Chlorpromazine: Effect on Food Intake and Glucose Distribution in Obese and Nonobese Mice

By P. D. CULLEN*, D. C. ARNEY†, and H. A. SWARTZ

Chlorpromazine, in doses of 1 mg./Kg., caused a significant reduction of food intake, weight loss, and oxygen consumption in both gold thioglucose-obese and normal nonobese mice as compared to saline-treated controls. Tissue activities after glucose-1-C-14 administration were reduced, but the blood levels were increased.

Gold thioglucose-obese mice have been employed in numerous studies involved with the etiology of obesity and hyperphagia. A summary of these studies, presented in a previous report (1), included evidence of the role and relationship of the lateral and ventro medial hypothalamic nuclei in food-intake regulation and satiety. The ventro medial nuclei, in response to blood glucose, have been shown to inhibit a lateral hypothalamic feeding center (2, 3) which results in an inhibition of food intake (4, 5). Stimulation of the feeding center results in hyperphagia and its destruction in a temporary cessation of food intake or aphagia. It is probable that any agent that could increase or block glucose uptake by the ventro medial nuclei could influence food intake. The role of centers in the frontal lobes cannot be overlooked, and is discussed in a review by Andersson and Larsson (6).

Selected anorectic agents were observed to reduce food intake in both normal nonobese and gold thioglucose-obese mice, with *d*-amphetamine and phenmetrazine the most effective of the agents employed (7). Reduction was much higher in the normal nonobese mice. These agents also caused a significant increase in residual hypothalamic activity following the administration of labeled glucose in the nonobese mice (1).

Interest arose over the effects of central depressants, and chlorpromazine was selected on the basis of its reported actions on hypothalamic centers, such as depression of emotional centers, sedative action, and tissue distribution (8–10).

EXPERIMENTAL

Food Intake and Weight Loss.—Gold thioglucose-obese mice were prepared as previously described (1, 7), and the treated and nontreated mice were maintained in separate groups for 3 to 4 months until a weight plateau was observed. At this time the mice were placed into individual cages with free access to food and water. Food was placed in hoppers suspended from the cage lid. After a period of 2 weeks to allow for adjustment to environment and isolation, the normal body-weight change and average daily food intake for a 7-day period was determined for both groups of mice. Food intake was determined by accurately weighing the food on the first and final day, and weight changes were determined from the initial and final body weights. The control values for both groups of mice were obtained from animals administered saline i.p. daily in volumes equivalent to those employed for the administration of chlorpromazine. Chlorpromazine,[1] as the hydrochloride, was administered in saline i.p. daily in a dose of 1 mg./Kg. (2.83 μ moles/Kg.). The observed values for both groups of mice are tabulated in Table I.

Oxygen Consumption.—Oxygen consumption for both groups of mice was determined by means of a spirometer (Minute Oxygen Uptake Spirometer, V68808 Aloe Scientific). Saline and chlorpromazine were administered as previously described 10 min. prior to placing the animals in the spirometer chamber. Following a 20-min. equilibration period, the oxygen consumption was determined for a 60-min. period, the values corrected to S.T.P. and the ml./hr./Gm. determined. From these values the Gm.-cal./hr./cm.^{2} were calculated. The values for both groups of mice are tabulated in Table II.

Glucose-1-C-14 Distribution.—The effect of chlorpromazine on residual tissue activity after the administration of glucose-1-C-14 was determined in both groups of mice. Saline was employed for the controls. The glucose-1-C-14 dose was 0.05 μc./Gm. and was administered i.p. in an aqueous solution of glucose 20 mg./ml., which gave a dose of 100 mg./Kg. Chlorpromazine and saline were administered as previously described 30 min. prior to the administration of the labeled glucose. The animals were sacrificed by decapitation 30 min. after the administration of the labeled glucose. Blood was collected in a porcelain container which was previously smeared with a saturated solution of sodium citrate to prevent coagulation, and a sample was obtained by means of micropipets. Tissue samples of adrenal, kidney, liver, and muscle were obtained, wiped free of external blood, and accurately weighed. The intact brain was removed, chilled in a deep freeze, and dissected so as to separate the cerebrum, diencephalon (thalamus and hypothalamus), and the hind brain. Weighed samples of each area were obtained and placed, along with the blood and other tissue samples, into individual counting vials containing 1 ml. of 1 *M* benzethonium chloride[2] base in methanol (11). The vials were sealed with screw caps and incubated at 55° for 24 hr. to facilitate solution, then cooled to room temperature, and 10 ml. of a liquid scintillation solvent system added (2,5-diphenyloxazole 0.4%, naphthalene 5.0%, cellosolve 300 ml., 1,4-dioxane 300 ml., and toluene to 1000 ml.) (12). The vials and contents were dark-adapted and temperature adjusted to −20° for 48 hr. A standard of the labeled glucose solution employed for the administration of the glucose-1-C-

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* Present address: Eli Lilly and Co., Greenfield, Ind.

