neurotransmitter acetylcholine in response to physiological stimuli (13) and is structurally similar to amylin, an endocrine metabolic regulator (6). We hypothesized that it might play a role in energy use and to investigate this possibility studied its effects on lipid metabolism. The data demonstrate that αCGRP increases fatty acid availability and use in key tissues including skeletal muscle, perhaps to meet the energy requirements generated by contraction.

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Abbreviations: AICAR, 5-Aminoimidazole-4-carboxamide 1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; CGRP, calcitonin gene-related peptide; FFA, free fatty acid; KHB, Krebs-Henseleit buffer; RAMP, receptor activity-modifying protein; TG, triglyceride.

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Materials and Methods

Animals

Male Wistar rats, weighing 200–250 g, were housed in a constant 12-h light, 12-h dark cycle and received standard rat chow or an in-house-derived high-fat diet [standard rat chow to which 30% (wt/wt) of lard had been added] and water *ad libitum*. All protocols were approved by the University of Auckland Animal Ethics Committee.

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Peptides

Rat amylin, rat αCGRP, rat amylin-(8–37), and human βCGRP and CGRP-(8–37) were all purchased from Bachem (Bubendorf, Switzerland). Full-length mouse adiponectin was produced by recombinant expression and characterized according to our previously published methods (14).

In vitro experiments

Animals were anesthetized using halothane; soleus muscles of both legs were excised and the tissue immersed in Krebs-Henseleit buffer (KHB) that was gassed continuously with 95% O_2-5% CO_2. Muscles were separated into two equal parts and rested for 20 min. They were then incubated in the various treatments (concentrations as indicated) for stated times. For acetylcholine experiments, incubation concentrations were 100 μM and 100 nM. Muscles were frozen in liquid nitrogen and stored for later analysis. For β-oxidation pulse-chase studies, the muscles were incubated in KHB containing 4% fatty acid-free BSA (ICP Bio, Auckland, New Zealand), 2 mM pyruvate, and 0.5 mM palmitate with 2 μCi [9,10-^{3}H] palmitate (GE Healthcare, Buckinghamshire, UK) and incubations performed as described (15), with minor modifications. In the short incubation study, muscles were stimulated with 1 μM αCGRP for 15 min, washed, and placed into fresh CGRP-free buffer, and fatty acid oxidation was then assessed at 60 min. Sixty-minute treated and control muscles were similarly treated but replaced into CGRP containing or KHB buffer as appropriate.

Lipid analysis

Total lipids were extracted from organs and tissues using a modified version of the method of Bligh and Dyer as described (16). Tissue TG levels were quantified using a GPO reagent set (Pointe Scientific, Canton, MI). Tissue free fatty acids (FFAs) were separated using bonded phase columns as described (17) and quantified using a colorimetric method (Boehringer, Ingelheim, Germany) adapted for microanalyses. For determination of lipid content by gas chromatography/mass spectrometry, separated lipids were processed for derivatization of methyl esters by the boron trifluoride-methanol method (18) and detected using a gas chromatograph/mass spectrometer (Hewlett Packard, Portland, OR).

cAMP analysis

Muscle strips were incubated for 10 min with peptide as indicated and then freeze dried and homogenized in 0.5 M $HClO_4$, heated, centrifuged, and the supernatant removed. The supernatant was then neutralized with NH_4OH, the samples refrigerated for 2 h, and centrifuged; finally cAMP content was determined using a commercial radioreceptor assay (Amersham, Buckinghamshire, UK), and values are expressed relative to dry tissue weight.

Western blot analysis of AMP-activated protein kinase (AMPK)

Phosphorylation and protein levels of AMPK were determined using anti-p-AMPK and anti-αAMPK antibodies (Cell Signaling, Beverly, MA) with detection via Qdot 605 Goat F(ab')2 antirabbit IgG conjugate (Invitrogen, Carlsbad, CA) per the manufacturer's instructions. Bands were quantified by Gel Doc XR (Bio-Rad, Hercules, CA) and Quantity One software using glyceraldehyde-3-phosphate dehydrogenase (Abcam, Cambridge, UK) as a loading control.

