# Further Evidence for the Inhibitory Action of Baclofen on a Prolactin-Releasing Factor (43265)

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*Abstract.* **The mechanism of action of a specific γ-aminobutyric acid B receptor agonist, β-*p*-chlorophenyl-γ-aminobutyric acid or baclofen, in its inhibitory action on prolactin release, was studied. Dose-response studies of the effect of baclofen on prolactin (PRL) secretion were performed in stressed male rats. Furthermore, the action of the drug was evaluated in (i) rats treated with haloperidol or α-methyl-*p*-tyrosine, (ii) stressed or suckled rats pretreated with sulpiride, and (iii) animals treated with serotonin, alone, or with α-methyl-*p*-tyrosine.**

**Baclofen showed a clear dose-dependent inhibition of prolactin secretion in males under stress. The drug was unable to inhibit the prolactin release induced by haloperidol or α-methyl-*p*-tyrosine, although it reduced the PRL secretion induced by serotonin. It also inhibited PRL release in sulpiride-pretreated stressed or suckled rats.**

**These results suggest that the dose-dependent effect of baclofen on PRL secretion is the consequence of an inhibition exerted on the prolactin-releasing factor component of the neuroendocrine responses evoked by stress or suckling, possibly acting at the serotonergic system.** [P.S.E.B.M. 1991, Vol 197]

It is accepted that prolactin (PRL) plays an important role in different physiologic events, and changes in serum hormone levels have been related to a variety of clinical conditions, as galactorrhea, amenorrhea, and anovulatory cycles. Although dopamine, of hypothalamic origin, seems to be the most important prolactin-inhibiting factor, other inhibitory and stimulatory neural factors regulating PRL secretion have been reported (1).

γ-Aminobutyric acid (GABA) seems to be involved in the regulation of PRL secretion since it directly inhibits the release of prolactin, its receptors are present in the anterior pituitary and tuberoinfundibular GABAergic neurons have been described (2–5).

Two pharmacologically distinct types of GABA receptors have been described based on their sensitivity to bicuculline (6). A novel receptor, named B, is insensitive to bicuculline and it is most effectively activated by the GABA analog β-*p*-chlorophenyl-GABA (baclofen), which appears to be inactive at the bicuculline-sensitive classical GABA A receptors. Previous studies from our laboratory have shown that baclofen was able to inhibit PRL secretion induced by a variety of stressful stimuli (ether, immobilization, cold, and swimming) (7). Moreover, it completely abolished the PRL and thyroid-stimulating hormone release evoked by suckling but it did not alter PRL secretion when the pituitary was isolated from the brain in male rats (8–10). Taken together our results indicate that baclofen blocks prolactin secretion when the hormone is dynamically stimulated by action on the rat brain. Thus, in the present work, a new series of experiments was designed to clarify the mechanism of action of the drug on the inhibitory/facilitatory mechanism(s) involved in the neural control of PRL secretion.

## Materials and Methods

Male and female Sprague-Dawley rats from the Instituto de Biologia y Medicina Experimental colony were housed in an air-conditioned room with lights on from 7 AM to 7 PM. They were given free access to laboratory chow and tap water.

**Dose-Response Studies.** Male rats (120–140 g body wt) were injected with saline or baclofen (Lioresal;

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Received July 19, 1990. [P.S.E.B.M. 1991, Vol 197]
Accepted March 27, 1991.

0037-9727/91/1973-0337$3.00/0

Ciba Geigy, Buenos Aires, Argentina) in graded doses (2.5, 5, and 10 mg/kg) intraperitoneally; 50 min later they were placed in immobilization cages, consisting of a 12- × 4-cm plastic cylinder, and 10 min thereafter they were decapitated. Trunk blood was collected for PRL radioimmunoassay (RIA) studies.

