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# Effect Of Infusing γ-Aminobutyric Acid Receptor Agonists and Antagonists into the Medial Preoptic Area and Ventromedial Hypothalamus on Prolactin Secretion in Male Sheep

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**We investigated the effects of γ-aminobutyric acid (GABA) agonists muscimol and baclofen ($GABA_A$ and $GABA_B$ agonists, respectively) and antagonists bicuculline methiodide (BMI, $GABA_A$ antagonist) or 2-hydroxysaclofen (SAC) and CGP 55845A ($GABA_B$ antagonists) on prolactin (PRL) secretion in castrated rams. The drugs were applied by microdialysis into either the medial preoptic area (mPOA) or ventromedial hypothalamus (VMH). Dialysis of baclofen into the mPOA significantly increased mean PRL ($p < 0.05$), whereas SAC caused a small, but significant decrease ($p < 0.01$). Dialysis of either muscimol or BMI into the mPOA had no effect on prolactin. In the VMH, baclofen significantly increased ($p < 0.01$) mean PRL but SAC and CGP 55845A were ineffective, whereas dialysis of either muscimol or BMI increased mean prolactin ($p < 0.01$). These results show that infusion into the mPOA of drugs that affect $GABA_B$ receptor alter PRL release, whereas infusion of a $GABA_A$ agonists and antagonist was without effect on PRL release. In contrast, infusion of both $GABA_A$ and $GABA_B$ agonists and a $GABA_A$ antagonist into the VMH altered PRL secretion. This suggest that GABAergic neurons in both regions participate in regulating PRL secretion, but by different receptor systems.**

**Key Words:** Prolactin; sheep; GABA; microdialysis.

## Introduction

It is well established that control of prolactin (PRL) secretion is exceedingly complex, involving several PRL stimulatory and inhibitory factors from the hypothalamus and the posterior pituitary as well as auto/paracrine control within the anterior pituitary itself. Although several hypothalamic peptides or amines can affect PRL secretion, dopamine is accepted as a primary PRL-inhibiting factor *(1–3)*, whereas thyrotropin-releasing hormone (TRH) and several other peptides may function as PRL-stimulating factors *(4–6)*. The secretion of these compounds is in turn regulated by others including γ-aminobutyric acid (GABA) *(5,7,8)*.

GABA can act on both the pituitary and hypothalamus to alter PRL secretion, although the physiologically important site appears to be the hypothalamus. Application of both GABA and GABA analog directly to the anterior pituitary will alter PRL release. However, biochemical evidence does not support local synthesis of GABA in the pituitary *(9)* and the concentration of GABA required to alter PRL release directly is severalfold higher than that found in hypophyseal-portal blood *(10)*.

In contrast, there is much evidence that GABA can act centrally to alter PRL secretion. Injection into the third ventricle of either GABA or the $GABA_A$ agonist muscimol repeatedly has been found to increase circulating PRL concentrations in ovariectomized, ovariectomized steroid-treated, and male rats *(11–15)*, although some investigators found that the effect of such injections is dependent on dose *(12,15)*. Localized implants *(13)* or microinjection of muscimol or GABA into the medial preoptic area (mPOA) or microinjection of these drugs into the mediobasal hypothalamus also increased PRL release *(16)*, whereas microinjection of the $GABA_A$ antagonists bicuculline or picrotoxin was without effect at either site *(16)*. In another study, microinjection of bicuculline methiodide (BMI) into the mPOA suppressed circulating PRL, but microinjection into the arcuate nucleus was ineffective *(17)*. Thus, the majority, but not all of the data suggest that in the rat, activation of $GABA_A$ receptors in either the mPOA or mediobasal hypothalamus elicits PRL release.