In vivo studies

Male Wistar rats were randomly assigned to experimental groups. Induction and maintenance of surgical anesthesia were by 3–5% halothane and 2 liters/min oxygen. The trachea was cannulated and the animal ventilated at 60–70 breaths/min with air supplemented with O_2. The respiratory rate and end-tidal pressure (10–15 cmH_2O) were adjusted to maintain end-tidal CO_2 at 35–40 mm Hg. Body temperature was maintained at 37 C throughout by a heating pad. The carotid artery and jugular vein were cannulated with saline-filled PE 50 catheters. Fluids (saline or peptide dissolved in saline) were delivered to the jugular vein. Infusion of saline or CGRP antagonist, as appropriate, was commenced 30 min before the start of αCGRP or saline infusion. Blood samples were taken at time zero (baseline reading), 30 min, and thereafter every 20 min until the end of the experiment at 90 min. The carotid artery line was connected to a blood pressure transducer. After 90 min a terminal blood sample was taken, and organs and tissues were removed and frozen until further analysis.

Plasma metabolites and hormones

Lactate and blood gas parameters were measured in heparinized whole blood using an arterial blood gas analyzer. The remaining blood was centrifuged for 15 min, 10 C at 3000 rpm, and the serum stored at −80 C. TG and FFAs were measured in serum as described above. Insulin, amylin, and αCGRP (Bachem) were analyzed with specific RIAs and catecholamines measured using an HPLC-based method (Endolab, Christchurch, New Zealand).

Calculations and statistical analysis

All results are presented as mean ± SEM except for EC_{50} values, which are presented as means (95% confidence intervals). Nonlinear curves were fitted according to a four-parameter logistic fit using Prism GraphPad 3.0 (GraphPad, San Diego, CA). The quantity of palmitate oxidized (nanomoles) was calculated from the specific activity of the incubation medium [radiolabeled palmitate (disintegrations per minute)/total palmitate (nanomoles)]. Data were analyzed by Student *t* tests or one-way ANOVA followed by Tukey's *post hoc* test, as appropriate. Differences whose corresponding *P* values were less than 0.05 were considered statistically significant.

Results and Discussion

Exogenous αCGRP dose-dependently elevates muscle FFA content

We first measured the effects of exogenous αCGRP on muscle FFA content after 1 h incubation of isolated rat soleus muscle strips. This revealed that αCGRP evoked a biphasic, concentration-dependent increase in muscle FFA content (Fig. 1A), for which the half-maximal response of the high-potency component was in the subpicomolar range, with an EC_{50} of 0.25 pM (0.13–0.50; mean ± 95% confidence interval). FFA content in skeletal muscle was also analyzed by gas liquid chromatography, with identification of individual FFAs by mass spectrometry (data not shown). We thereby verified that the FFA pool was accurately measured using our enzymatic method and that the quantities reported here are consistent with other literature reports (16, 19). Additionally, we incubated soleus muscle with physiological and supraphysiological acetylcholine concentrations and determined that this coreleased neurotransmitter did not itself evoke any changes in lipid content (data not shown). The calcitonin receptor-like-receptor/receptor activity-modifying protein (RAMP)-1 receptor complex shows the highest affinity to αCGRP of all the receptor combinations known. However, the binding characteristics for this receptor combination lie within the 0.5–1 nM range (20, 21), suggesting that the responses observed in our study are the result of a novel receptor complex.

By contrast, the half-maximal response for αCGRP (Fig. 1A) in the low-potency component of the biphasic curve was in the low nanomolar range [EC_{50} = 42 nM (8.5–90)], similar to EC_{50} values derived for βCGRP, (Fig. 1B) [EC_{50} = 4.8 nM (2.1–11.1)] and amylin (Fig. 1C) (EC_{50} = 7.8 nM (4.1–14.7)]. These similar concentration dependencies are consistent

