Longer-Term Fourth Ventricular 5-Thioglucose Infusion Increases Body Fat in the Rat

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Abstract. **5-Thioglucose (5-TG) has been shown to increase food intake after acute administration. To determine the longer-term effects of 5-TG on feeding and body composition, thirty-four female Sprague-Dawley rats were cannulated into the fourth ventricle and infused with artificial CSF and either 0.01 *M* 5-TG or 0.1 *M* 5-TG using osmotic pumps. Food intake and body weight were monitored daily. Rats were killed after 14 days of infusion. Carcass and fatpad weights were measured, and body compositions were determined. Food intake was not different during the first week of infusion; however, cumulative food intake was decreased in the 0.1 *M* 5-TG group during the second week as compared to the CSF control group. Body weight and carcass weight of this group also decreased as compared to the control. The group receiving the higher dose of 5-TG (0.1 *M*) had increased fatpad weights in all three depots examined (inguinal, retroperitoneal, and perimetrial depot); the group with lower dose of 5-TG infusion (0.01 *M*) increased the fatpad weights in the retroperitoneal and perimetrial depot, as compared to the CSF group. Data from the body composition analysis were consistent with the results of the fatpad weights. In conclusion, the present study demonstrated that chronic fourth ventricular 5-TG infusion increased body fat without increasing food intake, suggesting that energy expenditure is decreased under this condition. The results of this study indicate that glucose metabolism in the hindbrain is important in the control of energy expenditure, body fat deposition, and thus energy balance regulation.** [P.S.E.B.M. 1998, Vol 217]

Glucose analogs that induce glucoprivation have been found to increase feeding in many species (1–3). Two widely used glucose analogs are 2-deoxy-D-glucose (2-DG) and 5-thioglucose (5-TG). 5-Thioglucose differs from glucose by having sulfur substituted for the oxygen in the pyranose ring. It has been shown that 5-TG is more potent than other glucose analogs in eliciting glucoprivic feeding and hyperglycemia (4).

Most studies of glucoprivic feeding have used a single dose of the glucoprivic agent, examining only the short-term effects. The purpose of this study was to determine the longer-term effects of 5-TG infusion on feeding, body weight, and carcass composition.

Considerable evidence suggests that the sites of action for glucoprivic feeding are centrally located. Earlier studies suggested the presence of glucose-sensitive and glucose-responsive neurons within the hypothalamus (5, 6). More recent studies suggest that glucoreceptors may be located in the caudal hindbrain (7, 8). Therefore, in the present study, the fourth ventricle was chosen as the route of administration for 5-TG infusion because of its proximity to the caudal hindbrain.

Materials and Methods

Animals. Thirty-four, female Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 220–250 g, were kept in individual cages in a temperature-controlled room (23 ± 3°C) on a 12:12 hr light-dark cycle and with free access to ground lab chow (Purina 5012) and water.

All procedures involving animals were reviewed and

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This work was supported by NIH grant HD22226.

Portions of this study were previously presented at Experimental Biology 94, Anaheim, CA [He B, White BD, Martin RJ. Chronic 4th ventricular infusion of 5-thioglucose increases body fat in the rat. FASEB J 8:A172, 1994. (abs)].

Received May 19, 1997. [P.S.E.B.M. 1998, Vol 217]
Accepted August 24, 1997.

0037-9727/98/2172-0168$10.50/0

approved by the Institutional Animal Care and Use Committee at the University of Georgia.

Rats were cannulated in the fourth ventricle, and subcutaneous osmotic minipumps were connected to the cannula as described below. The osmotic pumps were filled with 0.01 *M* 5-TG (n = 12), 0.1 *M* 5-TG (n = 13) or the vehicle (n = 9), artificial cerebrospinal fluid (CSF), which contained 128.6 m*M* NaCl, 3.1 m*M* $KHCO_3$, 0.9 m*M* $MgCl_2$, 21.4 m*M* $NaHCO_3$, 1.2 m*M* $CaCl_2$ and 2.7 m*M* glucose (pH 7.4). Solutions had previously been filtered sterilized through a 0.22-μm filter (Millipore, Bedford, MA). All solutions were delivered to the rats at a rate of 0.5 μl/hr.