**Pharmacologically Treated Animals.** The endocrine action of baclofen was investigated in connection with the high levels of PRL elicited by pharmacologic treatments: (i) antidopaminergic agents, haloperidol (halopidol; Janssen, Buenos Aires, Argentina) and sulpiride (Sul: Vipral; Roemmers, Buenos Aires, Argentina), (ii) inhibition of catecholamine synthesis induced by $\alpha$-methyl-*p*-tyrosine (MPT; Sigma, St. Louis, MO); or (iii) the administration of serotonin (serotonin) creatinine sulfate; Sigma). Doses, routes, and times were selected according to previous experience from our laboratory.

Male rats (150–200 g body wt) were injected with baclofen (10 mg/kg ip); 15 min later haloperidol (0.1 or 0.25 mg/kg) was administered intraperitoneally. Saline-injected rats were used as controls. Animals were decapitated 60 min after baclofen injection and trunk blood was collected.

Another group of rats was injected intraperitoneally with MPT suspended in saline solution (250 mg/kg) 16 hr before the experiment. This dose effectively depletes brain catecholamines, achieving in this way a hyperprolactinemic state due to the decrease of hypothalamic dopamine. One hour before decapitation rats were injected with baclofen (10 mg/kg ip).

Male rats were pretreated with MPT (250 mg/kg ip) or saline 16 hr before the experiment. Baclofen (10 mg/kg ip) or saline was injected 60 min before decapitation. Forty-five minutes after baclofen administration, serotonin in a dose of 5 mg/kg (dissolved in saline) or saline was injected intraperitoneally. Animals were then decapitated and trunk blood was collected for RIA studies.

**Immobilization Stress and Sulpiride.** Male rats (120–140 g body wt) were injected with sulpiride (1 mg/kg) or saline. One hour later animals were injected with saline or baclofen (10 mg/kg ip), and 50 min after the last injection, a group of animals was placed in immobilization cages for 10 min and then the animals were decapitated (i.e., 2 hr after initiation of treatments). The other group of rats was sacrificed without having been disturbed. In both cases trunk blood was collected and sera were kept for RIA determinations.

**Sulpiride-Pretreated Suckled Dams.** Female rats were mated and placed in individual cages. After parturition, litters were reduced to eight pups per dam. Experiments were performed in lactating rats on Days 10–15 postpartum. On the morning of the experiment, dams were separated from their pups at 9.30 AM for 4 hr. Animals were preinjected with saline or sulpiride (0.5 or 1 mg/kg ip). One hour later baclofen (10 mg/kg ip) or saline was injected. Forty minutes thereafter pups were restored to their dams (suckled rats) and the time when the pups were attached to the nipples was recorded. Twenty minutes later these dams were decapitated (i.e., 2 hr after initiation of treatments). A group of unsuckled dams (pups not restored) was sacrificed 60 min after the second injection. Sera were collected for RIA studies.

**Serum PRL Determination.** Serum PRL was determined by RIA using the kit provided by NIDDK. Results are expressed in terms of $RP_3$ rat PRL. Intra- and interassay coefficients of variation were 8.1 and 11.4%, respectively. Baclofen by itself does not interfere in the RIA system used.

**Statistical Analysis.** The data were analyzed by one-way analysis of variance. When F was significant, Duncan's *t* test or Dunnett's test was used to compare means. Results were considered significant when $P < 0.05$.

## Results

**Baclofen Dose-Response Studies.** A clear dose-dependent response was observed in the inhibition of PRL evoked by baclofen in immobilization-stressed male rats. Although the decrease induced by 2.5 mg/kg was not significant, doses of 5 and 10 mg/kg increasingly lowered PRL levels (Fig. 1).

**Effect of Baclofen on PRL Secretion in Haloperidol- or MPT-Pretreated Rats.** Both doses of haloperidol (0.1 and 0.25 mg/kg) increased PRL secretion dose dependently in male rats ($P < 0.01$) (Fig. 2, left panel). Baclofen did not modify the rise of PRL evoked by either dose of the dopaminergic antagonist. Basal PRL levels were not modified by baclofen administration.