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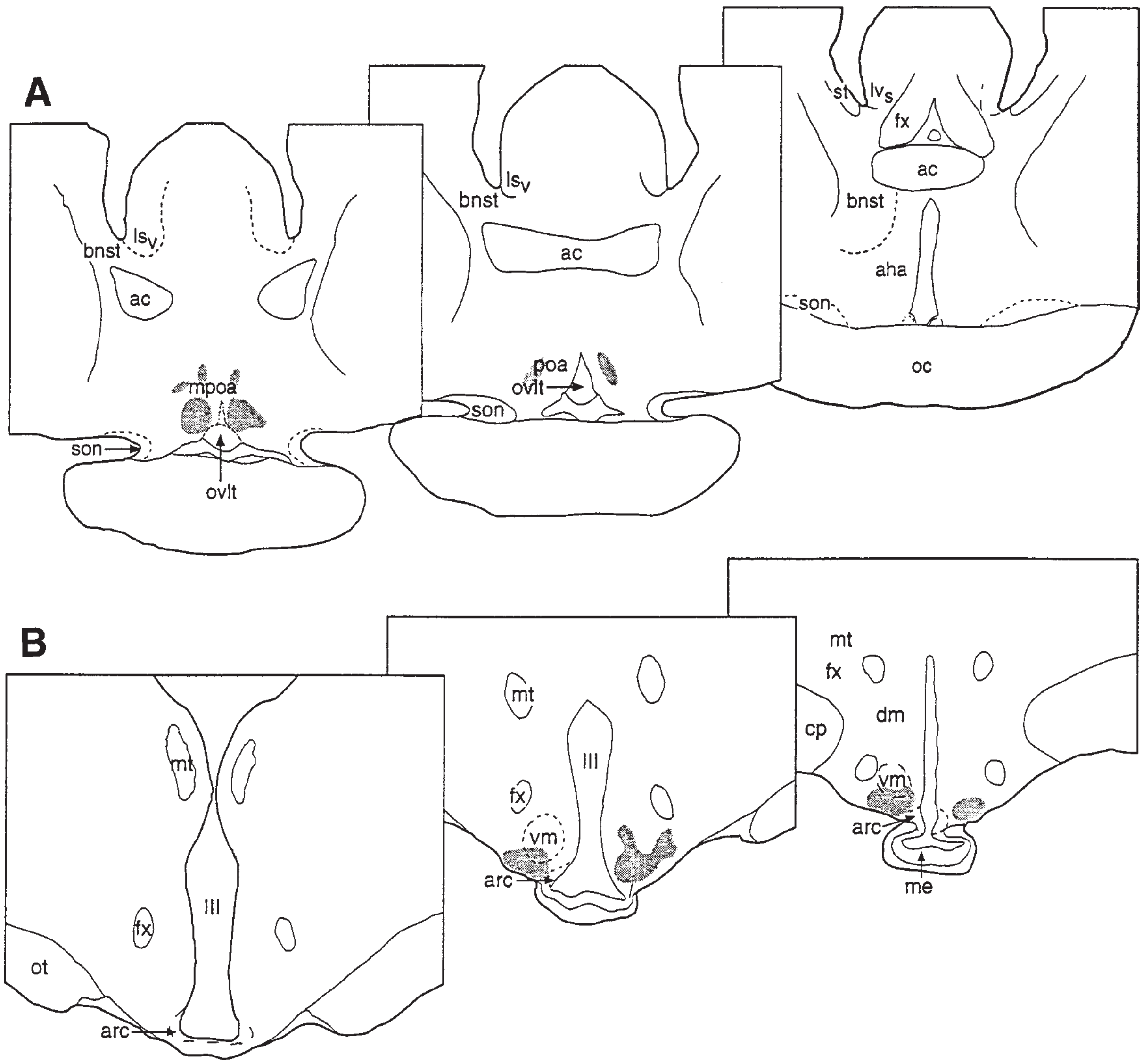


**Fig. 1.** Diagram showing composite location of probe placement in animals where the target was the mPOA (**A**) and for animals where the target was the VMH (**B**). The shaded areas represent the locations of the tips of the 2-mm dialyzing membrane length. AC, anterior commissure; AHA, anterior hypothalamic area; ARC, arcuate nucleus; BNST, bed nucleus of the stria terminalis; DM, dorsomedial nucleus; FX, fornix; ME, median eminence; MT, mamillothalamic tract; LSV, lateral septum; OVLT, organum vasculosum of the lamina terminalis; OC, optic chiasm; POA, preoptic area; SON, supraoptic nucleus; VM, ventromedial nucleus; III, third ventricle, OT, optic tract.

The role of the $GABA_B$ receptor system in the control of PRL release has received relatively little investigation. In male rats, SC injection of baclofen (a $GABA_B$ agonist) elevated PRL, whereas 2-hydroxysaclofen (a $GABA_B$ antagonist) was without detectable effect. These observations suggest that the $GABA_B$ system also may regulate PRL, but they do not address the site of this regulation. Accordingly, we investigated the relative roles of the $GABA_A$ and $GABA_B$ receptor systems in the mPOA and ventromedial hypothalamus (VMH) in regulating PRL secretion. We determined the effects of delivering $GABA_A$ and $GABA_B$ agonists and antagonists by in vivo retrodialysis into either the mPOA or VMH of castrated male sheep.