Food intake and body weight were measured daily. After 14 days, rats were killed by decapitation, and trunk blood was collected for the determination of serum glucose and insulin concentrations. The gastrointestinal tract was removed, and the carcass weight was determined. Fatpads (inguinal, retroperitoneal, and perimetrial depot) were excised, weighed, and returned to the carcass. Body composition analyses were performed as described below. Additionally, 10 μl of methylene blue dye was injected into the fourth ventricular cannula to verify cannula placement. Only data from rats with appropriate cannula placement were used in the statistical analysis.

Fourth Ventricular Cannulation. Each rat was anesthetized with a mixture of ketamine/acepromazine/xylazine (50 mg/kg ketamine; 3.3 mg/kg acepromazine; 3.3 mg/kg xylazine). The hair on the top of the head was shaved. The rat was placed in a stereotaxic apparatus, and a midline incision was made to expose the skull between bregma and occipital-interparietal suture. The cannula hole was drilled through the skull on the midline, determined from lambda, and 2 mm rostral to the occipital-interparietal suture. Four additional holes were made around the cannula hole, and small screws were inserted with the tip just penetrating the bone. A 22-gauge osmotic pump connector cannula (Plastics One, Roanoke, VA) was placed with the tip of the cannula 6.0 mm ventral to the surface of dura. The osmotic pump was connected to the cannula with polyethylene tubing and inserted into a pocket made under the skin between the scapulae. Dental cement was placed over the cannula and screws to fix the cannula to the skull. The rat was removed from the stereotaxic apparatus and placed in a warm shoebox cage to recover from anesthesia.

Determination of Serum Glucose and Insulin Concentrations. Serum glucose concentrations were determined enzymatically by the glucose oxidase method (Sigma Chemical, St. Louis, MO). Serum insulin concentrations were determined by an RIA kit (ICN Pharmaceuticals, Inc., Costa Mesa, CA).

Body Composition Analysis. The frozen carcasses were autoclaved in individual sealed beakers for 40 min at 120°C. When cool, each carcass was homogenized with three times of its own weight of water in a Waring blender (Fisher, Pittsburgh, PA). The slurry was then transferred to a large plastic container and homogenized for several minutes with a Polytron tissue homogenizer (Brinkman Instrument, Westbury, NY). Samples of homogenate for water, ash, and fat analysis were taken while the homogenizer was running. The percentage of carcass fat was determined in triplicate on 7-ml samples of homogenate by chloroform:methanol extraction. The percentage of water was determined by drying the samples for 48 hr at 85°C. The samples were subsequently placed in an ash oven set at 600°C overnight to determine the percentage of ash. Percent protein was determined by the difference. Total body water, fat, protein, and ash were determined by multiplying the ratio of each compartment by the carcass weight.

Statistical Analysis. Statistical analysis was performed using Abacus Concepts, SuperANOVA (Abacus Concepts, Inc., Berkeley, CA). Differences between treatments were determined by analysis of variance, and Fisher's protected LSD (Abacus Concepts) was used for multiple comparisons. In cases where the variances of the groups were not equal, the analysis was performed on the log values of the data. Differences were deemed statistically significant if $P < 0.05$.

Results

During the first week of infusion, cumulative food intake was not different between any of the three groups; however, during the second week, cumulative food intake was decreased ($P < 0.01$) in the animals receiving 0.1 *M* 5-TG as compared to animals receiving CSF and 0.01 *M* 5-TG (Fig. 1). Body weight of this group was also decreased ($P < 0.05$, Fig. 2). The differences in food intake and body weight between the 0.01 *M* 5-TG group and the CSF control were not statistically significant.

