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Ccl22/MDC, is a prostaglandin dependent pyrogen, acting in the anterior hypothalamus to induce hyperthermia via activation of brown adipose tissue

Olivia Osborn¹, Manuel Sanchez-Alavez¹, Jeffrey S. Dubins², Alejandro Sanchez Gonzalez¹, Brad Morrison¹, John R. Hadcock², and Tamas Bartfai¹

¹The Harold L. Dorris Neurological Research Institute, Department of Molecular and Integrative Neurosciences, The Scripps Research Institute, La Jolla, California, USA

²Pfizer Global Research, Groton, Connecticut, USA

Abstract

CC Chemokine ligand 22 (Ccl22) is a selective, high affinity ligand at the CC chemokine receptor 4 (Ccr4). We have identified cDNAs encoding both ligand and receptor of the Ccl22-Ccr4 pair in cDNA libraries of the anterior hypothalamus/preoptic area (AH/POA) by PCR. The AH/POA is the key brain region where endogenous pyrogens have been shown to act on warm sensitive neurons to affect thermogenesis in brown adipose tissue (BAT) and other thermogenically responsive tissues. We show that functional Ccr4 receptors are present in the AH/POA neurons as injection of Ccl22 into the POA but not to other hypothalamic nuclei induces an increase in core body temperature as measured by radiotelemetry. Indomethacin (5mg/kg s.c) pretreatment markedly reduced the hyperthermia evoked by POA injection of Ccl22 (10 ng/0.5ul) and thus suggests that this hyperthermia is mediated through cyclooxygenase activation and thus likely through the formation and action of the pyrogen prostaglandin E2. The temperature elevation involves a decrease in the respiratory exchange ratio and increased activation of the brown adipose tissue as demonstrated by ¹⁸F-FDG –PET imaging. We describe a novel role to the ligand Ccl22 and its receptor Ccr4 in the anterior hypothalamus in temperature regulation that depends on the synthesis of the endogenous pyrogen, prostaglandin E2.

Keywords

Ccl22; MDC; hyperthermia; brown adipose tissue; AH/POA

1. Introduction

Chemokines are a superfamily of structurally related peptides that act on G protein coupled, 7 transmembrane domain containing receptors that control leukocyte trafficking and participate in the activation and recruitment of specific cell populations to sites of inflammation and infection [1]. CC Chemokine ligand 22 (Ccl22), also known as

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Corresponding author's address, The Scripps Research Institute, 10550 North Torrey pines Rd, La Jolla, CA, 92037. olivial@scripps.edu.

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Macrophage derived chemokine (MDC) is synthesized specifically by cells of the macrophage lineage [2–3] and is a selective and high affinity ligand at the CC chemokine receptor 4 (Ccr4) [4]. Ccl22 is constitutively expressed in macrophages [5], dendritic cells [5–6], thymic epithelial cells [7] and intestinal epithelial cells [8]. Ccl22 expression is inducible by microbial products, cytokines and prostaglandin E2 [9–12]. Ccl22 is a potent attractant for type 2 T helper cells (Th2) cells that preferentially express the receptor Ccr4 [10] and support the humoral immunoglobulin E (IgE) associated immune responses [2,12]. Ccr4 mRNA is predominantly expressed in the thymus, spleen and in peripheral blood leukocytes including T cells, basophils, monocytes [13] macrophages and platelets [14]. Ccr4 is also expressed in both adipocytes [15] and muscle [16]. In the context of central nervous system, Ccr4 mRNA has been detected in astrocytes and microglia [17], and in hippocampal neurons [18].

