PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 183, 358–362 (1986)

β-(-4 Chlorophenyl) GABA (Baclofen) Inhibits Prolactin and Thyrotropin Release by Acting on the Rat Brain (42431)

VICTORIA A. R. LUX, GUSTAVO M. SOMOZA, AND CARLOS LIBERTUN

Instituto de Biología y Medicina Experimental, Obligado 2490, 1428 Buenos Aires, Argentina

Abstract. Baclofen, a GABA B agonist, inhibits prolactin release due to different kinds of stress. In the present study its effect was evaluated in several endocrine experimental situations to explore the specificity of this effect, as well as the site of action of the drug. Baclofen significantly inhibited prolactin and thyrotropin outputs induced by 25 min of suckling, without altering milk ejection or LH secretion. The effect was also tested in median eminence-lesioned rats and in *in vitro* incubations. Baclofen did not modify prolactin levels in rats in which brain control of the pituitary secretion was eliminated by destruction of the median eminence, and it did not inhibit prolactin or thyrotropin secretion from incubated hemipituitaries. It is postulated that baclofen inhibits prolactin and thyrotropin secretion by acting on GABA B receptors related to the brain control of pituitary secretion.

It has been demonstrated that GABA, a neurotransmitter which is concentrated in the hypothalamus, can modify hypophyseal secretions. It can inhibit ACTH and TSH secretion by respectively inhibiting CRF and TRH release (1–4). Prolactin and LH release can be either stimulated or inhibited (5–11). It has been shown to enhance GH release (12, 13). Recently, based on their sensitivity to bicuculline, two pharmacologically distinct types of GABA receptors have been described by Bowery *et al.* (14–16). A novel receptor named B appears to be unique in that it is insensitive to bicuculline and is most effectively activated by the GABA analog *p*-chlorophenyl GABA (baclofen), which appears to be inactive at bicuculline-sensitive classical GABA A binding sites.

Since previous results from our laboratory have shown that baclofen is able to inhibit the prolactin release caused by different kinds of stress (17, 18), experiments were undertaken to evaluate the effect of baclofen in another experimental model such as suckling. In an attempt to localizing the site of action of the drug, the effect was evaluated in median eminence-lesioned (MEL) rats, in which the hypophysis is separated from the hypothalamus, and also using incubations of hemipituitaries.

Materials and Methods. *Suckled rats.* Adult female Sprague–Dawley rats were housed with males in an air-conditioned room with controlled lighting (lights on from 0700 to 1900 hr) and given free access to laboratory chow and tap water. On Days 20–21 of pregnancy they were separated into individual cages. The date of parturition was recorded. Three days after delivery, each litter size was adjusted to eight pups. Experiments were performed when the litters were between 11 and 15 days old.

Rats were separated from their pups at 0900. At 1300 hr, baclofen, 10 mg/kg ip, or saline was injected. Fifty minutes later some rats of each group were decapitated, and blood was collected to obtain basal hormone values. Litters were then restored to the rest of the mothers and the time, when at least six of eight pups were attached to the nipples, was recorded. Twenty-five minutes later rats were decapitated, and blood was collected. Stomachs of the pups were dissected, and their content was extracted and weighed (19).

Median eminence-lesioned rats. In an attempt to elucidate the site of action of baclofen, its effect was tested in median eminence-lesioned rats. Adult male rats were anesthetized with Nembutal (33 mg/kg), and the median eminence of the tuber cinereum was destroyed, as described previously (20). Sham-operated rats, in which no current was passed, served as controls. Animals were used 10 days after the operation. On the day of the experiments, animals were weighed and injected ip with baclofen (10 mg/kg) or saline. After 1 hr, animals were rapidly decapitated. Blood was collected for hormone determinations, and the brain was inspected anatomically to determine the correct placement of the lesion.