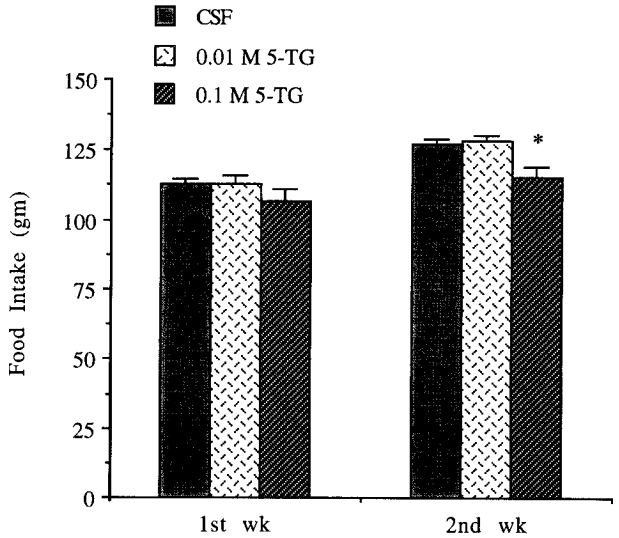

Figure 1. Cumulative food intake during 5-TG infusion. 5-TG (0.01 *M* or 0.1 *M*) or CSF was continuously infused into the fourth ventricle for 14 days; food intake was measured daily. Each value represents mean ± SEM of 9–13 observations. *$P < 0.01$ as compared to CSF group.

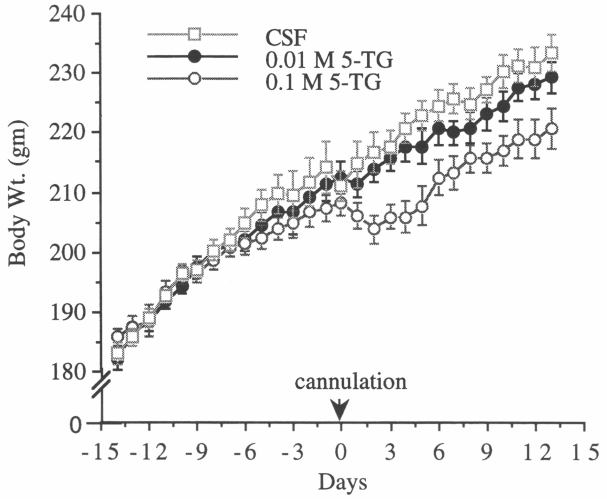

Figure 2. Body weight change during 5-TG infusion. 5-TG (0.01 *M* or 0.1 *M*) or CSF was infused into the fourth ventricle for 14 days. Body weight was monitored daily. Each point represents mean ± SEM of 9–13 observations.

Fatpad weights (inguinal, retroperitoneal, and perimetrial depot) all increased in the group receiving the higher dose of 5-TG (0.1 *M*) as compared to the group receiving CSF ($P < 0.01$), both on an absolute basis (grams) and as a percentage of the carcass weight (Fig. 3). Total fatpad weights were approximately 60% greater in animals treated with 0.1 *M* 5-TG as compared to animals treated with CSF. The lower dose of 5-TG (0.01 *M*) increased the fatpad weights for the retroperitoneal and perimetrial depots as compared to the CSF ($P < 0.01$). The differences in fatpad weights between the two 5-TG groups were not statistically significant.

Data from the body composition analysis were consistent with the results of the fatpad weights. Rats infused with 0.1 *M* 5-TG had more body fat than rats infused with CSF ($P < 0.05$, Table I). This was true whether expressed as absolute values or relative to the carcass weight. The lower dose of 5-TG also increased the amount of body fat as compared to CSF; however, this difference did not quite reach statistical significance ($P = 0.052$). The lean body mass (protein content) of the 0.1 *M* 5-TG-treated group was decreased as compared to the control, but the decrease in body weight of rats infused with 0.1 *M* 5-TG was due largely to a decrease of carcass water (Table I).

Serum glucose concentration (140.76 ± 4.3, 148.21 ± 2.99 and 150.93 ± 6.66 mg% in CSF, 0.01 *M* and 0.1 *M* 5-TG group, respectively) or insulin concentrations (64.76 ± 14.04, 67.62 ± 10.05 and 66.49 ± 15.36 μU/ml in CSF, 0.01 *M* and 0.1 *M* 5-TG group, respectively) were not significantly different among the three groups.