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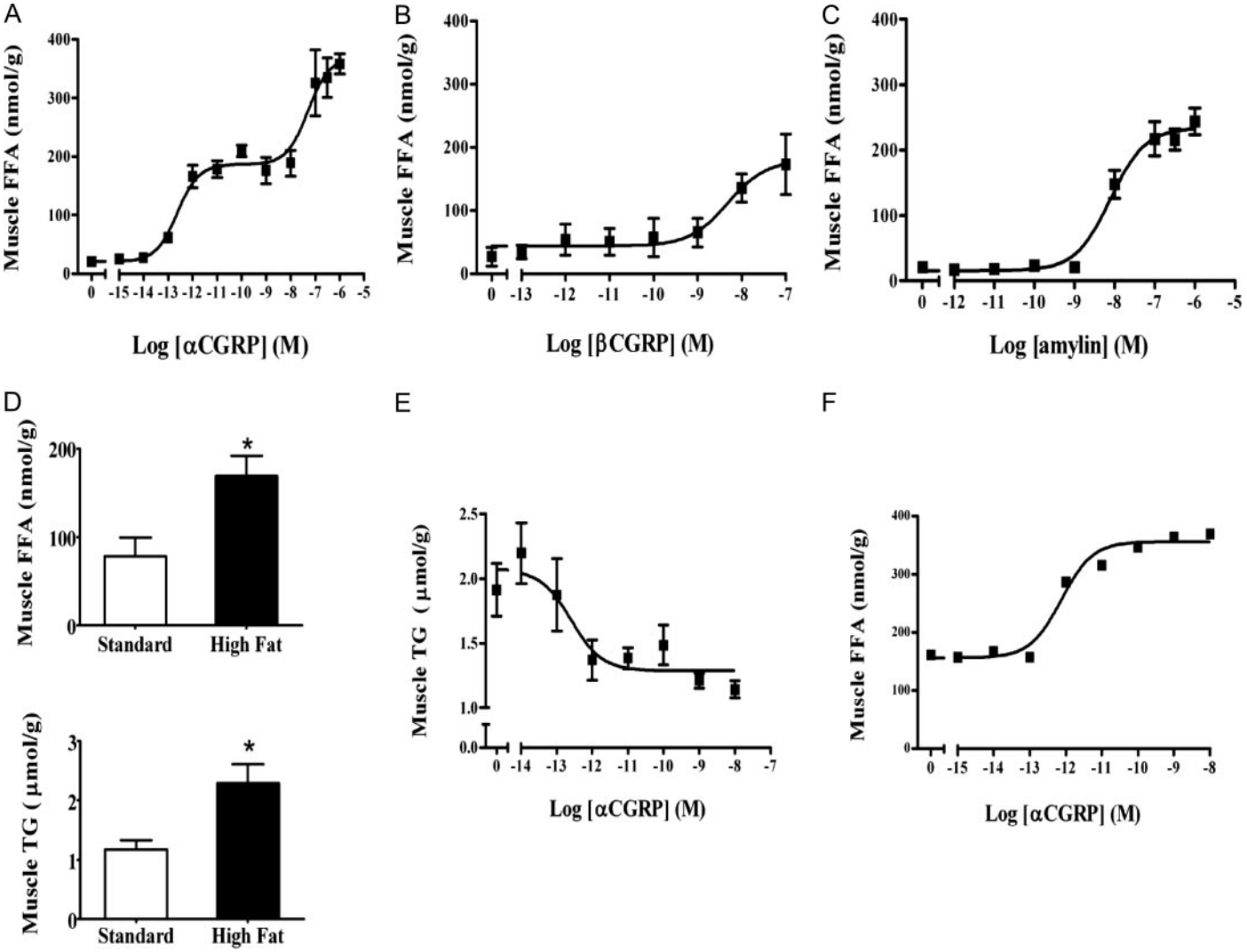

FIG. 1. Concentration-dependent effects of peptides on muscle lipid. FFA content in isolated rat soleus muscles from normal-fed Wistar rats were incubated with rat αCGRP (A); human βCGRP (B); or rat amylin (C). D, Comparison of the lipid levels in soleus muscles from animals fed a standard or high-fat diet. Effect of rat αCGRP on TG (E) and FFA (F) content in soleus muscles from high-fat-fed animals are shown. Each *point* represents the mean ± SEM (n = 10–16 individual muscle strips).

with the known and comparable actions of both CGRP and amylin as noncompetitive antagonists of insulin-stimulated glycogen synthesis in muscle (8). Additionally, this response closely parallels published potency orders for complexes of the calcitonin receptor and RAMPs (RAMP1 or -3), which display similar affinity for the CGRPs and amylin (22).

We next determined whether αCGRP-mediated increases in muscle FFAs were due to mobilization of intrinsic lipid stores by measuring muscle TG content after incubation with αCGRP. In soleus muscle isolated from standard chow-fed animals, there was no significant change in TG content in response to αCGRP, although a significant decrease in muscle glycerol content did occur ($P < 0.05$) (data not shown); this effect is most likely due to its conversion to glycerol 3-phosphate (23). Skeletal muscle from animals fed a 30% lard diet for 40 d showed increases in basal TG and FFA contents of 1.8- and 2-fold, respectively (Fig. 1D). Incubation of soleus muscle strips from these animals with αCGRP for 1 h revealed marked dose-dependent decreases in TG content to a maximal value of 50% (Fig. 1E), and concomitant increases in FFA content (Fig. 1F). Half-maximal responses were in the subpicomolar range [TG: EC_{50} = 0.25 pM (0.03–1.96), FFA: EC_{50} = 0.7 pM (0.4–1)], showing that these actions were of very high potency.

