Central administration of metformin into the third ventricle of C57BL/6 mice decreases meal size and number and activates hypothalamic S6 kinase

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METFORMIN IS A BIGUANIDE DRUG that is widely used to reduce blood glucose levels in both diabetic patients and mice (9, 26, 30). It has been proposed to reduce hepatic glucoseogenesis via activation of AMPK, or independently, via other signaling molecules, such as the mammalian target of rapamycin/S6 kinase (mTOR/S6K) (9, 13, 14, 35). In addition to the beneficial action of metformin in glucose regulation, it has also been shown to reduce body weight in humans and rodents (15, 26). The weight loss induced by metformin in humans and mice has mainly been attributed to a reduction in food intake (15, 19, 27, 30). Despite the clear evidence for reduced food intake after metformin treatment, the underlying mechanism of its anorectic action remains unclear.

The hypothalamus is a key player in the brain’s regulation of food intake (1, 21, 32). Various molecules involved in the regulation of food intake are integrated via signaling molecules in hypothalamic neurons to determine food consumption. AMPK and mTOR/S6K have been proposed as the main molecular sensors to monitor energy status in neurons (1, 33). Hypothalamic AMPK plays a critical role in the alterations in energy balance induced by hormones, including leptin, ghrelin, and insulin, and by dietary nutrients, including glucose and fatty acids (18). The change in AMPK activity requires either phosphorylation of AMPK at Thr172 or Ser485/491 (7, 20). Another important player in the regulation of energy balance is hypothalamic mTOR/S6K. Hormones (such as leptin) and dietary nutrients (including branched amino acids such as leucine) have been shown to decrease food intake via stimulation of hypothalamic S6K in rats and mice (2, 6). In addition, hypothalamic mTOR/S6K has been shown to interact with AMPK through phosphorylation sites at Ser485/491 (7). AMPK has also been hypothesized to regulate mTOR/S6K in the hypothalamus in a bidirectional, inhibitory manner (6).

The details of hypothalamic involvement in metformin-induced anorexia have not been clarified by previous studies (16, 19, 27). Some reports have shown that metformin treatment affects the hypothalamic neuropeptide Y (NPY) system in obese rats and mice, although the effect of metformin on neuropeptide systems requires further investigation (19, 27). A direct effect of metformin on hypothalamic AMPK was not observed in studies of food intake, despite the strong evidence for AMPK-mediated improvement of glucose regulation induced by metformin (16, 19, 29, 35). In addition to the association between the hypothalamus and metformin-induced anorexia, we have shown that peripheral administration of metformin may reduce food intake via neuronal activation in the hindbrain regions that mediate satiety signals from peripheral tissues (15). However, the potential involvement of the hypothalamus in the anorectic action of peripherally administered metformin cannot be excluded because it can easily cross
the blood-brain barrier (17, 19). Therefore, it remains to be
determined whether central administration of metformin can affect
food intake via regulation of the primary molecular
energy sensors in the hypothalamus.

In this study, we determined whether direct administration
of a range of doses of metformin into the third ventricle affects
the energy balance in mice by performing meal pattern analysis
and by comparing energy expenditure. In addition, we deter-
mined whether centrally administered metformin produced
either illness or food aversion by performing a conditioned
taste aversion (CTA) test in mice. We also investigated
whether centrally administered metformin could modulate key
energy sensors in the mediobasal hypothalamus, such as
AMPK and S6K, as well as signaling molecules related to
leptin and insulin pathways, such as the STAT3 and Akt.

MATERIALS AND METHODS

Animals. Male C57BL/6 mice were obtained from Orient (Seoul,
Republic of Korea) and were maintained in a room with controlled
temperature and humidity (22°C, 60% relative humidity) before the
experiments commenced. All animals were aged 8 wk upon arrival at
the laboratory and were housed individually in standard mouse cages
under a 12-h light-dark cycle (lights off at 1800). Water and standard
laboratory chow diet (5L79 Purina rat and mouse 18% Chow; Charles
River Laboratories, Wilmington, MA) were provided ad libitum. All
experimental procedures followed the guidelines on the ethical use of
animals after all animal protocols were approved by Institutional
Animal Care and Use Committee of Korea University.