Ccl22 and its' receptor Ccr4 are involved in a diverse range of pathologies. Ccl22 is highly-expressed in the lesions of T cell-mediated inflammatory diseases, such as atopic dermatitis, bronchial asthma, psoriasis and eosinophilic pneumonia [19–23]. Ccl22 and Ccr4 also play an important role in cancer growth and metastasis [24–28] and thus many Ccr4 receptor antagonists are being developed in the pharmaceutical industry [29–33] as well as an anti-Ccr4 antibody has been developed [26]. An association between Ccl22 and abdominal aortic aneurysm has also recently been described [34]. Ccl22 has been found in the cortico-spinal fluid of Multiple Sclerosis patients [35] but neither Ccr4 or Ccl22 expression or function have been reported specifically in the hypothalamus, prior to this report.

Cytokines such as Interleukin (IL)-1 α or IL-1 β , IL-6 and Tumor necrosis factor - α (TNF- α) or exogenous pyrogens i.e. LPS [36] have been shown to play a role in temperature regulation by triggering the febrile response. In response to these stimuli other endogenous pyrogenic mediators are generated and released within the brain such as prostaglandins, endothelin 1 [37] and corticotrophin releasing factor [38–39] that act either in a direct or indirect manner on thermosensitive neurons localized in the anterior hypothalamus /preoptic area (AH/POA) to regulate core body temperature. Several studies have demonstrated that some chemokines are also implicated in the generation of a febrile response: central injections of IL-8/CXCL8 [40], macrophage inflammatory protein-1 alpha and beta (MIP-1 α /CCL3 and MIP-1 β /CCL4) [41], and RANTES/CCL5 [42] administered into the AH/POA, evoke a febrile response characteristic of endogenous pyrogens. Furthermore, effects of MIP-1 α /CCL3 and MIP-1 β /CCL4 on the body temperature appear to be independent of the prostaglandin action, since the MIP-1-produced fever is not blocked by inhibitors of prostaglandin synthesis [43–44]. The hyperthermic effect of RANTES/CCL5 when injected directly into the AH/POA is however prevented by pretreatment with a prostaglandin synthesis inhibitor [45]. There are no reported studies examining the ability of Ccl22 acting in the AH/POA to induce a change in core body temperature.

Since the *ccr4* receptor and *ccl22* ligand were detected in the AH/POA we hypothesized that Ccl22 could modify thermogenesis by acting at Ccr4 receptors expressed on temperature sensitive neurons in the AH/POA and ultimately by affecting the uncoupling of mitochondrial respiration in BAT. The recent “re-discovery” of the importance of brown adipose tissue (BAT) in adult humans has brought this often neglected tissue back in the spotlight as a target tissue for the modulating whole body energy metabolism [46] and metabolic diseases such as diabetes and obesity and caused by certain antipsychotics [47].

2. Results

2.1 Effect of AH/POA injection of Ccl22 on core body temperature

Transcripts encoding *ccl22* and *ccr4* are found in the AH/POA (Fig 1A). Injection of Ccl22 (10 ng/0.5ul) directly to the pre-optic area induces an increase in core body temperature of mice (Fig 1B). Comparison of the area under the temperature curve (AUC) after AH/POA Ccl22 administration is significantly different, $P < 0.05$ when compared with the injection of vehicle, as shown in Fig 1C. This increase in core body temperature was not due to endotoxin contamination of the recombinant chemokine as heat treated Ccl22 did not elicit any hyperthermic effect (Fig 1B, C). In addition, the observed hyperthermic effect of Ccl22 injection to the AH/POA was not due to differences in locomotor activity of these mice and vehicle injected mice (Fig 1B–D). Ccl22 injection into the POA caused a significant dose-dependent hyperthermic response (Fig. 2A, B) that was not due to changes in locomotor activity (Fig 2C) between vehicle and Ccl22 injected mice.

