Effects of Gold Thioglucose and Bipiperidyl Mustard on Pituitary Prolactin and Growth Hormone Content of C3H Mice[1] (35374)

Y. N. SINHA AND W. P. VANDERLAAN
(Introduced by W. O. Weigle)
Division of Endocrinology, Scripps Clinic and Research Foundation, La Jolla, California 92037

A single injection of gold thioglucose (GTG) produces obesity in mice and rats (1). The treatment also produces lesions of variable degree in the hypothalamus. Since the hypothalamus is involved in the regulation of pituitary function, it is conceivable that the damage produced by GTG may encompass one or more of the releasing hormone centers and thereby affect the respective pituitary hormone secretion. Although thyroid-stimulating hormone (TSH) secretion was not found to be influenced (2), GTG-treated mice show mammary development (3), and an earlier onset of mammary tumors (4), suggesting stimulation of prolactin secretion. In addition, the obesity produced by GTG is associated with hyperphagia raising the possibility of alteration in growth hormone (GH) secretion. Another compound, bipiperidyl mustard (BM), also produces similar obesity and hypothalamic lesions and has the added property of being active at relatively small dosage (5).

Recently, simple techniques of gel electrophoresis have been developed for measuring GH and prolactin (6) which can be readily adapted to measuring both hormones simultaneously from individual mouse pituitary glands. It was of interest, therefore, to investigate the influence of these obesifying agents on the pituitary content of prolactin and GH of mice using these techniques.

Materials and Methods. Five- to 6-week-old C3H mice, maintained on Purina Lab chow and water *ad libitum*, were given, in groups of 10 to 20, a single ip injection of the drugs. The dose of GTG consisted of 1 mg/g of body weight and of BM of 0.015 mg/g of body weight. GTG was suspended in 0.85% NaCl in the concentration of 50 mg/ml; whereas BM was dissolved in 0.1 *N* borate buffer (pH 9.0), in the ratio of 0.5 mg/ml and incubated at 37° for 1 hr before injection.

Four and 8 weeks after the injection, the animals were sacrificed by decapitation. Pituitary glands were weighted and processed immediately since both prolactin and GH bands have been observed to diminish (Lewis and Sinha, unpublished observations), prolactin more readily than GH, during storage. The weights of the adrenal glands, ovaries, and uteri were recorded in some cases.

The electrophoresis was performed as outlined by Lewis *et al.* (6). A 10% acrylamide and 0.75% ethylene diacrylate was used in the lower gel. The individual pituitary glands were homogenized in a micro glass homogenizer in 50 μl of carbonate–bicarbonate buffer (pH 10), and another 50 μl of the buffer was added by letting the buffer run on the piston held over the mortar. The entire material was transferred to the gel column by a Pasteur pipet. The homogenizer was washed once with 100 μl of buffer and the washing was transferred to the column. Then 200 μl of two times concentrated upper gel solution was added to the sample, mixed, and the contents were photopolymerized.

The stained prolactin and GH bands were cut out with a razor blade and dissolved in 1.0 and 2.0 ml of 0.5 *M* KOH, respectively. The optical density was measured at 625 mμ in a DU spectrophotometer against a blank made up to an equivalent length of unstained gel dissolved in 0.5 *M* KOH. The quantity of the hormone was calculated from standard curves obtained by using crystalline prolac-

[1] This research was supported by NIH Grants AM 01328 and AM 5249.