Drugs. 1,1-Dimethylbiguanide hydrochloride (metformin; Sigma-
Aldrich, St. Louis, MO) was dissolved in artificial cerebrospinal fluid
(aCSF; Tocris Bioscience, Bristol, UK). Rapamycin (Calbiochem,
San Diego, CA) was dissolved in DMSO (Sigma-Aldrich). The
vehicles for each drug were used as controls.

Third ventricle cannulation. The animals were anesthetized with
intraperitoneal injection of pentobarbital sodium (50 mg/kg) and
underwent third ventricular cannulation, as previously described (4).
The mice were placed into a stereotaxic frame (David Kopf Instru-
ments, Tujunga, CA). A burr hole was drilled for the implantation of
a 26-gauge guide cannula (Plastics One, Roanoke, VA) into the third
ventricle. The tip of a guide cannula was placed just above the third
ventricle at the following coordinates (10): 0.825 mm posterior to
the bregma, midline, 4.8 mm below the skull surface. A 30-gauge dummy
cannula (Plastics One) was inserted into the guide cannula to maintain
patency. The animals were allowed to recover for at least 1 wk
following cannulation surgery before the experiments.

After the recovery period, cannula placement was verified as de-
scribed previously (3). Mice were administered 1 μg of NPY (Sigma-
Aldrich) at a volume of 1 μl for 30 s into the third ventricle (I3V)
during the middle of the light phase. Mice that ate more than 0.5 g of
food pellets in the subsequent 2 h were used for experiments.

Single I3V administration of metformin. For microinjection, the
dummy cannula was replaced with a 33-gauge injection cannula
(Plastics One), which was connected to a Hamilton syringe (Hamilton,
Reno, NV), driven by a remotely controlled infusion pump (model
102; CMA Microdialysis AB, Stockholm, Sweden). Mice were fed
prior to I3V administration of metformin (7.5, 15, or 30 μg). Control
solution (aCSF) or metformin was delivered into the third ventricle at
a volume of 2 μl for 1 min, and the injection cannula was withdrawn
1 min after the end of injection.

Meal pattern analysis. The effect of I3V administration of metformin
on spontaneous feeding behavior was evaluated by performing meal
pattern analysis, as described previously (15). Control or metformin (7.5,
15, or 30 μg) solutions were administered into the third ventricle of
the mice 1 h before the start of experiments. The mice were individually
placed into the open-field area of a cage (200 × 200 × 370 mm) after
administration of the solution. A food bin filled with food pellets and a
water bottle were both freely accessible to each mouse at all times. To
minimize food spillage, the wall of the chamber facing the food bin was
designed so that the spillage could return to the food bin. The weight of
the food bins was measured every second by using an automatic data
acquisition unit (National Instruments, Austin, TX). Raw data were analyzed
using custom-designed software written in LabVIEW 11 (National Instru-
ments). Meal pattern analysis was started at the beginning of the dark
phase (at 1800) after I3V administration of control or metformin solution,
and data were collected for 22 h. A meal was defined as the removal of
>0.01 g from the food bin for at least 10 s and following a 5-min intermeal
interval (IMI, the time elapsed between the end of one meal and the
start of the next). The meal continued until 5 min elapsed with no
consumption. Meal size was calculated as the change in the weight of
a food bin between the start and end of each meal. Mice that had
a tendency to gnaw the pellets until they were powdered were
excluded.

Energy expenditure. To determine whether changes in energy expen-
diture contributed to body weight change elicited by central administra-
tion of metformin, we assessed energy expenditure by indirect calorim-
etry (Oxylab; Panlab, Cornellà, Spain) after I3V administration of met-
formin (30 μg) in a separate experiment. A pair-fed (PF) group was
provided the same amount of food as the metformin-treated group every
12 h. The mice were adapted to the calorimeter chambers for 2 days prior to
I3V administration. Food and water were freely available, and resting
energy expenditure was recorded for the following 24 h. Oxygen
consumption (V\textsubscript{O\textsubscript{2}}) and carbon dioxide production (V\textsubscript{CO\textsubscript{2}}) were
measured continuously for 3 min at 30-min intervals. V\textsubscript{O\textsubscript{2}} was adjusted for
body weight to the power of 0.75, and respiratory quotient (RQ) was
calculated by dividing V\textsubscript{CO\textsubscript{2}} by V\textsubscript{O\textsubscript{2}} (31).