2.2 Ccl22 acts specifically at the AH/POA to elicit a hyperthermic response

The POA neurons send inhibitory signals through other hypothalamic nuclei, the dorsomedial hypothalamus (DMH) and the Raphe pallidus (RPa) to the brown adipose tissue (BAT) where adaptive thermogenesis occurs. Ccl22 acts specifically at the AH/POA where a significant hyperthermic effect ($p < 0.05$) was observed after Ccl22 injection compared with vehicle control (Fig 3A, B). The administration of Ccl22 to either the DMH or RPa nuclei within the hypothalamus did not elicit any significant elevation in temperature relative to vehicle control in these hypothalamic nuclei, respectively (Fig 3A, B). For example, 90mins after the administration of Ccl22, the hyperthermic effects of Ccl22 was $1.4 \pm 0.4^{\circ}\text{C}$ greater than vehicle applied to the POA, whereas the change in core temperature after microinjection of Ccl22 to the DMH or the RPa was $(0.6^{\circ}\text{C} \pm 0.2)$ and $(0.4^{\circ}\text{C}, p < 0.01)$ respectively (Fig 3A).

2.3 Indomethacin sc, but not LY294002 blocks the central Ccl22-induced hyperthermia

Prior administration (30 mins before Ccl22) of indomethacin (subcutaneous- sc, 5 mg/kg) significantly reduced hyperthermia induced by Ccl22 injection into the AH/POA whereas, the sc injection of indomethacin followed by saline or did not alter the basal temperature of the mice (Fig 4). Furthermore, pretreatment with PI3K-Inhibitor LY294002 had no effect on Ccl22 induced hyperthermia suggesting the PI3K pathway is not implicated in the hyperthermic effects of Ccl22 (Fig 5).

2.4. Effect of Ccl22 AH/POA injection on fatty acid utilization and brown adipose tissue activity

Mice treated with Ccl22 (10 ng/0.5ul) in the AH/POA show a decrease in respiratory exchange ratio compared with mice treated with vehicle, indicating a shift from carbohydrate utilization to an elevation of fatty acid utilization (Fig 6). A 2 hr profile quantification of PET/CT of ^{18}F -FDG uptake in rats injected with vehicle or Ccl22 (10 ng/0.5ul) shows an increase in BAT activity in Ccl22 treated mice (Fig 7). The PET study used rats because of better resolution, expression of receptor and ligand in this rodent is similar to that seen in mice.

3. Discussion

In this study, we have identified *ccr4* receptors and *ccl22* transcripts in the AH/POA, a region within the hypothalamus that is essential for the regulation of core body temperature [48] (Fig 1A). We validated the functional expression of Ccr4 in temperature sensitive AH/POA neurons by examining *in vivo* whether injection of the Ccr4-selective agonist Ccl22

has an effect on core body temperature. AH/POA microinjection of Ccl22 resulted in an increase in core body temperature in C57BL/6 wild type mice thus confirming the presence of functional Ccr4 in this region of the brain and attributing a novel role to the Ccr4 in temperature regulation. This is the first demonstration of a functional consequence of activation of Ccr4 by Ccl22 in the AH/POA. The injection of heat inactivated Ccl22 to the AH/POA did not cause an increase in CBT (Fig 1B, C) thus the possibility of endotoxin contamination of the Ccl22 being responsible for the observed changes in core body temperature was ruled out. The observed hyperthermic effects of Ccl22 observed in mice were not due to differences in locomotor activity (1D).

The AH/POA sends inhibitory signals through separate outflow pathways to other hypothalamic nuclei, the DMH and RPa, to mediate differential inhibitory control of the sympathoexcitatory drive determining brown adipose tissue (BAT) thermogenesis[49]. Here we show the hyperthermic effects of Ccl22 in the hypothalamus are elicited through the AH/POA nucleus and not at the level of the downstream DMH or RPa (Fig 3).