We investigated both sites because TRH perikarya are found in the mPOA, although the highest density is in the paraventricular nucleus *(18)*. The VMH is an important site, because the arcuate-nucleus contains many perikarya of the tuberoinfundibular dopaminergic *(19)* and tuberoinfundibular GABAergic systems *(20)*, both of which terminate in the median eminence, also a point of termination for TRH axons.

## Results

### *Histology*

A schematic representation of the extent of variation of probe placements is shown in Fig. 1. These schematic draw-

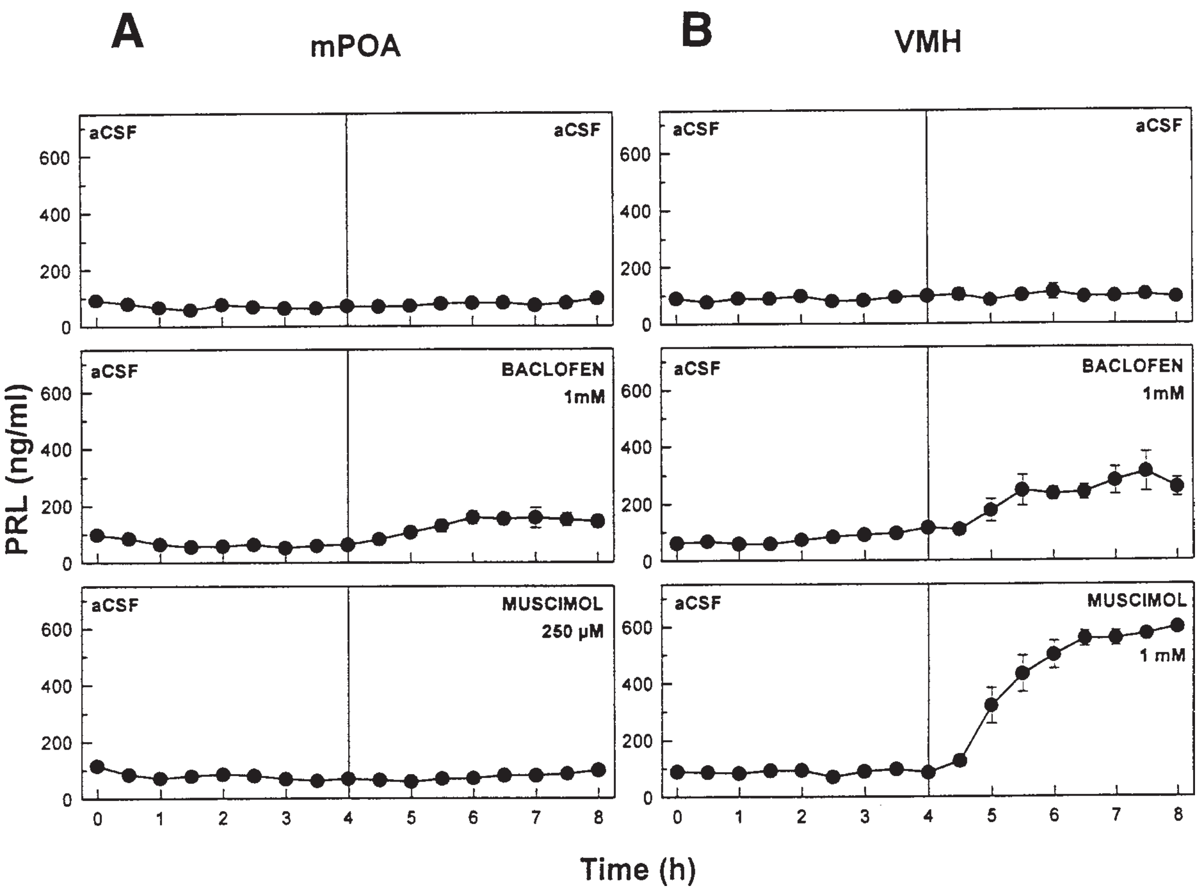


**Fig. 2.** Plasma PRL profiles of castrated rams in experiment species 1, subjected to three separate bilateral infusions of either aCSF only, aCSF-baclofen, or aCSF-muscimol into the mPOA **(A)** or the VMH **(B)**. Listed drug concentrations are those of the dialysis solutions. It was estimated that the total dose of baclofen and muscimol delivered at each bilateral site in the mPOA was 7.9 µg and 1.1 µg, respectively. In the VMH, the total dose of baclofen and muscimol delivered at each bilateral site was 7.9 and 4.5 µg, respectively. Drug delivery started at 4 h as indicated by the vertical line. Each symbol represents the mean ± SEM of PRL at that time-point. Error bars that fall within a symbol are not visible.