CTA. To determine whether I3V administration of 30 μg of met-
formin caused visceral illness, we performed a CTA test in lean mice.
Briefly, the animals were divided into three groups: an intraperitoneal
saline- and I3V control-treated (IP SAL/I3V CON) group, an intra-
peritoneal saline- and I3V metformin-treated (IP SAL/I3V M30)
group, and an intraperitoneal lithium chloride (a potent aversive agent,
0.15 M)- and I3V control-treated (IP LiCl/I3V CON) group. On the
conditioning day, after 22 h of water deprivation, the mice were I3V
administered control or metformin solution at a volume of 2 μl for
1 min and IP administered saline or equimolar LiCl (1% body weight).
Mice were given saccharin-flavored water (0.15% saccharin dissolved
in water) for 2 h immediately after the administration, and then, the
saccharin-flavored water was replaced with water. On the next day,
the mice were simultaneously given access to both saccharin-flavored
water and water after a 23-h water deprivation period. Fluid consump-
tion was recorded at 1, 2, 4, and 24 h, and preference ratios were
calculated by dividing saccharin-flavored water consumption by total
fluid consumption.

Double I3V administration of metformin and rapamycin. To de-
termine whether the anorexia induced by I3V administration of met-
formin was mediated by S6K activity in the hypothalamus, mice were
I3V administered either rapamycin (RAPA; 50 ng) or DMSO 1 h
before I3V administration of either metformin (M50, 50 μg) or
control (CON, aCSF). Each compound was delivered into the third
ventricle at a volume of 2 μl for 1 min. Food intake and change in
mouse body weight were measured in the home cages. In this experimental
design, we had to increase the dose of metformin to 50 μg to achieve the
same degree of anorexia as the single I3V admin-
istration study.

Western blot analysis. To investigate the involvement of key
energy sensors, such as AMPK and S6K, in the anorectic action of
centrally administered metformin, the 6 mice were killed 1 h after I3V
administration of the control solution or metformin (30 μg) in a
separate experiment. The mediobasal hypothalamus was quickly dis-
sected, according to the methods used in a previous study (34). Tissue
was homogenized and lysed with radioimmunoprecipitation assay
buffer (Triton X-100 1.25%, SDS 0.1%, 50 mM Tris-HCl, 150 mM
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NaCl, 5 mM EDTA, pH 7.6) supplemented with a mixture of phosphatase inhibitors, sodium fluoride (Sigma-Aldrich), and sodium pyrophosphate decahydrate (Sigma-Aldrich). A total of 20 μg of protein was separated by 10% SDS-PAGE, and then transferred electrophoretically to polyvinylidene difluoride membranes. The membranes were blocked in TBS (10 mM Tris, 150 mM NaCl, pH 8.0) with 5% skim milk or 1% BSA (Sigma-Aldrich) and incubated overnight at 4°C with a primary antibody against phospho-AMPKα (Thr172) (1:1,000), phospho-AMPKα1 (Ser485)/AMPKα2 (Ser491) (1:1,000), AMPK-α (1:2,000), phospho-p70 S6 kinase (Thr389) (1:1,000), p70 S6 kinase (1:2,000), phospho-STAT3 (1:500), STAT3 (1:1,000), phospho-Akt (1:1,000), Akt (1:2,000), or β-actin (1:4,000). All primary antibodies were purchased from Cell Signaling Technology (Beverly, MA). After washing in TBS containing 0.05% Tween-20 (TBST), membranes were incubated in HRP-conjugated secondary antibody (1:2,000, Cell Signaling Technology) for 1 h at room temperature. After further washing in TBST, the proteins were visualized using an ECL kit (Thermo Scientific, Rockford, IL). The intensity of the bands was analyzed using ImageJ software (National Institutes of Health, Bethesda, MD) and normalized to the intensity of an internal control (β-actin or alternate form of assayed protein).