The hyperthermic effect of Ccl22 applied directly into the AH/POA was blocked by systemic indomethacin pre-treatment suggesting that the hyperthermic effect of Ccl22 is mediated through the activation of cyclooxygenase and the subsequent production of PGE₂, a well characterized pyrogen [50]. The peak hyperthermic response to Ccl22 administered to the AH/POA was observed 90 mins after the pretreatment injection of aCSF that is compatible with Ccr4 mediated induction of COX2 and subsequent synthesis and release of PGE₂ is involved in the observed hyperthermia as suggested by the blocking effect of indomethacin. At this time point pretreatment with indomethacin reduced the Ccl22-induced temperature elevation by 1.68°C (from 37.85°C to 36.17°C) (Fig 4A).

Ccl22 has previously been shown to activate the PI3K pathway in a leukemic T cell line [51] and we investigated whether the observed hyperthermic effects of microinjection of Ccl22 into the AH/POA were mediated via the PI3K pathway. Pretreatment with PI3K inhibitor LY294002 (10 nanomoles in 0.5 µl) before Ccl22, both administered into the AH/POA 30 min apart, showed no attenuation of the Ccl22 induced hyperthermia (Fig 5). Therefore, the PI3K pathway appears not to play a role in the mediation of the temperature effects elicited by Ccl22. Antagonists to Ccr4 have been developed that bind to the human Ccr4 but had low affinity for the mouse Ccr4 and thus could not be used in this study.

Measurement of the respiratory exchange ratio (RER) demonstrated that AH/POA injection of Ccl22 was followed by a prolonged decrease in RER compared with vehicle treated mice, indicating a switch from glucose metabolism to elevated fatty acid utilization (Fig 6). Three hours after AH/POA injection of either vehicle or Ccl22 the RER was 0.86 ± 0.03 and 0.78 ± 0.01 , respectively.

Positron emission tomography (PET) and computed tomography (CT) imaging carried out on rats demonstrated that Ccl22 injected into the AH/POA stimulated glucose uptake in BAT as the Ccl22 treatment induced elevation in BAT glucose, fluorine-18 fluorodeoxyglucose (¹⁸F-FDG) uptake (Fig 7).

In summary, injection of Ccl22 into the preoptic area of the hypothalamus induced an elevation of core body temperature mediated by stimulation of BAT thermogenesis as shown by imaging and determination of RER. These data show how Ccl22, primarily known for its role in T cell-mediated inflammatory and neurodegenerative diseases, may act as central, PGE₂ dependent, stimulator of fatty acid oxidation in BAT. The demonstration that AH/POA neurons express functional Ccr4 and respond to Ccl22 with increased thermogenesis provides an important link between neuroinflammation and thermoregulation.

4. Materials and methods

4.1 Mice and diets

C57BL/6 wild type male mice used in this study were bred and maintained in the animal research facility at the Scripps Research Institute (The Scripps Research Institute, LA Jolla, CA). All procedures were approved by the Institutional Animal Care and Use Committee of the Scripps Research Institute. Mice were housed in groups of 4 and fed *ad libitum* with mouse breeder diet composed of 11% fat, 17% Protein, 3.5% fiber (S-2335 Mouse Breeder, gross energy kcal 4.39 kcal/g). Mice were individually housed in a Plexiglas cage in a room maintained at $25 \pm 0.5^{\circ}\text{C}$, relative humidity of 40–50% and a 12h dark/light cycle (lights on at 6:00 a.m). All injections were conducted between 9am and 10am.