Discussion

The results from this study indicate that longer-term continuous fourth ventricular 5-TG infusion increased body fat without increasing food intake. These data suggest that chronic 5-TG infusion caused a decrease in energy expenditure in the rats. This is consistent with studies showing

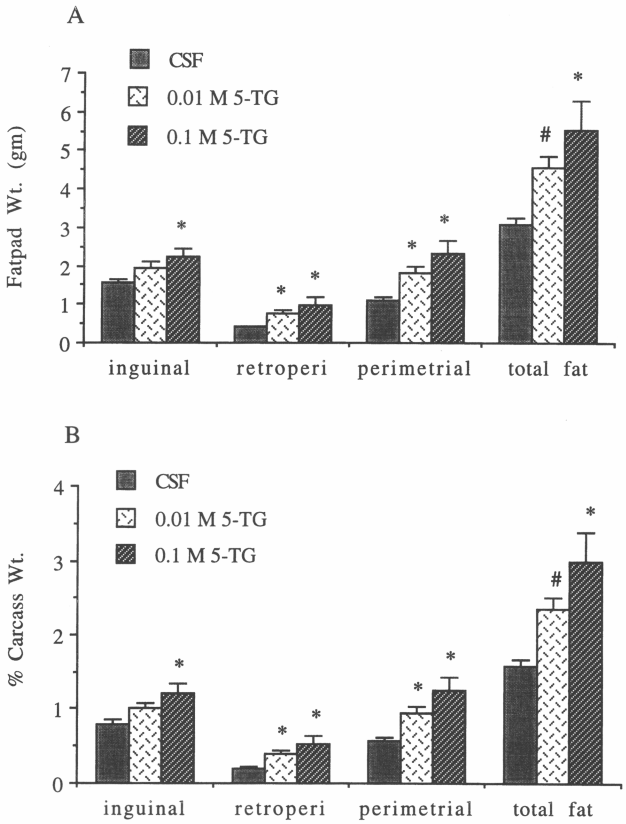

Figure 3. Effect of 5-TG infusion on fatpad weight. After 14 days of infusion of 5-TG (0.01 *M* or 0.1 *M*) or CSF into the fourth ventricle, rats were killed, and fatpads from inguinal, retroperitoneal, and perimetrial depots were excised and weighed. Fatpad weights on an absolute basis (A), fatpad weights expressed as percent of carcass weights (gram/gram × 100) (B). Each bar represents mean ± SEM of 9–13 observations. *$P < 0.01$, #$P < 0.05$ as compared to CSF group. The statistic was performed on the log values of the original data.

that central administration of another glucose analog, 2-deoxy-D-glucose (2-DG), lowers core temperature (9) and decreases brown adipose tissue (BAT) thermogenesis as measured by guanosine diphosphate (GDP) binding (10). The neurochemical factors mediating these responses are not known. Some evidence indicates that neuropeptide Y (NPY) may be a possible candidate. 2-Deoxy-D-glucose has been shown to elevate NPY content in the arcuate and suprachiasmatic nuclei of the hypothalamus (11). We have found administration of 2-DG to increase NPY gene expression in the caudal regions of the arcuate nucleus (unpublished observation).

Neuropeptide Y is well known for its powerful stimulatory effect on food intake (12–14). Chronic administration of NPY ultimately induces obesity, and the body energy gain in these rats is greater than can be accounted for by increased food intake alone (15), suggesting that NPY also reduces energy expenditure in addition to its feeding-stimulatory effect. Recent studies have provided firm evidence to support this hypothesis. NPY has been found to induce hypothermia when administered centrally (16, 17). And it has been shown that NPY inhibits BAT thermogenic activity as evidenced by decreased GDP binding (18), de-

Table I. Body Compositions

	Absolute values (grams)				Relative values (%)		
	CSF	0.01 *M* 5-TG	0.1 *M* 5-TG		CSF	0.01 *M* 5-TG	0.1 *M* 5-TG
Carcass wt.	194.92 ± 2.17	193.52 ± 2.01	184.91[a] ± 2.18				
Lipid	12.10 ± 0.24	14.44 ± 0.59	15.55[a] ± 1.28	Lipid	6.22 ± 0.16	7.46 ± 0.29	8.38[a] ± 0.66
Water	134.13 ± 1.79	131.16 ± 1.49	123.20[a] ± 1.61	Water	68.80 ± 0.28	67.78 ± 0.36	66.65[a] ± 0.65
Ash	7.60 ± 0.32	7.60 ± 0.20	7.67 ± 0.27	Ash	3.90 ± 0.17	3.93 ± 0.11	4.15 ± 0.14
Protein	41.10 ± 0.61	40.32 ± 0.55	38.49[a] ± 0.64	Protein	21.08 ± 0.16	20.83 ± 0.14	20.81 ± 0.24

Note. Each value represents the mean of 9–13 observations. Means are shown ± SEM. [a]represents a significant difference ($p < 0.05$) from the CSF group.

creases BAT uncoupling protein mRNA levels (19), and suppresses sympathetic nerve activity to BAT (20). Additionally, NPY increases white fat lipoprotein lipase activity and mRNA levels (18, 19).