Statistical analysis. Results are presented as means ± SE. All data from animal studies were analyzed by two-way repeated-measures ANOVA followed by Bonferroni’s multiple comparisons, one-way ANOVA followed by the Tukey post hoc test, or two-tailed Student’s t-test by using GraphPad Prism 5 software (GraphPad Software, San Diego, CA). For the Western blot analysis, the intensity of the bands in the groups was compared using two-tailed Student’s t-test. Statistical significance was accepted for P values of <0.05.

RESULTS

The effect of central administration of metformin on food intake and body weight. Compared with the administration of the control, administration of 15 and 30 μg of metformin (M15 and M30, respectively) into the third ventricle significantly reduced food intake after 22 h (Fig. 1A). Two-way repeated-measures ANOVA that was performed to analyze cumulative food intake showed significant effects of time (F4,172 = 173.89; P < 0.001) and doses of metformin (F3,43 = 3.67; P < 0.05). Bonferroni’s multiple comparisons revealed a significant difference in food intake between the control mice and the mice that received M15 and M30, but not 7.5 μg of metformin (M7.5), over a range of time points, including 4, 8, 12, and 22 h after treatment (Fig. 1A).

I3V administration of metformin was found to significantly reduce food intake at various time intervals over 0 – 8 h when analyzed on the basis of the time interval after metformin treatment by one-way ANOVA (F3,31 = 11.59, P < 0.001 for 0 –1-h time interval; F3,31 = 8.88, P < 0.001 for 1–2-h time interval; F3,31 = 12.19, P < 0.001 for 2– 4-h time interval; and F3,31 = 14.91, P < 0.001 for 4–8-h time interval). A Tukey post hoc test showed that compared with the control, M30 significantly reduced food intake at time intervals of 0–1 h, 1–2 h, 2–4 h, and 4–8 h, and M15 reduced food intake at intervals of 0–1 h, 1–2 h, and 2–4 h, indicating a potent effect on food intake of central administration of metformin.

Fig. 1. Administration of metformin into the third ventricle (I3V) decreases body weight and energy intake. I3V administration of metformin decreases cumulative food intake over the course of 22 h (A), food intake during designated time intervals (B), and body weight change after 24 h (C). I3V administration of metformin decreases both nocturnal meal size and number (D and E). Data are presented as means ± SE. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. CON. +P < 0.05, ++P < 0.01, and +++P < 0.001 vs. M7.5. BP < 0.05 vs. M15. CON, control artificial cerebrospinal fluid (aCSF)-treated group (n = 8); M7.5, 7.5 μg metformin-treated group (n = 9); M15, 15 μg metformin-treated group (n = 9); M30, 30 μg metformin-treated group (n = 9). I3V administration of metformin did not cause illness (F). Preference ratio was calculated by dividing the volume of saccharin intake by the total volume of fluid intake after I3V and intraperitoneal administrations. IP SAL/I3V CON, IP saline- and I3V control-treated group (n = 6); IP LiCl/I3V CON, IP lithium chloride- and I3V control-treated group (n = 6); IP SAL/I3V M30, IP saline- and I3V metformin-treated group (n = 5); IP, intraperitoneal administration. Data are presented as mean ± SE. *P < 0.05; **P < 0.01 vs. IP LiCl/I3V CON.
of centrally administered metformin on food intake during the early period of treatment (Fig. 1B).

Compared with the control, M30 significantly decreased body weight after 24 h \( (F_{3,31} = 4.50, P < 0.01; \) Fig. 1C). By 48 h after treatment, the decrease in body weight induced by M30 recovered to the level measured before metformin administration, indicating a transient effect of M30 on body weight (2.01 ± 1.14 g increase with 104.16% recovery).

Effect of centrally administered metformin on meal size and meal number. For a detailed analysis of changes in feeding behavior elicited by metformin treatment, we investigated patterns of meal size and meal number based on the time intervals after IV administration of the control solution or metformin.