0037-9727/86 $1.50

In vitro incubations of hemipituitaries. Male rats were decapitated during the morning, and the anterior pituitary was rapidly removed and hemidissected; each half was then placed in an incubation flask containing 1 ml of Krebs–Ringer bicarbonate buffer, with 14 m*M* glucose, pH 7.4. Incubations were carried out in a Dubnoff metabolic shaker under air at 37°C. After 30 min preincubation, the medium was removed, and the hemiglands were washed once with 1 ml buffer and finally replaced with 2 ml of fresh medium, with or without baclofen at concentrations of 1.10^{-6} or with 1.10^{-4} *M* or with dopamine (1.10^{-6} *M*) as control. After 4 hr of incubation, the medium was decanted, and 100 μl of the supernatant was diluted with phosphosaline buffer with 1% egg albumin and frozen until assayed. Hemipituitaries were then weighed. Results were expressed as nanograms of PRL per milligram of tissue.

Serum hormone determinations. PRL and TSH were determined by RIA using kits provided by the NIADDK; results were expressed in terms of RP_3 rat PRL standard and RP_2 rat TSH standard, respectively. LH was determined with the RIA developed by Niswender *et al.* (21), and results are expressed in terms of RP_2 rat LH.

Statistical analysis. The data were analyzed by one-way analysis of variance (to study variation between each group). When *F* was significant, Duncan's test was used to compare the means. Student's *t* test was employed to compare means of two different groups while Student's paired *t* test was employed to compare means in *in vitro* studies. Results were considered significant when *P* was <0.05.

Results. *Effect of baclofen in suckled rats.* There was no difference in basal PRL levels between saline- and baclofen-treated rats. After 25 min of suckling, baclofen completely suppressed serum PRL levels (Fig. 1), though the amount of milk ejected by both groups of rats was similar.

TSH secretion, which is also induced by suckling (22, 23), was also effectively inhibited by baclofen after this period of suckling (Fig. 2). No differences were observed in LH values, which remained in all cases at low basal levels.

Median eminence-lesioned rats. In this experimental model PRL titers were, as expected, significantly higher in MEL than in sham-operated rats. One hour after baclofen injection, serum prolactin levels remained unchanged in both groups (Fig. 3).

In vitro incubations of hemipituitaries. In this incubation system, baclofen did not mod-

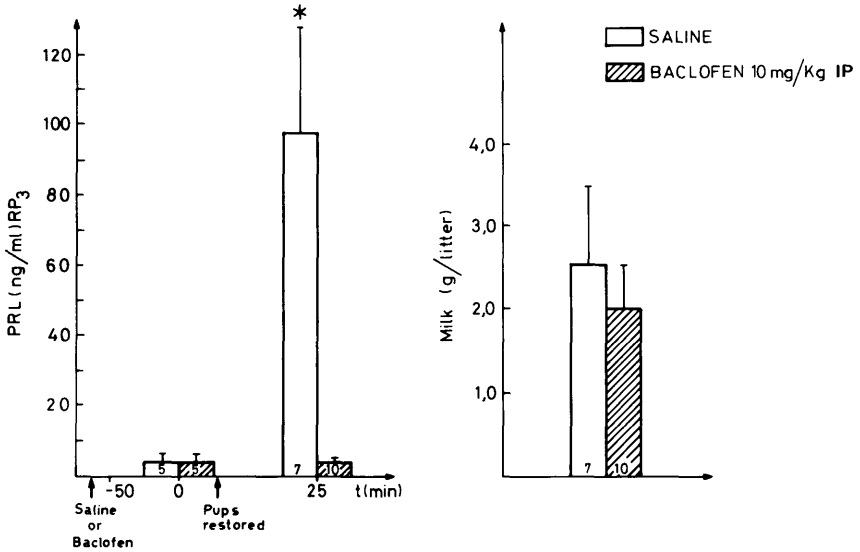

FIG. 1. Effect of baclofen on PRL secretion and milk yield after 25 min suckling. For this and following figures, numbers inside columns indicate number of rats per group. The height of the bar indicates the mean and the vertical line 1 SE. *$P < 0.05$ compared to saline controls.