Our observation that food intake was decreased in the present study may seem to disagree with a role for NPY in mediating 5-TG's effect. Yet studies have suggested that NPY may act at different brain sites to mediate different functions, including stimulation of ingestive behavior, regulation of metabolic fuels, and control of energy expenditure. It is proposed that the perifornical hypothalamus is the primary site for mediating the feeding-stimulatory effect of NPY, whereas the paraventricular nucleus (PVN) is the site that primarily controls NPY's autonomic and endocrine responses (21). It is possible that under the condition of long-term 5-TG infusion, NPY activity is elevated at specific brain regions, such as the PVN, that are mainly involved in the regulation of energy expenditure. It has been shown that injection of NPY into the PVN suppresses the sympathetic activity to BAT and reduces BAT thermogenesis (18–20).

It is possible that the decrease in food intake is a secondary effect of the increase in body fat. The fact that it took more than a week for the decrease in food intake to become significant supports this possibility. Additionally, changes in body fat were more sensitive to the dose of 5-TG than were changes in food intake. This is consistent with changes in food intake being secondary to changes in body fat. Recently, a fat specific gene (ob) has been sequenced and cloned (22). The protein product of this gene, termed OB protein or leptin, has been shown to decrease food intake (23–25). And evidence indicates that OB protein levels reflect body fat stores in the animal (26, 27). In the case of the present study, an increase in body fat in 5-TG-treated rats may be associated with an increase in the amount of OB protein released, which may act to decrease food intake. Interestingly, it has recently been suggested that a possible mechanism by which OB protein decreases food intake is through a decrease in hypothalamic NPY activity (28). Therefore, it seems that during long-term 5-TG infusion NPY activity might exhibit dynamic changes. Initially NPY activity might be increased by 5-TG infusion, which might be responsible for the decreased energy expenditure and increased body fat. As the body fat accumulated, OB protein levels might increase and thereby inhibit NPY activity, which would then decrease food intake.

Other possible mediators of 5-TG's longer-term effects include insulin and growth hormone. Insulin is an anabolic hormone and can promote energy storage as body fat. One study reported that blood insulin levels are elevated in mice treated with 5-TG for 3 weeks (29). However, we did not note a difference in blood insulin levels between 5-TG and control groups after 14 days of infusion. Studies have also shown that administration of 2-DG or 5-TG inhibit pulsatile growth hormone secretion in the rat (30, 31). Decreased growth hormone levels may enhance body fat accumulation. We did not measure growth hormone levels in this study so the possibility that growth hormone may have a role in the effects of long-term 5-TG infusion cannot be ruled out at this time.

Another possible candidate for 5-TG's action is corticotropin-releasing hormone (CRH). CRH is a component of the hypothalamo-pituitary-adrenal axis and is the major regulator of pituitary ACTH secretion (32). It has been shown that CRH decreases nocturnal- and starvation-induced food intake (33, 34). Increased CRH secretion and increased serum ACTH and corticosterone levels have been reported after 2-DG administration (35). It is possible that CRH secretion is increased after 5-TG infusion. Increased CRH and glucocorticoid levels could result in decreased feeding and energy repetition, that is, increased fat deposition and decreased lean body mass, as it is seen in Cushing's syndrome. However, without measurement of CRH and glucocorticoids, it is impossible to confirm this hypothesis.

In conclusion, the present study demonstrated that chronic fourth ventricular infusion of 5-TG increased body fat without increasing food intake, suggesting that energy expenditure is decreased under the condition of longer-term 5-TG infusion. Although the neurochemical mechanisms of this finding are not known, the results of the present study indicate that glucose metabolism in the hindbrain is important in the control of energy expenditure, fat deposition, and thus body energy balance regulation.

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