ings show the location of bilateral probes placed in the mPOA ($n = 7$) and VMH ($n = 8$) in experiment series 1, the mPOA ($n = 5$) and VMH ($n = 4$) in experiment series 2, and the VMH ($n = 6$) in experiment 3. Probe placements varied slightly within location, but were confined to either the mPOA or the VMH. Locations of probes in animals excluded from analysis owing either to misplacement or damage are not shown in Fig. 1.

*Experiment Series 1*

**Effects of Agonists in the mPOA.** Figure 2A illustrates temporal changes in plasma PRL profiles (mean ± SEM for each time-point) of all animals during treatment with artificial cerebrospinal fluid (aCSF), baclofen, or muscimol in the mPOA. Results of mean PRL during the first and last 4 h of infusion for all animals are summarized in Table 1. PRL concentrations were significantly higher ($p < 0.05$) after aCSF-baclofen than after aCSF-aCSF. Concentrations after aCSF-muscimol did not differ ($p > 0.05$) from those after aCSF-aCSF.

**Effects of Agonists in the VMH.** Figure 2B illustrates plasma PRL profiles (mean ± SEM for each time-point) of all animals during dialysis of aCSF, baclofen, or muscimol into the VMH. Results for all animals are summarized in Table 1. PRL concentrations were significantly higher ($p < 0.01$) after treatment with either aCSF-baclofen or aCSF-muscimol than after treatment with aCSF-aCSF.

*Experiment Series 2*

**Effects of Antagonists in the mPOA.** Figure 3A illustrates plasma PRL profiles (mean ± SEM for each time-point) of all animals during dialysis of 2-hydroxysaclofen (SAC) or BMI in the mPOA. Effects of drug treatment on mean PRL concentration for all animals are summarized in Table 2. SAC caused a small, but significant reduction in PRL release ($p < 0.01$). Although BMI affected the animals' behavior when dialyzed into the mPOA (incessant bleating and aggression), it did not significantly ($p > 0.10$) change PRL secretion.

**Effects of Antagonists in the VMH.** Figure 3B illustrates PRL plasma profiles (mean ± SEM for each time-point) of all animals during dialysis of SAC or BMI into the VMH. BMI dialysis caused marked hyperactivity. Thus, the

**Table 1**
Effect on Mean Plasma PRL Concentrations (ng/mL) of Dialyzing GABA Receptor Agonists Baclofen and Muscimol into Either the mPOA ($n$ = 7) or the VMH ($n$ = 8) of Castrated Rams[a]

| Dialysis site | Drug | Pretreatment[b] | Posttreatment[c] | Difference |
|---|---|---|---|---|
| POA | aCSF | 71 ± 9 | 79 ± 11 | 8 ± 12 |
| | Baclofen[d] | 67 ± 5 | 135 ± 18 | 67 ± 18[f] |
| | Muscimol[e] | 79 ± 12 | 76 ± 11 | -3 ± 16 |
| VMH | aCSF | 89 ± 9 | 98 ± 15 | 9 ± 9 |
| | Baclofen[d] | 78 ± 8 | 231 ± 26 | 153 ± 24[g] |
| | Muscimol[e] | 89 ± 11 | 464 ± 26 | 375 ± 21[g] |

[a]Results from experiment series 1 shown here are expressed as mean ± SEM. [b]During 4 h dialysis of aCSF. [c]During dialysis of aCSF or drug. [d]1-m$M$ concentration at both sites. [e]250 μ$M$ in mPOA and 1 m$M$ in VMH. [f]$p$ < 0.05 compared to aCSF difference within site. [g]$p$ < 0.01 compared to aCSF difference within site.