One-way ANOVA followed by the Tukey post hoc test revealed that compared with the control, M15 and M30 significantly decreased both meal size \( (F_{3,31} = 10.83, P < 0.001) \) and meal number \( (F_{3,31} = 13.73, P < 0.001) \) during the first 1 h after treatment. Afterward, compared with the control, M30 significantly reduced meal size at the time intervals of 1–2 h \( (F_{3,31} = 5.99, P < 0.01) \) and 2–4 h \( (F_{3,31} = 3.91, P < 0.05) \), and reduced meal number at intervals of 1–2 h \( (F_{3,31} = 8.52, P < 0.001) \) and 4–8 h \( (F_{3,31} = 3.90, P < 0.05) \) (Fig. 1D and E). These results indicated that administration of metformin into the third ventricle decreased food intake by reducing both meal size and meal number during the early period of treatment and that the reduced food intake was maintained until 22 h after treatment.

Effect of centrally administered metformin on CTA. CTA was produced in the IP LiCl/IV CON group, but not in the IP SAL/IV CON group or the IP SAL/IV M30 group. The preference ratio in the IP LiCl/IV CON group was significantly lowered compared with that in the other two groups \( (F_{2,14} = 7.352, P < 0.01) \). However, no significant difference was observed in the preference ratios between the IP SAL/IV CON group and the IP SAL/IV M30 group (Fig. 1F). These results indicated that central administration of metformin was not associated with aversion or illness.

Effect of centrally administered metformin on energy expenditure. Nocturnal RQ ratios were significantly lower in the M30-treated and PF groups than in the control mice \( (F_{2,21} = 9.39, P < 0.01; \) Fig. 2A). However, no differences in diurnal RQ ratios were observed between the groups (Fig. 2B). Nocturnal and diurnal energy expenditure expressed as oxygen consumption \( (\hat{V}O_2) \) adjusted for calculated body mass was also not significantly different between the groups (Fig. 2, C and D). However, compared with the control-treated mice and the PF group, the M30-treated group showed significantly reduced energy expenditure and RQ ratios during the first 3 h after IV administration \( (F_{2,21} = 8.90 \) and \( F_{2,21} = 10.59, \) respectively, all \( P < 0.01; \) Fig. 2, E and F).

Effect of centrally administered metformin on hypothalamic energy sensors. To investigate the direct effect of metformin on key hypothalamic energy sensors, we compared changes in phosphorylation of AMPK and S6K 1 h after IV administration of metformin at the dose and time point that potently reduced food intake in the meal pattern study. M30 slightly decreased phosphorylation of AMPK at Thr172 in the mediodasal hypothalamus, which was associated with an increase in AMPK activity \( (P = 0.092; \) Fig. 3A). However, metformin significantly increased the phosphorylated form of AMPK at Ser485/491 in the mediobasal hypothalamus, which inhibited AMPK activity \( (P < 0.05; \) Fig. 3B).

Next we determined whether hypothalamic S6K, another key energy sensor, was associated with metformin-induced
anorexia. M30 significantly increased S6K phosphorylation at Thr389 in the mediobasal hypothalamus (P < 0.001; Fig. 4A). Furthermore, M30 decreased both Akt and STAT3 phosphorylation in the mediobasal hypothalamus (P < 0.05; Fig. 5, A and B), indicating that the hypothalamic leptin and insulin signaling pathways may not be directly involved in metformin-induced anorexia.

Effect of rapamycin pretreatment on metformin-induced anorexia. We chose a subthreshold dose of rapamycin (50 ng) that did not influence body weight or food intake in this experiment. Compared with both the DMSO- and CON-treated (DMSO/CON) group and the RAPA- and CON-treated group, the DMSO- and M50-treated (DMSO/M50) group showed a significant decrease in food intake and body weight 24 h after I3V administration of metformin. Pretreatment with rapamycin, a selective mTOR inhibitor, mitigated the weight loss and anorexia observed in the DMSO/M50 group (F3,37 = 14.59 and F3,37 = 9.59, respectively, all P < 0.001; Fig. 4, B and C), suggesting an important role for hypothalamic mTOR/S6K signaling in metformin-induced anorexia.