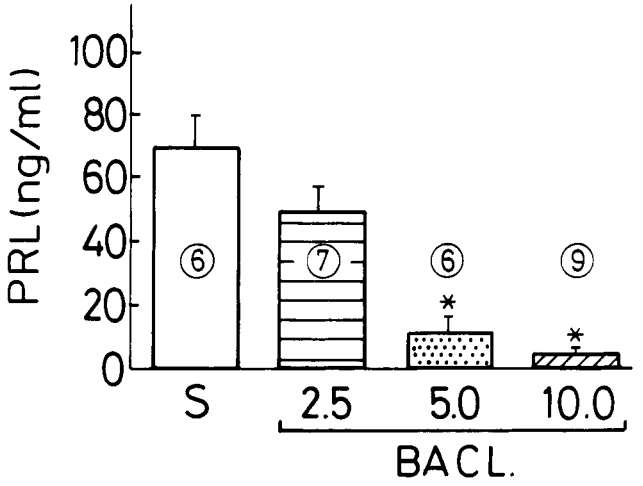


**Figure 1.** Dose-response effect of baclofen (BACL) (2.5, 5, and 10 mg/kg) on prolactin secretion induced by immobilization stress in male rats. For this and the figures that follow, numbers inside or above columns indicate number of rats per group. The height of the bar indicates the mean and the vertical line 1 SE. *$P < 0.05$.

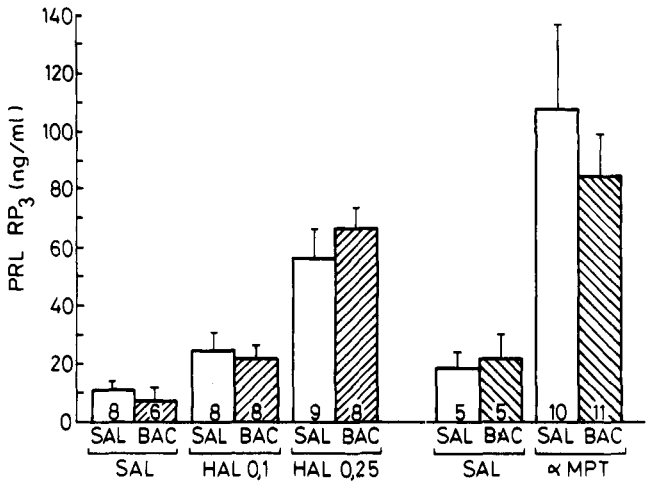


**Figure 2.** Effect of baclofen (BAC) on PRL secretion in male rats pretreated with haloperidol (HAL) (0.1 or 0.25 mg/kg ip) (left panel) or $\alpha$-methyl-*p*-tyrosine (MPT) (250 mg/kg ip) (right panel).

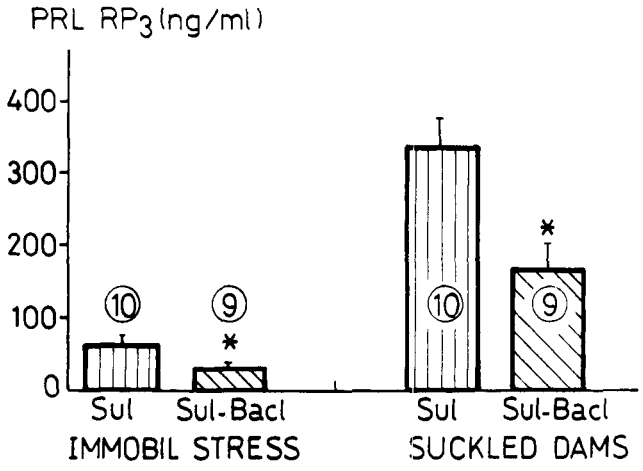


**Figure 3.** Effect of baclofen (BACL) on the prolactin secretion induced by immobilization stress (left panel) or suckling (right panel) in sulpiride (Sul)-pretreated animals (see text).