αCGRP-mediated muscle lipid effects are mediated via CGRP receptors

To further confirm the specificity of this newly described action of αCGRP, we investigated its specific blockade. We incubated soleus muscles with 100-fold molar excess concentrations of the NH_2-terminally truncated peptides of CGRP [CGRP-(8–37)] and amylin [amylin-(8–37)]. Both peptide fragments effectively blocked the effects of exogenous αCGRP on TG and FFA content (Fig. 2, A and B). This result is interesting because much higher concentrations of amylin-(8–37) were required to block cAMP responses in COS-7 cells transfected with $CGRP_1$ (calcitonin receptor-like-receptor/

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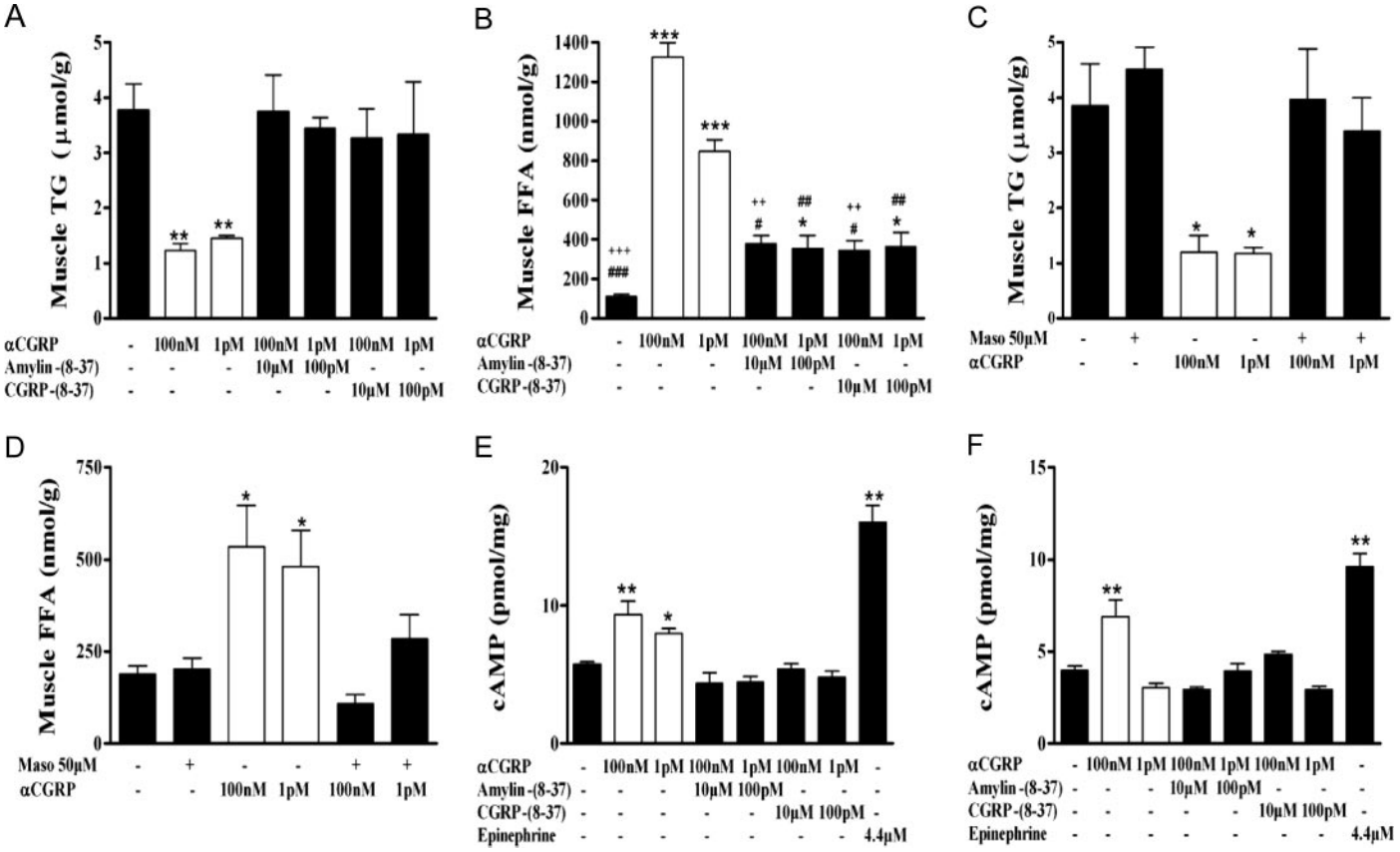