DISCUSSION

The aim of this study was to determine the direct hypothalamic effect of metformin on food intake and key energy sensors, such as AMPK and S6K, by its direct administration into the third ventricle in the mice. In the meal pattern analysis, we showed that I3V administration of metformin significantly reduced food intake in a dose-dependent manner, which manifested as a reduction in both meal size and meal number during the early period of treatment. Of particular importance was the observation that I3V administration of metformin at a dose eliciting a profound reduction in food intake did not produce aversion or sickness in the mice. Furthermore, we provided evidence that this reduction in food intake was accompanied by increased phosphorylation of AMPK (at Ser485/491) and S6K (at Thr389) in the mediobasal hypothalamus and that metformin-induced anorexia was attenuated by inhibition of mTOR/S6K signaling within the hypothalamus. These results suggest the involvement of key energy sensors, such as S6K, in the anorectic action of centrally administered metformin.

The findings of our study clearly show evidence of metformin’s action in the hypothalamus. Despite the endeavors of previous researchers, indications of the involvement of the hypothalamus in metformin-induced weight loss, as well as the details of the underlying mechanism have been conflicting (16, 19, 27). Moreover, acute administration of metformin into the lateral cerebral ventricle had no effect on food intake in rats (29). However, since it is possible that metformin can affect the hypothalamus by crossing the blood-brain barrier (17, 19, 21), it was more reasonable to investigate metformin’s effects on hypothalamic regulation of food intake by administering it into the third ventricle (1, 12). In this study, we found that I3V administration of metformin adjacent to the hypothalamus decreased food intake in mice. In addition, I3V administration of metformin decreased both meal size and meal number, mainly during the nocturnal period. Energy intake is a function of meal size and number, which are associated with meal termination and initiation, respectively. These factors have been shown to have reciprocal compensatory action via independent mechanisms (28). Therefore, the decreases in both meal size and meal number support a potent inhibitory action of central metformin on food intake. Moreover, it should be noted that the reduced food intake was mediated via physiological changes rather than by toxic effects of metformin treatment, as shown by the results of the CTA test (8).

Overall, nocturnal and diurnal energy expenditures were unchanged, but rather transiently decreased during the early period after metformin treatment. Consistent with the reduced food intake, the nocturnal RQ ratio was lower in the metformin-treated group, implying that fat was used as an energy substrate. These results suggest that the change in the energy expenditure did not contribute to the body weight loss observed after metformin treatment.
Activation of AMPK induced by metformin improves glucose regulation via an increase in fatty acid oxidation, glucose uptake, and a decrease in lipogenesis in liver and skeletal muscle (11, 35). Hypothalamic AMPK activity contributes to the regulation of food intake, i.e., activation of AMPK in the hypothalamus increases food intake in vivo and increases NPY gene expression in the hypothalamus (2, 6). This might be related to the previous finding that inhibition of AMPK activity following activation of S6K has been shown to suppress food intake (7, 20).

Interestingly, we observed that the phosphorylation of critical signaling molecules related to leptin and insulin action, including STAT3 and Akt, decreased in the mediobasal hypothalamus after I3V administration of metformin compared with that of the control. Leptin and insulin are considered adiposity signals and are used to monitor energy levels in the body (32). Central administration of leptin or insulin decreases food intake via activation of hypothalamic STAT3 or Akt, which mediates their anorectic action (22–24). Our study suggests that the key molecules related to leptin and insulin signaling might not directly participate in metformin-induced anorexia. Furthermore, the decrease in hypothalamic Akt phosphorylation induced by metformin may be associated with the negative feedback control of enhanced mTOR/S6K signaling (25), although the detailed interaction and role of these signaling molecules needs further investigation.

**Perspectives and Significance**

Our study showed that central administration of metformin into the murine third ventricle near the hypothalamus, a key region for the regulation of food intake, decreased food intake mainly via reduction in both the size and number of meals. Notably, while metformin treatment caused profound anorexia, it did not produce taste aversion in mice. In addition, our study suggests that metformin-induced anorexia may be mediated by an increase in S6K phosphorylation at Thr^{172}, and presumably by subsequent AMPK phosphorylation at Ser^{485/491}, although the interaction of these specific pathways should be investigated in future studies. Furthermore, we postulate that key molecules related to leptin and insulin action, STAT3 and Akt, are not directly associated with metformin-induced anorexia. Therefore, we have provided novel data to help clarify the mechanism underlying the anorectic action of centrally administered metformin. Further detailed studies of the mechanism of metformin-induced anorexia based on these results may enhance their clinical application.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

REFERENCES