In MPT (250 mg/kg)-pretreated rats, PRL levels rose significantly above basal levels (Fig. 2, right panel). Baclofen in a dose of 10 mg/kg had no effect on these high PRL levels.

**Effect of Baclofen on PRL Secretion in Sulpiride-Pretreated Stressed or Suckled Rats.** When sulpiride-pretreated male rats were injected with baclofen and were then stressed by immobilization, baclofen was able to lower PRL levels significantly (Fig. 3, left panel). Similar results are obtained with suckled dams. When these animals were pretreated with sulpiride and were then suckled by their pups, baclofen significantly decreased PRL secretion (Fig. 3, right panel).

Sulpiride pretreatment effectively raised PRL secretion in male rats or in suckled dams (male rats—saline: 6.7 ± 0.5 (nine rats) vs Sul (1 mg/kg): 26.5 ± 4.9 (10 rats) ng/ml, $P < 0.05$; suckled dams—saline: 12.2 ± 2.2 (eight rats) vs Sul (0.5 mg/kg): 284.8 ± 44.1

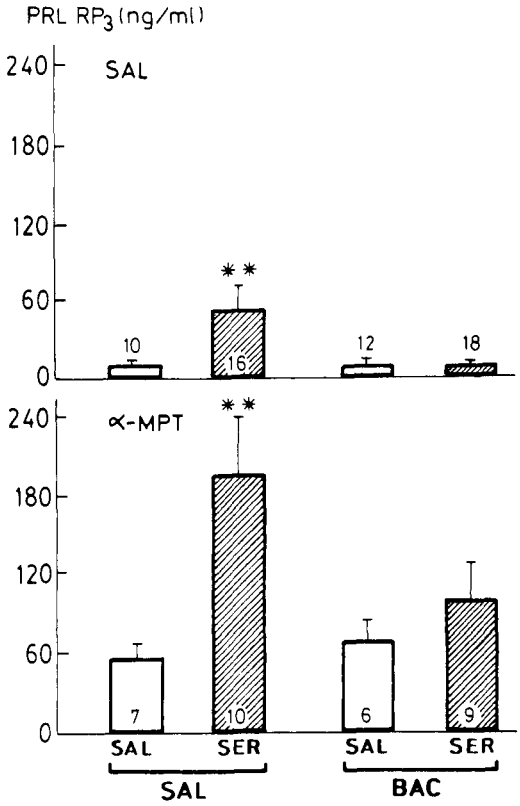


**Figure 4.** Effect of baclofen (BAC) on the prolactin secretion induced by serotonin (SER) (5 mg/kg) in male animals pretreated with saline (upper panel) or with MPT (lower panel).

(10 rats) ng/ml, $P < 0.05$). Higher doses of sulpiride did not further increase PRL secretion in suckled rats (Sul 1 mg/kg: 312.3 ± 49.1 (nine rats) ng/ml; Sul 10 mg/kg: 257.9 ± 35.3 (eight rats) ng/ml).

**Effect of Baclofen on PRL Secretion in Serotonin-Induced Hyperprolactinemia.** In saline-pretreated animals, serotonin administration induced a significant rise in PRL levels (Fig. 4, upper panel). Baclofen treatment completely blunted this rise, having no effect on basal levels.

In MPT (250 mg/kg)-pretreated animals (Fig. 4, lower panel), basal levels were significantly elevated with respect to saline-pretreated animals. In these rats baclofen did not modify PRL titers as shown in a previous experiment (Fig. 2, right panel). When serotonin was administered to MPT-pretreated rats, PRL titers rose significantly above the high levels achieved by MPT. In these animals baclofen significantly lowered PRL titers to MPT-pretreated levels, though not attaining the low values observed in saline-pretreated rats.

