Inhibition of Prolactin Release by a Thalidomide-Related Compound (CG 603) (36417)

M. GELATO, S. K. QUADRI, AND J. MEITES[1]

Department of Physiology, Michigan State University, East Lansing, Michigan 48823

CG 603 is a cyclic imide. Some cyclic amides like thalidomide are central nervous system depressants (1). Barbiturates block ovulation in the rat (2, 3), and recently this laboratory reported that a single injection of Na pentobarbital (Pb) at 1 PM on the day of proestrus prevented the pronounced rise in serum LH on the late afternoon of proestrus (4). Serum prolactin was elevated 30 min after the Pb injection, but the late afternoon surge of serum prolactin was blocked completely. It was of interest, therefore, to determine the effects of a single injection of CG 603 given on the early afternoon of the day of proestrus on serum prolactin levels.

Materials and Methods. Mature, 3 to 4 month old virgin female Sprague–Dawley rats (Carworth Farms, Portage, MI), weighing 210–245 g each, were housed in a temperature controlled (25 ± 1°) and artificially illuminated (lights on from 7 AM to 9 PM daily) animal room. They were given Wayne Lab Blox pellets and water *ad libitum*. At least two consecutive estrous cycles were followed on all rats by examining daily vaginal smears, and only rats with regular 4 or 5 day cycles were used.

Individual blood samples (.5 to 1.0 ml) were removed by heart puncture under light ether anesthesia at 12 noon on the day of proestrus. This method of blood collection was found not to alter normal serum prolactin values in rats (4). A dose of 5, 10 or 25 mg CG 603[2]/100 g body wt was injected ip in .85% NaCl at 12:30 AM, and the rats were bled at 1, 2, 3 and 5 PM. Control rats were injected only with .85% NaCl. The animals were killed by guillotine immediately after the 5 PM bleeding and their pituitaries were removed and weighed. The pituitaries were frozen at —20° and stored for assay. The blood samples were stored at 2.5° and 24 hr later the clots were removed and the serum was frozen at —20° until assayed. The pituitaries were homogenized in .01 *M* phosphate buffer saline (pH 7.0) with a Sonifier cell disruptor (Heat Systems—Ultrasonics Inc., Plainview, NY) immediately before assay.

Prolactin in individual serum samples and pituitaries were measured by radioimmunoassay (5). Each serum sample was assayed at two dilutions and each pituitary sample was assayed at four dilutions. The samples were averaged and expressed in terms of a standard rat prolactin preparation (NIAMD-RP-1). The hormone used for iodination (H-10-10-B, 28 IU/Mg) was provided by Dr. S. Ellis, NASA Research Center, Moffett Field, CA. Sample means and standard error of means were calculated within each experimental group, and significance of differences between groups was determined by Student's *t* test (6).

Results. Table I shows that serum prolactin rose progressively in the control rats to the highest value at 5 PM. A single ip injection of each of the three doses of CG 603 at 12:30 PM significantly reduced serum prolactin levels as compared to control values at each time period. The lowest dose of CG 603 (5 mg/100 g body wt) partially suppressed the rise in prolactin, and at 5 PM values were less than half of that in the control rats. The 10 mg dose of CG 603 was more effective than the 5 mg dose, and the 25 mg dose completely prevented any rise in serum pro-

[1] Aided in part by NIH research Grants AM 04784 and CA 10771.

[2] Cyclic imide, CG 603, (NSC 1293967) was kindly provided by Dr. D. Jane Taylor, Endocrine Evaluation Branch, General Labs and Clinics, National Cancer Institute, Bethesda, MD.

TABLE I. Serum (ng/ml) and Pituitary (μg/mg) Prolactin Levels Before and After CG 603[a] Injection.

Treatment	Pretreatment 12 AM	Pretreatment 1 PM	Posttreatment 2 PM	Posttreatment 3 PM	Posttreatment 5 PM	Pituitary μg/mg
Controls (17)[b]	48.5 ± 5.7[c]	57.1 ± 5.7	105.4 ± 14.5	141.3 ± 10.7	185.7 ± 11.7	0.55 ± 0.02
CG 603 (8),	39.6 ± 6.3	46.9 ± 7.3	51.0 ± 8.3	55.9 ± 10.8	80.0 ± 13.0	0.76 ± 0.03
5 mg/100 g body wt	NS	NS	$p < .05$	$p < .001$	$p < .001$	$p < .001$
CG 603 (13),	36.6 ± 6.8	36.0 ± 5.4	35.8 ± 7.7	63.1 ± 7.6	67.6 ± 12.1	0.72 ± 0.03
10 mg/100 g body wt	NS	$p < .05$	$p < .001$	$p < .001$	$p < .001$	$p < .001$
CG 603 (12),	33.8 ± 5.5	24.3 ± 4.7	24.7 ± 4.5	27.6 ± 6.1	60.2 ± 10.2	0.79 ± 0.02
25 mg/100 g body wt	NS	$p < .001$[d]	$p < .001$	$p < .001$	$p < .001$	$p < .001$

[a] CG 603 was injected at 12:30 PM.
[b] Number of rats per group.
[c] Standard error of mean.
[d] Significance of difference from control value.

lactin until 5 PM when values were only about one-third of those in the controls. Pituitary concentrations of prolactin in all the CG 603 treated rats were elevated significantly above control values ($p < .001$), reflecting the reduced release and increased accumulation of prolactin in these pituitaries.

Discussion. A single injection of CG 603 during the early afternoon of proestrus inhibited the rise in serum prolactin during the late afternoon. Unlike Pb (4), CG 603 did not elicit an initial rise in serum prolactin nor did it completely suppress the late afternoon rise in prolactin. CG 603 does not evoke an initial excitatory response like Pb, which may account for its failure to produce an initial increase in serum prolactin. On a dose basis, CG 603 is less potent than Pb, since an injection of 31.5 mg Pb/kg completely suppressed the rise in serum prolactin on the afternoon of proestrus (4).

The mechanism(s) of action of CG 603 on prolactin release remains to be elucidated. Wuttke *et al.* (7) observed that Pb directly inhibited pituitary prolactin release *in vitro*, but depressed hypothalamic PIF activity when injected systemically. This suggests that Pb inhibits prolactin release by a direct action on the pituitary, and that its brief initial stimulatory effect on prolactin release is mediated via the hypothalamus. Whether CG 603 also suppresses prolactin release by a direct action on the pituitary remains to be determined. It has been reported that CG 603 depresses adrenal cortical function in rats (8), and this may influence its action on prolactin release.

CG 603 inhibits growth of 7, 12-dimethylbenzanthracene-induced mammary adenocarcinomas in rats (9). This is probably mediated through its suppression of pituitary prolactin release, since prolactin has been shown to be an essential hormone for mammary tumor development and growth in the rat (10).

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Received Dec. 7, 1971. P.S.E.B.M., 1972, Vol. 140.