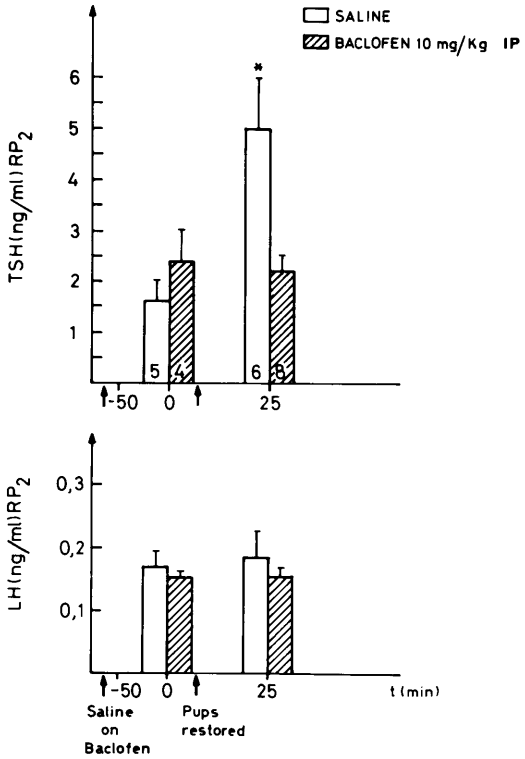

FIG. 2. Effect of baclofen on TSH and LH secretion after 25 min suckling.

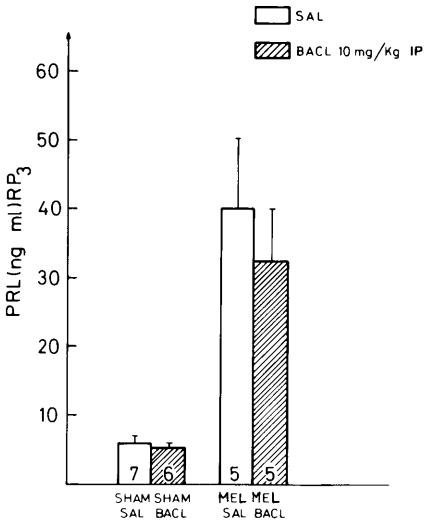

FIG. 3. Effect of baclofen (BACL) on PRL release in median eminence-lesioned (MEL) rats.

ify PRL medium titers; in contrast, dopamine clearly inhibited prolactin output (Fig. 4). TSH secretion was not affected either by baclofen or by dopamine present in the incubation medium (not shown).

Discussion. The present results indicate that baclofen prevents the rise in serum prolactin and in TSH due to suckling in the rat. Our previous results indicate that gabaergic drugs are able to inhibit prolactin secretion and milk ejection induced by suckling (7, 19). Racagni *et al.* (24) have suggested that the tuberoinfundibular gabaergic system could play a role in controlling prolactin secretion during continuous suckling stimulation. Also, some findings are in favor of an inhibitory action of GABA on TRH release (3, 4). TRH has been postulated as one releasing hormone involved in prolactin secretion induced by suckling, and it has been documented that it also promotes TSH release in the rat (22, 25–28). Therefore it is possible that during suckling, baclofen acting on GABA B sites could be dampening prolactin and TSH outputs by inhibiting TRH secretion. No effect in milk yield after 25 min of suckling was found due to acute baclofen treatment; thus it is improbable that the drug has an important action on oxytocin secretion. LH release also was not modified.

In order to investigate the site of the endocrine action of baclofen, two experimental models, one *in vivo* and one *in vitro*, were performed. It had been shown that GABA can

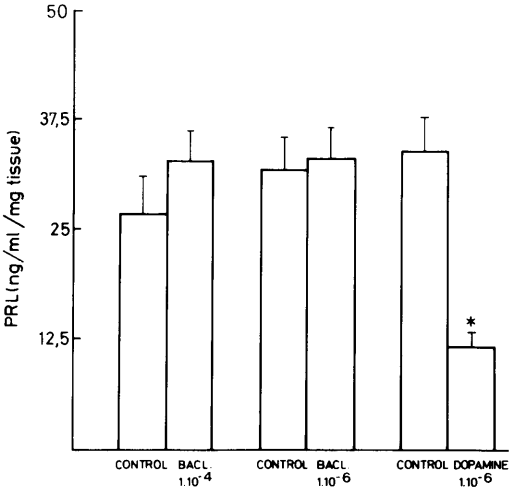

FIG. 4. Effect of baclofen on PRL release in *in vitro*.