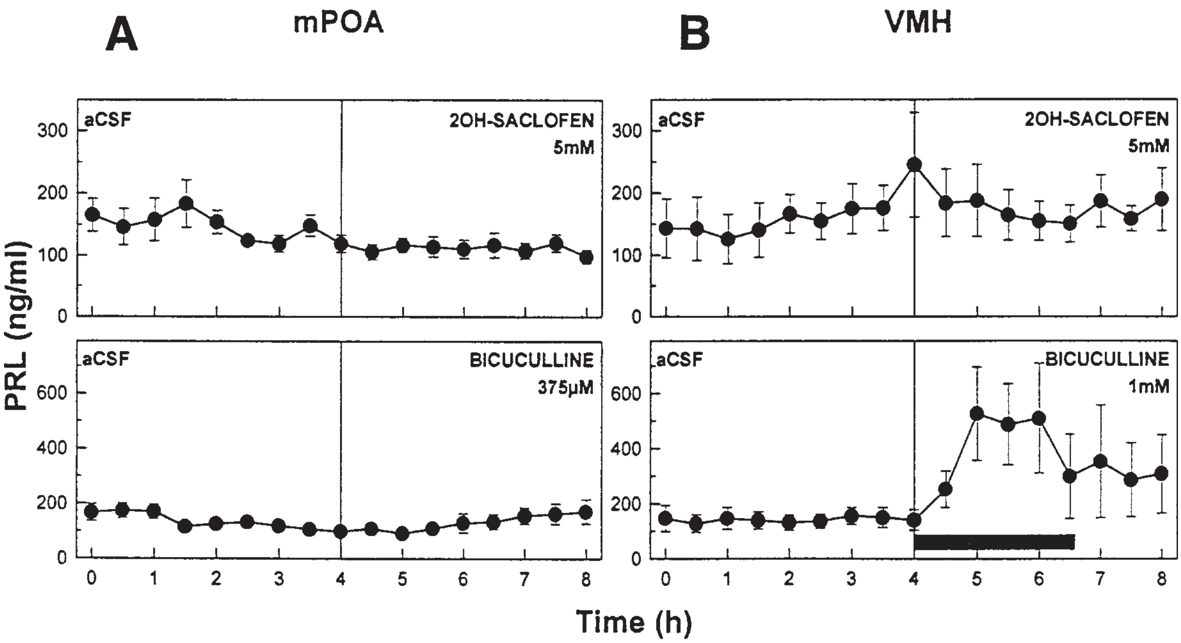


**Fig. 3.** Plasma PRL profiles of castrated rams in experiment series 2, subjected to two separate bilateral infusions of either, aCSF-SAC or aCSF-BMI into the mPOA (**A**) or VMH (**B**). Listed drug concentrations are those of the dialysis solution. It is estimated that the total dose of SAC delivered at each bilateral site was 51 μg, but for BMI, it was 7.3 and 12.21 μg in the mPOA and VMH, respectively. Drug delivery started at 4 h as indicated by the vertical line. Note that dialysis BMI into the VMH was carried out only for 2.5 h as depicted by the solid bar. Each symbol represents the mean ± SEM of PRL at that time-point. Error bars that fall within a symbol are not visible.

dialysis of this drug was terminated after 2.5 h and the concentration in the subsequent experiment was lowered to 375 μ$M$; however, SAC dialysis and blood collection from both groups were continued for 4 h. Effects of treatment on mean PRL concentration for all animals are summarized in Table 2. SAC did not change ($p$ > 0.01) mean PRL. BMI increased ($p$ < 0.05) PRL release in all animals.

*Experiment 3*

**Effects of Antagonists Dialysis in the VMH.** Figure 4 shows PRL plasma profiles (mean ± SEM for each time-point) of all animals during dialysis of aCSF, 3-[1-(S)-3,4-dichlorophenyl)-ethyl] amino-2-(S)-hydroxy-propyl-*p*-benzyl-phospinic acid (CGP) and BMI into the VMH. Effects of treatment for all animals are summarized in Table 3. PRL concentrations were elevated significantly ($p$ < 0.01) by aCSF-BMI as compared to aCSF-aCSF. PRL concentrations after aCSF-CGP did not differ ($p$ > 0.05) from that of aCSF-aCSF.