† Present address: Long Hospital, Indiana University Medical Center, Indianapolis.

[1] Chlorpromazine HCl supplied by Smith Kline & French Laboratories, Philadelphia, Pa.

[2] *p*(Diisobutylcresoxyethoxyethyl) - dimethylbenzyl - ammonium chloride. Marketed as Hyamine by the Rohm and Haas Co.

TABLE I.—WEIGHT LOSS AND DAILY FOOD INTAKE IN GOLD THIOGLUCOSE-OBESE AND NONOBESE MICE[a]

	Obese Mice		Nonobese mice	
	Saline	Chlorpromazine	Saline	Chlorpromazine
Init. wt. Gm. $\bar{X}$	56.83	53.33	30.5	32.66
Wt. loss Gm. $\bar{X}$	0	1.5	+0.83	0.72
S_x	1.49	0.96	0.55	0.92
Wt. loss[b] Gm./Gm. $\bar{X}$	0	0.028	+0.027	0.022
S_x	0.026	0.018	0.018	0.028
Food intake daily Gm. $\bar{X}$	6.21	4.36	4.38	3.93
S_x	0.83	0.27	0.28	0.27
Food intake[b] Gm./Gm. $\bar{X}$	0.109	0.081	0.14	0.12
S_x	0.014	0.005	0.009	0.008
Food intake[c] reduction %	...	29.8	...	10.3
Gm./Gm.[b] $\bar{X}$	...	0.028	...	0.02

[a] Values are arithmetical means of 6 animals for a 7-day period. [b] Gm./Gm. values calculated from Gm. initial body weights. [c] Food intake reduction values calculated from saline controls.

TABLE II.—OXYGEN CONSUMPTION AND ENERGY OUTPUT[a]

		Obese Mice		Nonobese Mice	
		Saline	Chlorpromazine	Saline	Chlorpromazine
Init. wt. Gm.	$\bar{X}$	44.3	46.08	28.5	26.1
Body surf. cm.^{2}[b]	$\bar{X}$	125.1	128.9	93.1	88.1
O_2 ml./Gm./hr.	$\bar{X}$	3.4	2.8	3.5	2.86
S_x		0.41	0.29	0.17	0.37
Gm.-cal./hr./cm.^{2}[c]	$\bar{X}$	5.94	4.93	5.4	4.04
S_x		0.66	0.65	0.26	0.46

[a] Values are arithmetical means of 6 animals. [b] Body surface calculated from $A = \frac{W^{2/3}}{K}$ (K assumed to be 10) (15). [c] Calculated from $\frac{\text{ml./Gm./hr.} \times \text{init. wt.} \times 4.8}{\text{body surf. in cm.}^2}$ (15).

TABLE III.—PER CENT RESIDUE OF ADMINISTERED RADIOACTIVE DOSE OF GLUCOSE-1-C-14 PER GRAM OF DRY TISSUE AND MILLILITER OF BLOOD[a]

Tissue	Saline Treated[b] $\bar{X}$	S_x	Chlorpromazine Treated[b] $\bar{X}$	S_x
Gold Thioglucose-Obese				
Blood	1.87	0.03	2.36	0.07
Adrenal	8.89	1.15	7.73	1.43
Kidney	11.53	0.47	12.63	1.09
Liver	19.41	0.64	18.56	0.93
Muscle	3.86	0.75	2.14	0.79
Cerebrum	18.25	2.25	16.31	2.41
Hind brain	12.97	1.32	10.21	1.92
Hypothalamus	19.92	1.33	17.20	2.17
Normal Nonobese				
Blood	0.94	0.1	2.16	0.31
Adrenal	7.84	0.74	7.82	3.80
Kidney	18.83	1.46	14.21	1.21
Liver	18.27	1.08	16.30	1.31
Muscle	11.04	1.75	6.60	2.40
Cerebrum	23.60	2.45	20.61	3.12
Hind brain	22.73	0.98	16.87	2.71
Hypothalamus	11.63	0.88	9.81	1.60

[a] Each value is the arithmetical mean of 5 individual animals. [b] Administered 30 min. prior to administration of glucose-1-C-14.