FIG. 2. αCGRP can evoke increases in cAMP and can be inhibited by NH_2-terminally truncated CGRP and amylin peptide fragments. TG (A) or FFA (B) content in high-fat-fed soleus muscle after treatment with specific receptor antagonists are indicated. Effects of masoprocol on TG (C) and FFA (D) content in soleus muscle from high-fat-fed animals are also shown. cAMP content in soleus from high-fat-fed (E) and normal-fed (F) animals are indicated. *, Significant difference, compared with untreated ($P < 0.05$); **, $P < 0.01$; *** $P < 0.001$; ++, significant difference, compared with 100 nM αCGRP ($P < 0.01$), +++, $P < 0.001$; #, significant difference, compared with 1 pM αCGRP ($P < 0.05$); ##, $P < 0.01$; ###, $P < 0.001$. Each *bar* represents the mean ± SEM (n = 10–16 individual muscle strips).

RAMP1) (24) or amylin (1a) [calcitonin (a)/RAMP] (22) receptors, further suggesting the presence of a novel receptor phenotype.

It is currently unclear what the components of the high potency receptor complex might be, and it is therefore possible that these receptors might be differentially sensitive to the effects of antagonists, thus explaining why the effects of αCGRP on muscle lipid are not completely blocked as observed in Fig. 2B.

Additionally, the effects of αCGRP were inhibited by coincubation of soleus muscles with the lipoxygenase inhibitor, masoprocol (nordihydroguaiaretic acid) (Fig. 2, C and D), a compound reported to lower isoproterenol-induced lipolysis by decreasing the phosphorylation of hormone-sensitive lipase (25).

Evidence that αCGRP effects on muscle lipid are evoked via receptor-mediated increases in cAMP

We next investigated αCGRP signaling in soleus muscle at concentrations corresponding to the two components of the biphasic dose-response curve. αCGRP (100 nM) significantly increased cAMP content in muscle from both high-fat-fed (Fig. 2E) and standard chow-fed rats (Fig. 2F), whereas 1 pM αCGRP significantly increased cAMP only in soleus muscle from high-fat-fed animals. Thus, high-fat-feeding appears to sensitize the αCGRP-mediated cAMP response and increase TG breakdown (Fig. 1E) and FFA availability (Fig. 1F). This may reveal a role of endogenously released αCGRP in the sensitization of muscle lipid metabolism in the context of conditions of high-fat feeding.

Increases in cAMP content were inhibited by coincubation with 100-fold molar excesses of either of the peptide fragments used above, consistent with a previous report that αCGRP-mediated cAMP stimulation in skeletal muscle is CGRP receptor-mediated (26).

Interestingly, we observed that high-fat feeding induced an increase in basal muscle cAMP content (5.7 ± 0.2 *vs.* 3.9 ± 0.2 pmol/mg, $P < 0.01$). This finding is novel to our knowledge and complements our previous experiments, which showed that high-fat feeding elevated the phosphorylation of P20, a common intracellular target for insulin and several of its antagonists [Fig. 5 in Wang *et al.* (27)]. Together the increases in cAMP and changes in P20 could contribute to the insulin resistance observed after high-fat feeding.

αCGRP stimulates β-oxidation in vitro

To further investigate the regulatory role of αCGRP in muscle energy use, we used pulse-chase methodologies to

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measure fatty acid β-oxidation. αCGRP-mediated increases in muscle FFA content were significantly lower than expected from concomitant disappearance of muscle TG (data not shown). This finding suggested that muscle FFAs were undergoing further metabolism once released from TG by hormone action. Theoretically, FFAs can undergo one or more of three possible fates in muscle once freed from TG: release via the cell membrane; reesterification into TG; or β-oxidation in mitochondria. In this study, FFA concentrations were below the detection limit of the assay in muscle incubation medium ($\sim 10^{-6}$ M), indicating that measurable amounts of FFAs were not released from the muscle after αCGRP-evoked stimulation. Furthermore, because im TG concentrations decreased after hormone treatment, it is unlikely that reesterification was taking place. Administration of 1 μM αCGRP for 1 h stimulated fatty acid oxidation *in vitro* (Fig. 3A) to a degree similar to that induced by full-length mouse adiponectin (89 nM), an established activator of fat oxidation (14, 28). Additionally, treatment of isolated soleus muscle with 100 nM αCGRP for 2 h significantly increased rates of lipid oxidation (Fig. 3B). We also compared the effects of 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR; 2 mM), a commonly used experimental stimulator of fatty acid oxidation, with that of αCGRP and found that the response was again similar [control, 1.95 ± 0.13; αCGRP, 2.79 ± 0.21 ($P = 0.0031$ *vs.* control); AICAR, 2.95 ± 0.28 ($P = 0.0051$ *vs.* control] (data not shown). Interestingly, we also found that αCGRP increased fatty acid oxidation when applied to skeletal muscle for 15 min (Fig. 3C).