## Discussion

This is the first study to evaluate the role of both $GABA_A$ and $GABA_B$ receptors in both the mPOA and VMH on PRL secretion. Dialysis into the mPOA of either the $GABA_A$ agonist muscimol or the antagonist BMI did not affect PRL release. In contrast, dialysis of the $GABA_B$ receptor agonist, baclofen, and the antagonist, SAC, significantly increased

**Table 2**
Effect on Mean Plasma PRL Concentrations (ng/mL) of Dialyzing GABA Receptor Antagonists SAC or BMI into Either the mPOA ($n$ = 5) or the VMH ($n$ = 4) of Castrated Rams[a]

| | Dialysis site | | | |
|---|---|---|---|---|
| | mPOA | | VMH | |
| Drug | Pretreatment[b] | Posttreatment[c] | Pretreatment[b] | Posttreatment[c] |
| SAC[d] | 146 ± 16 | 111 ± 11[g] | 163 ± 35 | 172 ± 40 |
| BMI[e] | 134 ± 13 | 133 ± 24 | 141 ± 29 | 456 ± 115.5[f] |

[a]Results from experiment series 2 shown here are expressed as mean ± SEM. [b]During 4 h dialysis of aCSF. [c]During dialysis of drug, 4 h of SAC and 2.5 h of BMI. [d]5 m$M$ concentration at both sites. [e]375 µ$M$ in mPOA, 1 m$M$ in VMH. [f]$p < 0.05$ vs pretreatment. [g]$p < 0.01$ vs pretreatment.

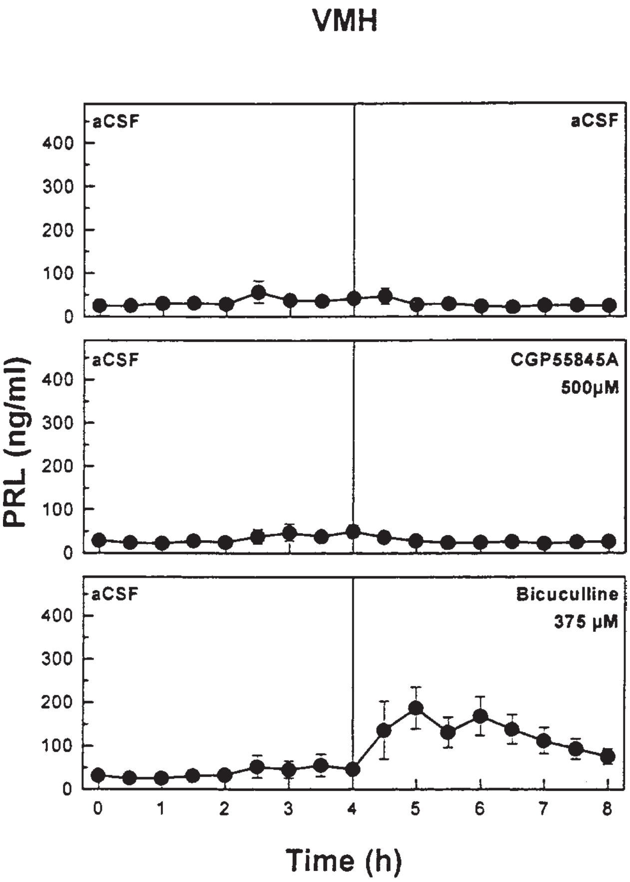


**Fig. 4.** Plasma PRL of castrated rams in experiment 3, subjected to three separate bilateral infusions of either, aCSF-aCSF, aCSF-CGP 55845A or aCSF-BMI into the VMH. Listed drug concentrations are those of the dialysis solution. It is estimated that the total dose of CGP 55845A and BMI delivered at each bilateral site was 8.3 and 7.3 µg, respectively. Drug delivery started at 4 h as indicated by the vertical line. Each symbol represents the mean ± SEM of PRL at that time-point. Error bars that fall within a symbol are not visible.

**Table 3**
Effect on Mean Plasma PRL Concentrations (ng/mL) of Dialyzing GABA Receptor Antagonists CGP (500 µ$M$) or BMI (375 µ$M$) into the VMH of Castrated ($n$ = 6) Rams[a]

| Drug | Pretreatment[b] | Posttreatment[c] | Difference |
|---|---|---|---|
| aCSF | 35 ± 7 | 29 ± 4 | –6 ± 3 |
| CGP | 33 ± 8 | 27 ± 3 | –7 ± 6 |
| BMI | 38 ± 12 | 130 ± 35 | 92 ± 29[d] |