14 was prepared by adding an aliquot of the solution to 10 ml. of the liquid scintillation solvent system. The activity of each blood and tissue sample and the glucose-1-C-14 standard was determined by means of a liquid scintillation detector and associated β spectrometer (Ekco Detector model N664 and Scaler model N610A). Quenching of the true count rate of each sample was corrected by use of an internal standard of hexadecane-1-C-14 (13). Blank samples were prepared to determine the background count. The per cent of the administered radioactive dose per Gm. of dry tissue and per ml. of blood was calculated for each animal. The mean values are tabulated in Table III. The dry tissue weights were calculated from the known moisture contents, determined as previously described (1).

RESULTS AND DISCUSSION

The administration of chlorpromazine caused a significant ($p > 95$) loss of body weight, reduction of food intake, oxygen consumption in ml./Gm./hr., and lowered Gm.-cal./hr./cm.^{2} in both the gold thioglucose-obese and the normal nonobese mice, as compared to their respective controls.

The actual weight loss was greater in the obese mice than with the nonobese mice (1.5–0.72 Gm.), but when calculated as Gm./Gm. of the initial body weight or per cent, the values were 0.028 Gm./Gm. or 2.8 % of the obese and 0.022 Gm./Gm. or 2.2 % for the nonobese mice. Food intake was reduced 1.85 Gm. (29.8%) or 0.028 Gm./Gm. initial body weight for the obese mice and 0.45 Gm. (10.3%) or 0.02 Gm./Gm. initial body weight for the nonobese mice.

Oxygen consumption in ml./Gm./hr. was similar in both groups of mice, but when calculated on the basis of body surface and expressed as Gm.-cal./hr./cm.^{2}, the obese mice had higher values than the non-

obese and in both groups the administration of chlorpromazine caused a significant ($p > 95$) reduction in the observed values. This effect is not expected with weight loss and is indicative of a reduced metabolic rate and/or depression of the C.N.S. The latter was not observed, and in fact, there was no visual difference in activity between the saline controls and the chlorpromazine-treated animals in either groups of mice. It is known, however, that the release of TSH to affect the secretion of the thyroid hormone is influenced by hypothalamic centers (14), and the action of chlorpromazine to lower the metabolic rate could be due to an action on this sequence, and in particular on the hypothalamic nuclei involved.

The mechanism by which chlorpromazine affected food-intake reduction was of sufficient magnitude to result in significant weight losses in both groups of mice even with the reduction of caloric output. As both the weight losses and food intake reduction values were similar in the obese and nonobese mice when compared on the Gm./Gm. initial body weight basis, it is probable that the mechanism was similar in both groups of mice. The effect of chlorpromazine on the residual tissue activity following administration of labeled glucose was studied as a possible clue to the mechanism of food-intake reduction and in general, a slight reduction in residual tissue activity was observed in all tissues as compared to the controls, with the exception of kidney tissue from the gold thioglucose-obese mice. Blood levels were significantly higher ($p > 95$) in both groups of mice. The lowered tissue levels, coupled with the increased blood levels are in agreement with the observed reduced oxygen consumption and energy output; and the increased blood levels, undoubtedly associated with elevated blood sugar, would be expected to have activated the ventro medial nuclei to inhibit the lateral hypothalamic feeding center (4, 5). This action is not supported, however, by the brain residual tissue activities, as chlorpromazine was observed to cause lower levels in all the brain tissues examined, and if activation of the ventro medial nuclei had occurred, the hypothalamic levels should have been higher than in the controls. Furthermore, the gold thioglucose-obese mice with destroyed or partially destroyed ventro medial nuclei had a similar food-intake reduction as the nonobese on the Gm./Gm. basis. The lower tissue activities in all brain tissue indicate a general mild depression, and this could include the lateral hypothalamic feeding center or centers in the forebrain. Sedation would not be expected to cause sufficient reduction in food intake to cause weight loss as observed, particularly with the reduction in the metabolic rate. Thus, it would appear that the action of chlorpromazine to reduce food intake involves a specific central action. The data presented do not permit a conclusion as to centers involved, but do exclude action through activation of the ventro medial nuclei and increased glucose uptake by these cells. Hypothalamic activity can be postulated, however, for both food intake reduction and lowering of the metabolic rate, on the basis of previous reports as to the tissue distribution of chlorpromazine and action on other hypothalamic centers (8–10).

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