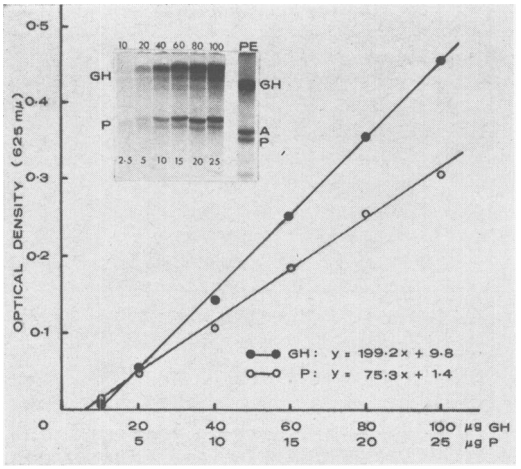

FIG. 1. Standard curves for GH and prolactin obtained by colorimetric measurement of alkali-soluble diacrylate gel columns: Known amounts of purified GH and prolactin were mixed and electrophoresed simultaneously. Abbrev.: A = albumin; GH = growth hormone; P = prolactin; PE = pituitary extract.

tin (NIH-P-S5)[2] and growth hormone (NIH-GH-B16)[2] preparations.

Results. The standard curves for prolactin and GH are shown in Fig. 1. The NIH preparations of prolactin and GH separated into three and four components, respectively, although in the pituitary extracts both hormones showed up as a single band. Thus, all the three bands of prolactin and four of GH were cut out and dissolved. As is apparent from the curves, both prolactin and GH can be detected in this system over a wide range of values.

Nineteen of 20 mice injected with GTG survived. Only 5 of 10 mice showed marked obesity in 4 weeks, whereas 8 of 9 animals became obese at 8 weeks (Table I). Obesity was judged by body weight, and grossly there were in the obese mice enormous adipose deposits in subcutaneous tissues, intraperitoneally, and about the viscera. At both stages, GTG-treated animals possessed more prolactin ($p<0.01$) than the control (9.2 vs 6.7 and 9.8 vs 6.9 μg/mg, respectively). The GH content of the pituitary glands did not change ($p>0.05$). Among the GTG-treated mice, onset of obesity was not the prime factor in altering prolactin; the concentrations changed both in those developing obesity and in those which remained lean. At 8 weeks, GTG-treated mice possessed heavier uteri than the controls ($p<0.05$). The weights of the pituitary gland, adrenals and ovaries did not vary ($p>0.05$).

Twenty-six of 30 mice injected with BM survived. Nine of 10 became obese at 4 weeks and all at 8 weeks (Table II). Prolactin content increased significantly ($p<0.01$) both at 4 (9.4 vs 7.6 μg/mg) and 8 (12.7 vs 9.1 μg/mg) weeks. GH did not change ($p>0.05$) much at 4 weeks (48.8 vs 46.7 μg/mg) but increased 30% ($p<0.01$) at 8 weeks (66.7 vs 51.3 μg/mg). At 4 weeks, there was no difference in the pituitary gland size but the adrenals, ovaries, and uteri were heavier (Table III). At 8 weeks, all organs in the BM-treated group weighed more than the controls.

The incidence of obesity from GTG and from BM and the gross distribution of fat was in keeping with previous findings (1, 5).

Discussion. Although the prolactin bands did not appear very distinct below 5 μg in the

[2] A gift from the National Institutes of Health.

TABLE I. Prolactin and GH Content of Mouse Pituitaries After Gold Thioglucose Administration.

Treatment[a]	Interval (weeks)	No. of animals	Body wt (g)		Prolactin		Growth hormone	
			Initial	Final	Conc (μg/mg)	Content (μg/pit.)	Conc (μg/mg)	Content (μg/pit.)
Saline	4	10	18.3	21.4	6.7 ± 0.4	11.5 ± 0.9	43.4 ± 1.4	73.9 ± 4.4
GTG	4	10	19.2	26.2	9.2 ± 0.4[b]	16.7 ± 1.7[b]	45.0 ± 1.7	80.7 ± 2.9
Saline	8	9	18.6	23.1	6.9 ± 0.4	14.5 ± 1.1	51.7 ± 2.1	106.4 ± 5.7
GTG	8	9	18.7	33.6	9.8 ± 0.5[b]	19.9 ± 1.7[b]	49.1 ± 2.6	99.7 ± 9.2

[a] Normal saline or gold thioglucose (1.0 mg/g of body wt) was administered ip in one injection.
[b] $p<0.01$.