act directly on the hypophysis to lower PRL levels and that GABA receptors are under brain control (20, 29); in consequence, we studied the effect of baclofen in median eminence-lesioned rats. In this model, in which the pituitary is surgically separated from the brain, hormone values are dramatically altered. Serum PRL concentration is very high, due to lack of the tonic inhibition of the brain, while the concentrations of the other adenohypophysea hormones are low because of the absence of their respective hypothalamic-releasing hormones (20). Baclofen was unable to alter prolactin serum levels in median eminence-lesioned rats with respect to sham-operated animals. This suggested that its effect on PRL secretion must be located at some other site, possibly at the hypothalamic level. These results were reinforced by those achieved in the *in vitro* model. Here, baclofen was once again ineffective in changing PRL or TSH levels, though a clear decrease in the PRL level was obtained with dopamine. Thus, a direct action of baclofen on the hypophysis is improbable.

Baclofen has central depressant properties and is able to interact with some neurotransmitters (17, 18). The role of these actions, at the molecular level, in the endocrine effect of the drug, should be studied further.

In conclusion, since baclofen is active at the GABA B site, whereas it is devoid of activity at the classical GABA A site, and the drug is not active on the isolated pituitary, it is possible to postulate that the drug is acting on a bicuculline-insensitive GABA receptor, possibly at the brain level. It is able to inhibit prolactin and TSH levels, in some circumstances, in which hormone output has been stimulated, as in stress or suckling. This action could involve a decrease in TRH release or in other facilitatory factors, and/or an increase in dopaminergic inhibition of prolactin release.

1. Makura GB, Stak E. Effect of gamma amino butyric acid (GABA) and GABA antagonist drugs on ACTH release. Neuroendocrinology **16**:178–182, 1974.
2. Burden J, Hillhous WE, Jones MT. The inhibitory action of GABA and melatonin on the release of corticotrophin-releasing hormone from the rat hypophysiotropic area in vitro. J Physiol **239**:116–117, 1974.
3. Jordan D, Poncet C. Veis Seire M, Mornex R. Role of GABA in the control of thyrotropin secretion in the rat. Brain Res **268**:105–110, 1983.
4. Mattila J, Mannisto PT. Modification of gabaergic activity and thyrotropin secretion in male rats. Acta Pharmacol Toxicol **47**:241–248, 1980.
5. Miodszouwski R, Grandison L, Meites J. Stimulation of prolactin release in rats by GABA. Proc Soc Exp Biol Med **151**:44–46, 1976.
6. Vijayan E, McCann SM. The effect of intraventricular injection of gama-aminobutyric acid (GABA) on prolactin and gonadotrophin release in conscious female rats. Brain Res **155**:35–43, 1978.
7. Libertun C, Arakelian MC, Larrea GA, Foglia VG. Inhibition of PRL secretion by GABA in female and male rats. Proc Soc Exp Biol Med **161**:28–31, 1979.
8. Schally AV, Redding TW, Arimura A, Dupont A, Linthicum GL. Isolation of γ aminobutyric acid from pig hypothalami and demonstration of its prolactin release-inhibiting (PIF) activity "in vivo" and "in vitro." Endocrinology (Baltimore) **100**:681–691, 1977.
9. Lamberts SWJ, MacLeod R. Studies on the mechanism of the GABA mediated inhibition of prolactin secretion. Proc Soc Exp Biol Med **158**:10–13, 1978.
10. Pass KA, Ondo JG. The effect of γ aminobutyric acid on prolactin and gonadotropin secretion in the unanesthetized rat. Endocrinology (Baltimore) **100**: 1437–1442, 1977.
11. Rivier C, Vale W. Effects of γ aminobutyric acid and histamine on prolactin secretion in the rat. Endocrinology (Baltimore) **101**:506–511, 1977.
12. Martino JB, Durand D, Gurd W, Faillace G, Audet J, Brazeau P. Neuropharmacological regulation of episodic growth hormone and PRL secretion in male rats. Acta Pharmacol Toxicol **47**:241–248, 1980.
13. Vijayan E, McCann SM. Effects of intraventricular injection of gamma-aminobutyric acid (GABA) on plasma growth hormone and thyrotropin in conscious ovariectomized rats. Endocrinology (Baltimore) **101**: 1888–1893, 1978.
14. Bowery NG. Baclofen: 10 years on. Trends Pharmacol Sci **3**:400–403, 1982.
15. Bowery NG, Hill DR, Hudson AL, Doble A, Middlenis DN, Shaw J, Turnbull M. (−) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature (London) **283**:92–94, 1980.
16. Hill DR, Bowery NG. ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline insensitive GABA B sites in the rat brain. Nature (London) **290**:149–152, 1981.
17. D'Eramo JL, Somoza GM, Kertesz E, Libertun C. Baclofen, a GABA derivative, inhibits stress-induced prolactin release in the rat. Eur J Pharmacol **120**:81–85, 1986.
18. Lux VAR, D'Eramo JL, Somoza GM, Libertun C. Participation of GABA B binding sites on brain control of pituitary secretion: Effect of baclofen. In: Racagni G, Donoso AO, eds. GABA in Neuroendocrine Function. New York, Raven Press, in press.