αCGRP stimulates phosphorylation of 5′-AMPK-activated protein kinase

The protein kinase, AMPK, acutely stimulates fatty acid oxidation via inhibitory phosphorylation of acetyl-CoA carboxylase (29). We hypothesized that αCGRP-mediated stimulation of fatty acid oxidation is mediated through AMPK. Using quantitative Western blotting, we determined that 1 h exposure of skeletal muscle to αCGRP (1 μM) stimulated phosphorylation in the α-subunit of AMPK, similar to that induced by 2 mM AICAR (Fig. 3D). There was no difference in the total amount of nonstimulated αAMPK protein between treated and untreated muscle. We next determined the effect of αCGRP on skeletal muscle AMPK phosphorylation using two lower concentrations, the first (100 nM) sufficient to stimulate maximally both the low- and high-potency phases and the second (50 pM) sufficient to maximally stimulate only the high-potency phase. Figure 3E shows that 100 nM αCGRP significantly increased phosphorylation of AMPK, whereas phosphorylation after 1 h stimulation with 50 pM αCGRP was not significantly different to control.

These findings show that the effects on skeletal muscle lipid at higher concentrations of αCGRP occur via pathways involving both cAMP and AMPK. In contrast, effects on muscle lipolysis at αCGRP concentrations within the high-