4.2 Brain dissection and PCR confirmation

To obtain POA, DMH and RPa tissue a brain slicer (EM Corporation, Chestnut Hill, MA) was used to produce 1-mm slices. Under chilled conditions, each brain was sliced quickly, and the slices were placed in a *RNAlater* (Ambion, Austin, TX), which prevents RNA degradation. On completion of the slicing, the various brain regions were microdissected under a dissecting microscope, using the mouse brain atlas (Franklin and Paxinos) for accurate dissection of brain regions including the POA, DMH and RPa. Microdissected tissues were rapidly frozen and stored at -80°C . To detect the presence of the *Ccr4* and *Ccl22* transcripts the LightCycler FastStart DNA Master-Plus SYBR Green I reaction mix (Roche, USA) was used according to manufacturers' instructions using primers (*Ccr4*-F TGC CTT AAC CCC GTC ATT TA, *Ccr4*-R TCA CGG AAG TCA TGA TCC AC or *Ccl22*-F ATT GAC AGT GCC AGC CAC A; *Ccl22*-R GAG GAC AAG GAC CCA AGT CA) at an annealing temperature of 60°C and cycling conditions 10 mins at 95°C followed by 40 cycles of 95°C for 05 secs, 60°C for 05 seconds, 72°C for 8 seconds and a final extension of 68°C for 10 minutes. Samples were run on a 2% agarose gel (Fisher Scientific).

4.3 Telemetry and indirect calorimetry studies

C57BL/6 wild type male mice aged 14–16 weeks were surgically implanted with radio telemetry devices (TA-F20, Data Sciences, Inc.) into the peritoneal cavity for core body temperature (CBT) and locomotor activity (MA) measurement. Mice were then placed into a stereotaxic apparatus and a guide cannula (26 Ga, 10 mm length) was positioned aiming at the AH/POA (anterior–posterior from bregma, +0.34 mm; lateral, 0mm and ventral, 3.8 mm from skull). The cages were placed onto the receiver plates (RPC-1; Data Sciences) and radio signals from the implanted transmitter were continuously monitored and recorded. For combined metabolic and telemetry studies the cages were placed in receivers plates (RPC-1; Data Sciences) integrated in the Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, OH).

A volume of 0.5 μl of Recombinant Mouse *Ccl22* (439-MD-025/CF (R&D, Minneapolis, MN, USA). or vehicle (artificial cerebro-spinal fluid) were administered directly to either the POA, DMH or RPa using an injector (33 GA, protruding 0.4 mm beyond the tip of the cannula, total length 10.4 mm) through an implanted cannula (26 GA, 10 mm length) connected to plastic tubing and a microsyringe (10 μl) in a volume of 0.5 μl over a period of 5 min to allow diffusion ($n = 5$ mice per group). Coordinates for the POA cannula were anterior-posterior [AP] from bregma = -0.38 mm anterior to the bregma, lateral [Lat] = midline, ventral [V] = 3.8 mm, cannula 26 GA, 10 mm length). Coordinates for the DMH were AP= 1.58 mm posterior to the bregma, Lat = 0.25 mm; V= 4.6 mm from the surface of the brain. Coordinates for the RPa were AP= 6.12 mm posterior to the bregma, Lat= 0.0 mm; V= 5.8 mm from the surface of the brain).

For combined treatments, we applied a pretreatment with either the PI3 kinase inhibitor LY294002 (Sigma, St. Louis, MO, USA), 10nM microinjection to AH/POA or indomethacin (I7378 Sigma, St. Louis, MO, USA), 5mg/kg in 150ul volume of saline injected subcutaneously, followed by a 20-min interval when Ccl22 (10 ng in 0.5ul) was injected to the AH/POA.

After AH/POA Ccl22 administration indirect calorimetry was performed using a computer-controlled, open-circuit system (Oxymax System) that is part of an integrated Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, OH). Respiratory exchange ratio (RER) was calculated as the ratio of carbon dioxide production (VCO_2) to oxygen consumption (VO_2). Energy expenditure measures (VO_2 , VCO_2 and heat formation [$(3.815 + 1.232 \cdot RER) \cdot VO_2$]) are corrected for estimated effective metabolic mass per Kleiber's power function.