TABLE II. Prolactin and GH Content of Mouse Pituitaries After Bipiperidyl Mustard Administration.

Treatment[a]	Interval (weeks)	No. of animals	Body wt (g)		Prolactin		Growth hormone	
			Initial	Final	Conc (μg/mg)	Content (μg/pit.)	Conc (μg/mg)	Content (μg/pit.)
Buffer	4	10	19.6	21.6	7.6 ± 0.6	12.1 ± 1.3	46.7 ± 2.9	74.1 ± 6.9
BM	4	10	21.3	27.6	9.4 ± 0.4[b]	16.2 ± 1.6[b]	48.8 ± 1.4	83.9 ± 7.2
Buffer	8	10	20.4	24.9	9.1 ± 0.6	16.0 ± 1.7	51.3 ± 2.2	89.6 ± 7.5
BM	8	16	21.9	38.4	12.7 ± 0.7[b]	27.7 ± 1.6[b]	66.7 ± 3.1[b]	145.1 ± 5.9[b]

[a] Borate buffer or bipiperidyl mustard (0.015 mg/g of body wt) was administered ip in one injection.
[b] $p < 0.01$.

case of prolactin and 20 μg for GH, all the pituitary glands contained prolactin and GH well over these limits. Moreover, the assay can be further sensitized, if necessary, by reducing the volume of KOH used. The use of a soluble gel by Lewis *et al.* (6) has provided a very simple technique to quantitate adenohypophyseal prolactin and GH. The prolactin band of the mouse pituitary emerges quite distinctly on this gel containing 10% acrylamide. This method, for the first time, has allowed measurement of prolactin from individual mouse pituitaries. Yanai *et al.* (7) attempted using 7.5% acrylamide but reported, in agreement with the experiences of Cheever *et al.* (8), unsatisfactory separation of prolactin and albumin bands.

BM treatment increased the weights of all the organs measured, whereas only the uterine weight was increased by GTG administration. Other workers (9), however, have reported increase in the weights of other organs including the adrenals and testes in GTG-obesity, and the increase have been attributed to lipid as well as nonlipid constituents. Waxler and Enger (9) have also reported an increase in the weight of the femur and thymus and an increase in the body protein in the GTG-obese mice, suggesting that an enhancement of GH secretion may occur.

Both GTG and BM treatment increased the prolactin content of the pituitary glands. The effect was measurable at 4 weeks. GTG-treated mice which did not look obese at 4 weeks also possessed elevated pituitary prolactin content. Since mammary gland growth is stimulated in GTG-treated mice (3), it would appear that prolactin secretion is enhanced as a result of GTG or BM treatment. This augmented secretion of prolactin may be responsible for the early onset of mammary tumors observed by Waxler *et al.* (4) since prolactin is believed to be closely related to mammary tumorigenesis in mice (10).

In contrast, GH concentration was markedly elevated only in BM-treated animals at 8 weeks. GTG-treated animals did not show any significant change. But lack of change in the pituitary content of a hormone, especially GH, cannot be interpreted to

TABLE III. Organ Weights of Mice After Bipiperidyl Mustard Administration.

Treatment[a]	Interval (weeks)	No. of animals	Pituitary gland (mg)	Adrenal glands (mg)	Ovaries (mg)	Uteri (mg)
Buffer	4	10	1.6 ± 0.06	4.8 ± 0.46	6.5 ± 0.6	56.4 ± 5.9
BM	4	10	1.7 ± 0.11	6.5 ± 0.24[c]	10.5 ± 1.1[c]	81.8 ± 9.9[b]
Buffer	8	10	1.7 ± 0.10	5.3 ± 0.36	12.3 ± 1.4	93.1 ± 13.5
BM	8	16	2.2 ± 0.04[c]	8.6 ± 0.45[c]	15.9 ± 0.7[c]	113.4 ± 8.5