[a]Results from experiment 3 shown here are expressed as mean ± SEM. [b]During 4-h dialysis of aCSF. [c]During 4-h dialysis of drug. [d]$p < 0.01$ compared to aCSF difference.

and decreased PRL secretion, respectively. In the VMH, both the $GABA_A$ agonist muscimol and the antagonist BMI increased PRL secretion. Dialysis into the VMH of the $GABA_B$ agonist baclofen increased PRL secretion, whereas the antagonists SAC and CGP 55845A were ineffective. These results indicate that in the mPOA of the wether, either stimulation or blockade of $GABA_B$ receptors alters PRL secretion, whereas altering $GABA_A$ receptors has little effect. In contrast, in the VMH, perturbation of either $GABA_A$ or $GABA_B$ receptors stimulated PRL release, indicating that both receptor types are involved in regulating PRL release. Because effects differed with brain region, our discussion will deal with the mPOA and VMH in that order.

The finding that in the mPOA baclofen, a $GABA_B$ receptor agonist, increased mean PRL concentrations whereas the $GABA_B$ receptor antagonist decreased PRL suggests that $GABA_B$ receptors in the mPOA mediate a stimulatory effect of GABA. These results are consistent with the observations that electrochemical stimulation *(21)* of the mPOA or intraventricular infusion of GABA-stimulated PRL release in the rat *(12)*. The neuronal systems through which this effect is mediated are not known. Elevation of PRL could be achieved either by increased secretion of a stimulatory factor, such as TRH, or inhibition of dopamine. To date there is relatively little information on the role of GABAergic receptors in the mPOA on secretion of either of these PRL regulators.

We were unable to demonstrate that mPOA $GABA_A$ receptors affect PRL release in the wether. Our finding that muscimol was ineffective in the mPOA contrasts with observations that muscimol injected or implanted into the mPOA/anterior hypothalamus of ovariectomized or male rats increased PRL release *(13,16)*. The ineffectiveness of BMI also is consistent with observations by Willoughby et al. *(16)*, but inconsistent with those of Rettori et al. *(17)*, who found that microinjections of BMI into the mPOA of male rats decreased PRL release. These contradictory results possibly may be explained by the differences in species, methodology, or drug concentration. The $GABA_A$ agonist and antagonist regimens we used effectively changed luteinizing hormone (LH) secretion *(22,23)* in these same animals. Thus, the failure of $GABA_A$ agonists and antagonists to alter PRL when applied to the mPOA is not likely a reflection of inadequate drug regimens. Instead, it appears to reflect either a minor or no role for $GABA_A$ receptors at this site in regulating prolactin in this species.

In contrast, $GABA_A$ receptors in the VMH appear to be involved in regulating PRL. The observation that muscimol in the VMH increased PRL release is consistent with observations that GABA or muscimol injections into the third ventricle (e.g., *11,14*) or muscimol injections into the mediobasal hypothalamus of rats increased PRL release *(16)*. The massive release of PRL caused by muscimol infusion into the VMH in this study could have been brought about either by direct inhibition of GABA release itself, by release of a PRL-stimulating factor, or by the inhibition of the tuberoinfundibular dopaminergic system. The third mechanism is supported by observations that intraventricular injections of muscimol into rats decreased dopamine turnover in the mediobasal hypothalamus accompanied by increased PRL secretion *(14)*. The concept that GABA alters PRL by acting on dopaminergic neurons *(24)* also is supported by observations that GABAergic neurons synapse with arcuate nucleus dopaminergic neurons *(25)* and that [$^3$H]-dopamine release in synaptosomal preparations is inhibited by muscimol treatment. This indicates that $GABA_A$ receptors are present on dopaminergic terminals *(26)* and that their activation decreases dopamine release.