To determine the cannula injection site 2 μ L of the tetrasodium salt of copper(II)phthalocyanine-tetrasulfonic acid 2.5% was injected, then mice were sacrificed under deep isoflurane anesthesia and perfused transcardially with PBS and the brain was removed. The brain was fixed in 4% paraformaldehyde for 24 hrs before being placed in 30% sucrose. Brains were sectioned, and 35 μ m sections were cut using a cryostat. Free-floating sections were mounted on gelatin-coated slides and stained with Cresyl violet. Cannula injection site was determined at the point of maximal gliosis and the presence of the dye in the POA, DMH and RPa according to the mouse brain atlas (Franklin and Paxinos). Coronal sections were manually redrawn directly from mounted sections using video-assisted microscopy. Verification of cannula placement was confirmed and data from animals with incorrectly placed cannulas were excluded from the final analysis (supplemental figure 1).

4.4 Positron emission tomography (PET) and computed tomography (CT) imaging

Positron emission tomography (PET)/computed tomography (CT) imaging in this study was carried out with 2-[18 F]fluoro-2-deoxyglucose (18 F-FDG) on 12-week-old male Sprague Dawley rats (mean body wt 250 g). Rats are known to have a relatively large amount of BAT in the interscapular region and thus are often used in research for BAT activation [52]. Each rat received 18.5 MBq of 18 F-FDG via intraperitoneal injection, and 30 min later they were anesthetized with isoflurane (5% induction, 1–2% maintenance) and imaged with a combined PET/CT scanner (Siemens Medical Solutions) designed for small rodents at baseline and after microinjection of Ccl22 into the preoptic area (AP= -0.9, lat = midline, V= 8.0mm from dura) at 15, 30, 60, and 180 min. In brief, CT for attenuation correction and 5-min emission PET were performed, followed by thin-slice (1.5 mm thick) CT. PET and thin-slice CT images were reconstructed as 35-cm field-of-view images, and the PET and CT image sections at the same location were manually fused using image analysis software (Photoshop 6.0; Adobe Systems). The mild 18 F-FDG uptake in skin and the contours of normal organs were used as landmarks. The PET and CT images were also intrinsically registered because of their acquisition on the dedicated PET/CT scanner. 18 F-FDG uptake in interscapular BAT was evaluated using the PET, CT, and fused PET/CT images. Animal's body temperature was maintained at 37°C by a heat lamp (Temperature Controller RET-3 Temperature probe and HL-1 Heat Lamp; Physitemp Instruments, Clifton, NJ) in between periods of recording to avoid hypothermia during isoflurane anesthesia. PET data were analyzed by visual interpretation of coronal, sagittal, and transverse slices alone and in cross-referenced situations. When 18 F-FDG PET uptake increase was observed, two levels were identified in comparison with normal activity: moderate (more or less twice the activity in a reference region) or intense (markedly higher than the reference activity). PET and conventional imaging were interpreted separately, and the results were then compared with each other and, in certain cases, with the physiological information on hyperthermia.

Bone scans and CT images were read independently by two nuclear medicine physicians and by two radiologists, respectively.

4.5 Statistical Analysis

The two tailed independent student's *t*-test was used to compare the effect of Ccl22 treatment with vehicle. When more than 2 treatment groups were compared a one-way analysis of variance (ANOVA) with Newman-Keuls post hoc testing was used to assess statistical significance. All results are expressed as mean $\pm$ S.E. A value of $P < 0.05$ was considered statistically significant. Area under the curve analysis was performed using GraphPad Prism 5.03.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

Ccl22	CC Chemokine ligand 22
MDC	macrophage derived chemokine
Ccr4	chemokine receptor 4
PGE 2	prostaglandin E 2
IL-1	Interleukin 1
AH/POA	anterior hypothalamus /preoptic area
DMH	dorsomedial hypothalamus
RPa	Raphe pallidus
¹⁸F-FDG	fluorine-18 fluorodeoxyglucose
PET	Positron emission tomography
CT	computed tomography

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Figure 1. Effects on CBT and RER of the Ccl22 at the Ccr4 receptor identified in warm sensitive neurons administered to the AH/POA in WT C57BL/6 mice