[a] Borate buffer or bipiperidyl mustard (0.015 mg/g of body wt) was administered ip in one injection.
[b] $p < 0.05$.
[c] $p < 0.01$.

mean *no* alteration in secretion because the mouse pituitary gland contains enormous amounts of GH (almost 5% of the total wet wt) compared to the levels present in the plasma and moderate changes in secretion rate may not be reflected in the pituitary levels. This is supported by the fact that BM-treated mice did not show any appreciable change in the pituitary content of GH at 4 weeks although they were obese by that time and if they were secreting more GH at 8 weeks, it is not unreasonable to believe that they were at 4 weeks as well.

If GH secretion was indeed enhanced as a result of BM treatment (and probably GTG as well), the mechanism of stimulation remains unclear. Destruction of the hypothalamic center regulating the hormone secretion can explain stimulation of prolactin, since the hypothalamus exerts an inhibitory influence on prolactin secretion. But in the case of GH, destruction of the center might adversely affect GH secretion. It is possible, however, that a mild irritation of the neurons by BM or GTG may result in the stimulation of GH-releasing hormone (GH-RH) and thereby GH secretion. Another possibility is that the levels of blood substrates resulting from the increased consumption of feed by these animals may itself modify the GH secretion.

It is generally held that hyperphagia is the primary cause of obesity in GTG-treated mice (1). The results of these experiments have shown that the pituitary content of at least one metabolic hormone, prolactin, which is capable of stimulating body weight gain and appetite (11), is definitely altered. There are indications, from data on BM treatment, that GH secretion may also be modified. However, what precise roles these hormones play in the induction of obesity remain to be determined.

Summary. The effects of a single injection of gold thioglucose (GTG) and bipiperidyl mustard (BM) on the pituitary prolaction and GH contents of C3H mice were studied. Virtually all mice receiving the drugs became obese in 8 weeks. GTG and BM increased pituitary prolactin concentration 37 and 24% at 4 weeks and 42 and 40%, respectively, at 8 weeks ($p<0.01$). GH concentration was enhanced significantly (30%; $p<0.01$) only by BM treatment at 8 weeks. BM increased the weights of the pituitary glands, adrenals, ovaries, and the uteri; whereas only the uterine weights were increased by GTG treatment. The data suggest that prolactin and/or GH may be related to the induction of obesity by GTG and BM.

We thank Dr. R. J. Rutman for providing bipiperidyl mustard, and Mrs. Eileen F. VanderLaan for technical help. The assistance of Dr. U. J. Lewis in the gel electrophoresis technique and other aspects of the research is greatly appreciated.

1. Deter, R. L., and Liebelt, R. A., Tex. Rep. Biol. Med. **22**, 229 (1964).

2. Schindler, W. J., and Liebelt, R. A., Endocrinology **80**, 387 (1967).

3. Browning, H. C., Larke, G. A., and Gibbs, W. E., Neuroendocrinology **1**, 93 (1965/66).

4. Waxler, S., Tobar, P., and Melcher, L. R., Cancer Res. **13**, 276 (1953).

5. Rutman, R. J., Lewis, F. S., and Bloomer, W. D., Science **153**, 1000 (1966).

6. Lewis, U. J., Litteria, M., and Cheever, E. V., Endocrinology **85**, 690 (1969).

7. Yanai, R., Nagasawa, H., and Kuretani, K., Endocrinol. Jap. **15**, 365 (1968).

8. Cheever, E. V., Seavey, B. K., and Lewis, U. J., Endocrinology **85**, 698 (1969).

9. Waxler, S. H., and Enger, M., J. Nutr. **54**, 209 (1954).

10. Muhbock, O., and Boot, L. M., Biochem. Pharmacol. **16**, 627 (1967).

11. Riddle, O., J. Nat. Cancer Inst. **31**, 1039 (1963).

Received July 24, 1970. P.S.E.B.M., 1971, Vol. 136.