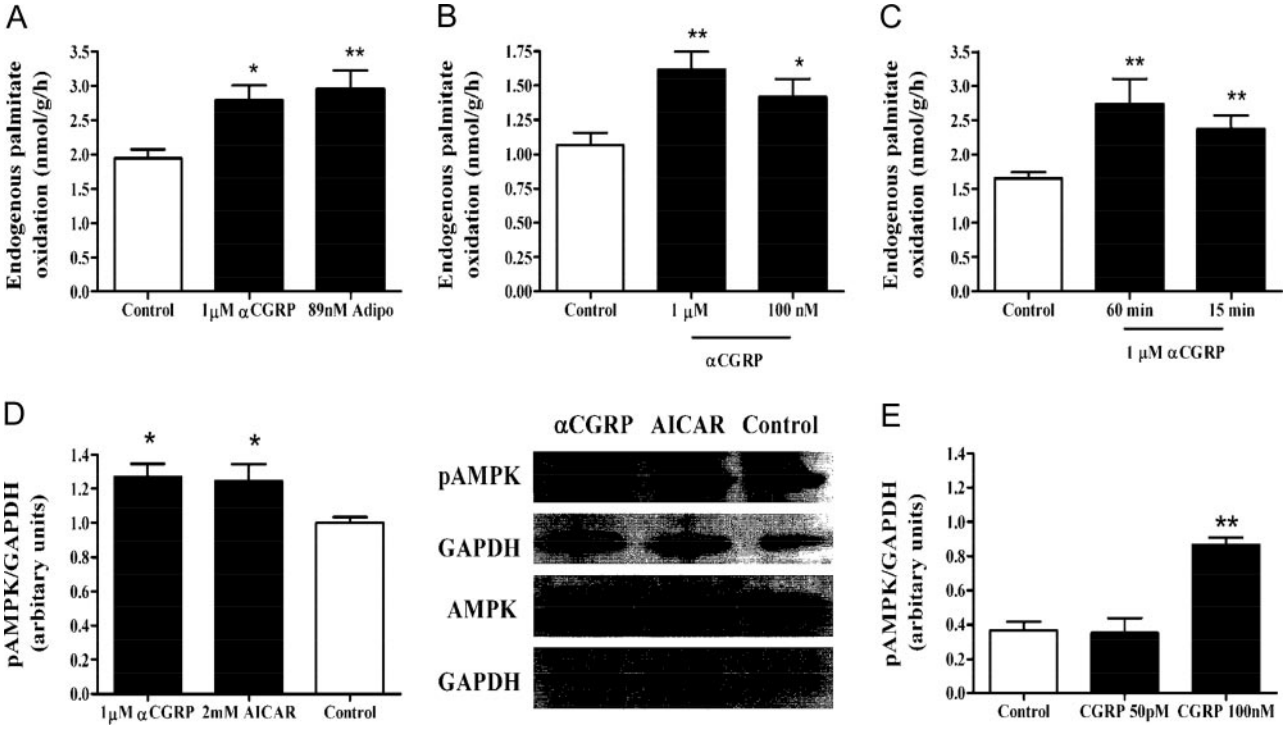

FIG. 3. αCGRP increases fatty-acid oxidation and AMPK signaling in skeletal muscle**.** Fatty-acid oxidation was determined in isolated soleus muscles in the presence of 1 μM rat αCGRP, 89 nM mouse full-length adiponectin, or modified KHB buffer (control) (A) and 1 μM rat αCGRP, 100 nM rat αCGRP, or control (B); and modified KHB buffer or 1 μM rat αCGRP (C). Each *bar* represents the mean ± SEM (n = 12 individual muscle strips). Representative immunoblots of AMPK Thr172 phosphorylation (pAMPK) and total protein content (D) and densitometric quantification show increased pAMPK levels (D and E). Each *bar* represents the mean ± SEM (n = 6 individual muscle strips). *, Significant difference, compared with untreated ($P < 0.05$), **, $P < 0.01$.

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potency phase apparently occur via a potentially novel mechanism independent of cAMP and AMPK.

αCGRP evokes specific effects on in vivo lipid metabolism

We also examined the tissue specificity of αCGRP treatment on *in vivo* organ lipid content after its infusion into anesthetized male Wistar rats in the presence or absence of a 100-fold molar excess of the αCGRP receptor antagonist, CGRP-(8–37) (Table 1). At an infusion rate of 100 pmol/kg·min, plasma [αCGRP] was significantly elevated after 1 h, compared with saline infusion (325 ± 75 *vs.* 6.3 ± 0.8 pM, $P < 0.001$), whereas plasma concentrations of insulin, epinephrine, norepinephrine, and amylin were unchanged (data not shown). Analysis of tissue-lipid content revealed 3-fold increases in FFA ($P < 0.01$) and 2-fold decreases in TG ($P < 0.01$) content in soleus muscle but not in heart, epididymal adipose tissue, or kidney. Additional acute effects on lipid content were observed in liver, in which FFAs increased 7-fold ($P < 0.01$) and TG decreased 2.5-fold ($P < 0.05$); plasma TG underwent a concomitant 7-fold decrease ($P < 0.01$) and serum FFAs a 4-fold increase ($P < 0.01$) (Table 1). These effects were eliminated by coinfusion of CGRP-(8–37). In addition to its use via lipolysis and β-oxidation, TG may also be exported in the form of very low-density lipoprotein and used elsewhere in the body. Interestingly, infusion of CGRP-(8–37) alone caused changes in the reverse direction in muscle and liver TG content (both $P < 0.05$), consistent with blockade of an endogenous CGRP agonist, presumably αCGRP.

These current studies show that, at least *in vitro*, αCGRP at concentrations sufficient to stimulate the low potency phase elicits skeletal muscle TG breakdown via a pathway that is apparently coupled to cAMP production. This pathway can be blocked by both peptide fragments of CGRP and amylin, and an inhibitor of hormone-sensitive lipase-mediated lipolysis. In addition to TG breakdown, αCGRP increased FFA partitioning toward β-oxidation and activated AMPK in muscle. Interestingly, recent evidence shows that AMPK can be activated by increased FFA supply within muscle cells and that this is independent of changes in the energy status of the cell (30).

There are several other endogenous compounds that have been demonstrated to increase lipid use, including adiponectin and other adipokines such as leptin. These, along with exercise and the antidiabetic drug metformin, all activate AMPK, and it is now clear that this enzyme plays a crucial role in the regulation of energy expenditure (31). The physiological significance of the low-potency phase in fatty acid oxidation is unclear because αCGRP concentrations at the neuromuscular junction after stimulation are unknown. Nevertheless, the maximal αCGRP effect is similar to that of leptin- and AICAR-induced activation of AMPK, which reach significance 60 min after administration (32, 33). Compounds that activate the AMPK pathway, as αCGRP appears to, could improve the lipid profile of patients with obesity and type 2 diabetes.