A. PCR analysis showing presence of the transcripts encoding the Ccr4 receptor and Ccl22 ligand in the AH/POA. B. Administration of Ccl22 (10 ng in 0.5ul) to the AH/POA results in an increase in core body temperature. Administration of heat treated (98°C for 30 mins) Ccl22 does not elicit hyperthermic effects and thus the observed hyperthermic effects of Ccl22 were not due to endotoxin contamination. Comparison of the area under the temperature curve (AUC) after AH/POA Ccl22 administration is significantly different, $P < 0.05$ when compared to injection of vehicle or heat inactivated Ccl22, as shown in Fig 1C. This increase in temperature observed after Ccl22 injection to the AH/POA was not due to changes in locomotor activity (Fig1D).

Figure 2. Microinjection of Ccl22 to the AH/POA causes a dose dependent hyperthermic response

A. Profile of the dose dependent effects on core body temperature of POA injection of Ccl22 (10ng/0.5ul = white, 5ng/0.5ul = grey, vehicle = black) (n= 6 mice per group) for 6 hours after injection. B. AUC analysis shows the significant increase in temperature with increasing doses of Ccl22 either 5ng or 10ng ($P < 0.05$) compared with vehicle control. C. The hyperthermic effect of Ccl22 was not due to differences in locomotor activity. Data are presented as mean $\pm$ SE.

Figure 3. Ccl22 acts specifically at the AH/POA to elicit a hyperthermic response

Graph showing the differential effects on CBT of the injection of 10 ng of Ccl22 in the AH/POA, the DMH and the RPa (POA = circles; DMH=triangles; RPa=diamonds, white = Ccl22; black = vehicle control) for 6 hours after injection (n=3 mice per group), arrow indicates time of injection. B. AUC analysis shows injection of Ccl22 in the AH/POA ($P<0.05$) causes a significant elevation in temperature compared with vehicle control but injection of Ccl22 to the DMH or RPa does not elicit a significant increase in temperature compared with vehicle control injected to the same regions respectively. D. This increase in temperature observed after Ccl22 injection to the AH/POA was not due to changes in locomotor activity. Data are presented as mean $\pm$ SE.

Figure 4. The hyperthermic effect of Ccl22 was blocked by pretreatment with the prostaglandin synthesis inhibitor indomethacin

A. Administration of the prostaglandin synthesis inhibitor indomethacin (INDO) 5 mg/kg sc. completely inhibited the hyperthermic effect of Ccl22. **B.** AUC analysis of temperature 1–6 hrs after injection showed indomethacin pretreatment significantly decreased the hyperthermic effects of Ccl22 ($p < 0.05$) and that indomethacin treatment alone did not have any effects on temperature in these mice. **C.** The observed temperature effects of Ccl22 were not due to differences in locomotor activity.

A

B

C

Figure 5. The hyperthermic effect of Ccl22 was not affected by pretreatment with the PI3 kinase inhibitor LY294002

Fig 4A. Pretreatment with the PI3kinase inhibitor (PI3KI) LY294002, 10nM injected to the AH/POA had no effect on the hyperthermic effect of Ccl22 microinjection to the AH/POA, as shown by AUC analysis (B). C. No significant differences in locomotor activity were observed between these treatment groups.

Figure 6. AH/POA injection of Ccl22 increased fatty acid utilization

A. 6 hr profile of respiratory exchange ratio of mice treated with vehicle of Ccl22 (10 ng/0.5ul) in the AH/POA, demonstrating that Ccl22 injection decreased RER, indicating an elevation of fatty acid utilization.

Figure 7. AH/POA injection of Ccl22 increased brown adipose tissue activity

2 hr profile of PET/CT of ^{18}F -FDG uptake in rats injected with vehicle or 10 ng/0.5ul of Ccl22 in the AH/POA. The top panel shows the representative PET/CT, squared in dotted lines is the area investigated following treatment with Ccl22 or vehicle shown below. The anatomical position of BAT is indicated with an arrow.