## Discussion

Previous studies have shown that baclofen did not modify PRL secretion in basal conditions, and it was postulated that it was only active when a prolactin-releasing neuroendocrine reflex was activated (stress,

suckling) (7–9). In the present work, a clear dose-dependent response was observed in the inhibition of PRL secretion in immobilization-stressed animals. The decrease in plasma PRL could be due to an effect of the GABAergic system on the dopaminergic inhibitory tone or, alternatively, the GABAergic system could be inhibiting a prolactin-releasing mechanism. Thus, the action of baclofen could involve the inhibition of the secretion of a PRL-releasing factor (PRF), since it has been described that both in stress and in suckling, releasing factors play a key role in the PRL rise (11, 12). On the other hand, dopamine, a well-known and potent prolactin-inhibiting hormone (13), decreases its hypothalamic turnover in these same situations (14–16), which means that the inhibitory effect of baclofen could also be due to the reduction of this decrease in dopamine release.

When baclofen was administered to haloperidol- or MPT-pretreated animals (17), it had no effect on PRL secretion. This would suggest that when hyperprolactinemia is the consequence of interference with the dopaminergic system and, apparently, no PRF is involved, baclofen is not effective. Furthermore, it has been shown that baclofen, like lactation and stress, induces a reduction in dopamine turnover (18) and in behavior studies an antidopaminergic effect was revealed, an action similar to that observed with haloperidol (19). Therefore, if the reduction in dopamine turnover caused by baclofen had a direct role in controlling the lactotrophs its action would be expected to be hyperprolactinemic rather than hypoprolactinemic as actually observed.

Sulpiride, an antagonist of dopamine receptors, induced a significant increase in PRL levels in male rats. When sulpiride was preinjected and stress was applied, a further significant increase in PRL serum levels was observed. If these animals were also treated with baclofen, a significant decrease in PRL levels was determined. This shows that even when the dopaminergic system was blocked, the inhibitory effect of baclofen on PRL secretion induced by stress could still be observed, suggesting that baclofen was acting at a PRF released by stress. A similar study was performed with suckled dams. When sulpiride-preinjected rats were exposed to suckling, a small but not significant increase in PRL titers was observed. This is probably the consequence of the special state of the pituitary gland in this physiologic condition. This gland is very sensitive to PRL-releasing stimuli and even a low dose of sulpiride is capable of releasing very high levels of PRL. Thus, the additive increase in PRL, that is the consequence of a decrease in dopamine (or dopamine blockade) and the effect of a releasing factor, as observed in MPT-pretreated thyrotropin-releasing hormone-injected lactating rats (14, 20) could not be observed. Even so, when these rats were treated with baclofen, PRL levels were reduced significantly, suggesting again that the effect of baclofen was on the PRF component of PRL release induced by suckling (8).

Supporting this mechanism, baclofen completely abolished PRL release evoked by serotonin administration. If serotonin-treated rats were also preinjected with MPT, an additive effect on PRL secretion was also observed. In these rats baclofen was able once again to lower PRL titers only to MPT-pretreated levels. Serotonin facilitatory pathways controlling PRL secretion at a central level have been described (21) in a variety of situations including stress and suckling, probably through vasoactive intestinal peptide or thyrotropin-releasing hormone secretion (20, 22–28). Thus, when analyzing the present results, one could postulate that baclofen may interfere with serotonergic transmission and thus modify the secretion of a PRF; as a consequence, this component of PRL release would be abolished in stress or suckling. It has recently been postulated that GABA B receptors may play an important neuromodulatory role in serotonin function (6, 29–31).

Our results suggest that the dose-dependent effect of baclofen on PRL secretion is probably the consequence of an inhibition exerted on a prolactin-releasing component of the neuroendocrine responses evoked by suckling and stress, possibly at a serotonergic step.

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad de Buenos Aires, Fundación Antorchas (Argentina) and J. S. Guggenheim Memorial Foundation (New York, NY).

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