At αCGRP concentrations sufficient to stimulate the high-potency phase, the breakdown of TG occurs via a mechanism that, at least in skeletal muscle, is not coupled to cAMP production or mediated via the AMPK pathway. Further studies will be required to fully elucidate the signaling path-

TABLE 1. Tissue FFA and TG content and plasma lipid profile after αCGRP infusion

	Saline	αCGRP	CGRP-(8–37)	CGRP-(8–37) + αCGRP
FFA content				
Heart	0.8 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	0.7 ± 0.2
Liver	0.1 ± 0.01	0.7 ± 0.1[a]	0.06 ± 0.04	0.1 ± 0.01
Epididymal adipose	2.1 ± 0.5	2.7 ± 0.7	2.2 ± 0.6	2.0 ± 0.3
Soleus muscle	0.2 ± 0.02	0.6 ± 0.1[a]	0.1 ± 0.02	0.2 ± 0.04
Kidney	0.8 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.7 ± 0.2
TG content				
Heart	2.9 ± 0.4	2.6 ± 0.4	3.1 ± 0.4	3.7 ± 0.5
Liver	4.3 ± 0.3	2.2 ± 0.5[a]	5.8 ± 0.4[b]	3.7 ± 0.3
Epididymal adipose	5.1 ± 1.2	5.0 ± 0.9	4.9 ± 1	5.2 ± 1.3
Soleus muscle	3.7 ± 0.4	1.5 ± 0.2[a]	4.3 ± 0.4[b]	3.6 ± 0.5
Kidney	2.8 ± 0.5	2.4 ± 0.4	2.5 ± 0.4	2.3 ± 0.5
Plasma				
TG				
Before	1.3 ± 0.01	1.4 ± 0.03	0.5 ± 0.01	1.1 ± 0.02
After	1.5 ± 0.02	0.7 ± 0.02[c]	1.4 ± 0.05[c]	1.4 ± 0.05
$\Delta_{(post-pre)}$	0.2 ± 0.05	−0.7 ± 0.06[a]	0.9 ± 0.09[a]	0.4 ± 0.02
FFA				
Before	0.45 ± 0.01	0.6 ± 0.05	0.7 ± 0.01	0.6 ± 0.01
After	0.32 ± 0.03	1.1 ± 0.02[c]	0.4 ± 0.05[d]	0.5 ± 0.02
$\Delta_{(post-pre)}$	−0.1 ± 0.02	0.4 ± 0.01[a]	−0.3 ± 0.06[b]	−0.1 ± 0.02
Lactate				
Before	1.6 ± 0.05	1.5 ± 0.2	2.0 ± 0.05	1.75 ± 0.01
After	1.9 ± 0.05	3.0 ± 0.25[c]	1.98 ± 0.03	2.20 ± 0.05[d]
$\Delta_{(post-pre)}$	0.3 ± 0.02	1.7 ± 0.3[a]	0.02 ± 0.01[b]	0.4 ± 0.03

Tissue levels of FFA and TG, micromoles per gram, and plasma metabolite levels, expressed as millimoles per liter; there were no significant differences in baseline values between groups. Values depict means ± SEM (n = 10 animals at each point).

[a] Significant difference, compared with saline ($P < 0.01$).

[b] Significant difference, compared with saline ($P < 0.05$).

[c] Significant difference, compared with plasma prevalue ($P < 0.01$).

[d] Significant difference, compared with plasma prevalue ($P < 0.05$).

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ways of αCGRP on muscle lipid metabolism, particularly at these lower concentrations. Further studies to determine the dose responsiveness of the αCGRP-induced effects on lipid *in vivo* and determine whether αCGRP alters lipid metabolism after stimulation of the neural supply to muscle would provide further support for the physiological significance of this mechanism. As mentioned, there is a lack of obvious phenotype in the αCGRP knockout mouse model. However, this knockout model has yet to be characterized in sufficient depth because there are no available reports pertaining to the effects of high-fat feeding nor have lipid measurements been reported. Consequently, determination of the effects of a high-fat dietary challenge on the lipid profile of these knockout mice will be of significant interest.

In summary, the present findings provide the first evidence that αCGRP, released from motor neurons, could act as a neurohormone that coordinates the availability of intramyocellular lipid to meet muscle energy requirements created by concomitant activation of contraction by acetylcholine.

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Competing Interest Statement: The authors declare association with Protemix Corp., Auckland, New Zealand.

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