19. Arakelian MC, Foglia VG, Libertun C. Prolactin and milk ejection during the first 20 minutes of suckling in the rat: blockade by nembutal and aminooxiacetic acid. Horm Metab Res **16**:154, 1984.
20. Fiszer de Plazas S, Becú D, Mitridate de Novara A, Libert C. γ Aminobutiric acid receptors in the anterior pituitary and brain areas after median eminence lesions. Endocrinology (Baltimore) **111**:1974–1977, 1982.
21. Niswender GD, Modgley AR Jr, Monroe SE, Reichert LE. Radioimmunoassay of rat luteinizing hormone with antiovine LH serum and ovine $LH^{131}I$. Proc Soc Exp Biol Med **128**:807–811, 1968.
22. Blake CA. Stimulation of pituitary prolactin and TSH release in lactating rats and proestrous rats. Endocrinology (Baltimore) **94**:503–508, 1974.
23. Burnet FR, Wakerley JB. Plasma concentration of prolactin and thyrotropin during suckling in urethane-anesthetized rats. J Endocrinol **70**:429–437, 1976.
24. Racagni G, Apud JA, Cocchi D, Locatelli V, Iuliano E, Casanueva F, Muller E. Regulation of PRL secretion during suckling: Involvement of the hypothalamo-pituitary gabaergic system. J Endocrinol Invest 7:481, 1984.
25. Mena F, Martínez-Escalera G, Clapp C, Aguayo D, Grosvenor C. Regulatory and control mechanisms of PRL secretion within the pituitary of the lactating rat. In: Mena F, Valverde CM, eds. Prolactin Secretion. New York, Academic Press, p161, 1984.
26. Plotsky PM, Neill JD. Interactions of dopamine and thyrotropin-releasing hormone in the regulation of prolactin release in lactating rats. Endocrinology (Baltimore) **111**:168–173, 1982.
27. de Greef WJ, Vissier TJ. Evidence for the involvement of hypothalamic dopamine and thyrotropin releasing hormone in suckling-induced release of prolactin. J. Endocrinol **91**:213–223, 1981.
28. Grosvenor CE, Mena F. Evidence that thyrotropin-releasing hormone and an hypothalamic prolactin releasing factor may function in the release of prolactin in the lactating rat. Endocrinology (Baltimore) **107**: 863–868, 1980.
29. Grandison L, Guidotti A. γ-Aminobutiric acid receptor function in the rat anterior pituitary: Evidence for control of PRL release. Endocrinology (Baltimore) **105**:754–759, 1979.

Received June 5, 1986. P.S.E.B.M. 1986, Vol. 183.
Accepted September 3, 1986.