In both experiments 2 and 3, dialysis of BMI into the VMH also stimulated PRL release—an effect similar to that of muscimol. This observation contrasts with the findings that BMI was ineffective in changing PRL release when injected into the mediobasal hypothalamus of rats *(16)*. The difference may be owing to a lower concentration of drug used in that study. However, such parallel effects of $GABA_A$ agonists and antagonists also have been found in other systems. Both BMI and muscimol suppressed LH when infused into the VMH of castrated rams *(22,23)*. Both elevated LH when injected into the POA of some ovariectomized, estrogen-treated ewes, but both suppressed LH when injected into the POA of ovariectomized ewes *(27)*. Similarly injection of either GABA or bicuculline into the POA of ovariectomized rats reduced circulating LH *(28)*. The stimulatory effects of both BMI and muscimol on PRL appears paradoxical, but possibly may be explained by actions of BMI on transmitter systems in addition to the GABAergic. Although BMI is a competitive antagonist of $GABA_A$ receptors, it also can affect other neuronal systems, including the *N*-methyl-*d*-aspartate (NMDA) receptor for glutamate. For example, an electrophysiological study showed that low doses of BMI (10–100 μ*M*) decreased channel open time of the NMDA receptor, and at higher doses of 100 and 200 μ*M*, suppressed whole-cell NMDA-evoked responses *(29)*. BMI also reduced NMDA- and kianate-induced membrane currents *(30)* and decreased NMDA-evoked release of [$^3$H]-DA *(31)*. More importantly, NMDA and kianic acid administration to male rats decreased circulating PRL concentrations *(32)*, and iv injection of NMDA resulted in a prolonged suppression of PRL in ewes *(33)* and mares *(34)*, whereas blockade of NMDA receptors in ovariectomized (OVX) ewes stimulated PRL release *(35)*. Those studies suggest that NMDA receptors may be involved in the control of PRL release. If BMI decreases channel open time of the NMDA receptors in the VMH, PRL release could be affected. Thus, one explanation for the stimulatory effects of BMI on PRL is that it may antagonize NMDA receptors located on dopaminergic neurons in the arcuate nucleus. This would result in decreased dopamine release accompanied by increased PRL secretion. This explanation is consistent with available observations, but additional studies are needed, and caution is indicated regarding the use of BMI as $GABA_A$ blocker.

The specific role of $GABA_B$ receptors in the VMH appears to be complex. Application of the $GABA_B$ agonist baclofen in the VMH increased PRL. However, the antagonists SAC and CGP had no effect in the VMH, in contrast to the mPOA, where SAC suppressed PRL. These observations are consistent with those of Wagner et al. *(36)*, who reported that ip injections of baclofen into rats increased PRL, whereas SAC was without effect. The mechanism by which activation of $GABA_B$ receptors elevates PRL is not clear, although it also could be through stimulation of PRL-stimulatory factors, such as TRH, or via inhibition of dopamine release. The observation that elevated PRL induced by systemic injection of baclofen was accompanied by a decrease in DOPAC, a dopamine metabolite, concentrations in the median eminence *(36)* of rats is consistent with the latter possibility.

There are at least three possible explanations for the ineffectiveness of infusing CGP into the VMH on PRL release. One is that the drug was inactive at the concentration delivered. This seems unlikely given the high potency, binding affinity, and proven actions in other systems *(37–39)*. The second is that in experiment 3, PRL already was relatively suppressed by the short day photoperiod and therefore resistant to further suppression. The third possibility is that the VMH $GABA_B$ receptors were not activated under the conditions of this study, hence the ineffectiveness of CGP on PRL release. In fact, it has been suggested that activation of $GABA_B$ autoreceptors in the hippocampus requires strong stimulation to overcome the GABA reuptake system and/or low-affinity presynaptic $GABA_B$ receptors *(40)*. Also, it must be noted that in experiment 2, SAC also was ineffective in the VMH at the same dose that suppressed PRL when applied to the mPOA.

Although the effects of these treatments on changes in LH secretion have been reported *(22,23)*, it is of interest to compare and contrast the responses of prolactin and LH. Infusion of either muscimol or BMI into the VMH suppressed LH, but elevated PRL. In the POA, BMI and muscimol also both tended to suppress LH, whereas BMI elevated PRL and muscimol was without effect. These results demonstrate although that these two drugs had similar effects on a given hormone, they also had opposite effects on the two hormones. This also suggests that GABA regulation of PRL and LH is mediated via distinct neuronal pathways. Interestingly, baclofen administration into the POA had no detectable effect on LH, but elevated PRL, whereas baclofen in the VMH elevated both prolactin and LH. Thus, modulation of the $GABA_A$ receptors had opposite effects on PRL and LH, whereas stimulation of the $GABA_B$ receptors appeared to have similar effects on these two hormones.

In summary, our results show that infusion into the mPOA of drugs that affect $GABA_B$ receptors alters PRL release, whereas infusion of $GABA_A$ agonists and antagonist had little effect on PRL. In contrast, infusion of both $GABA_A$ and $GABA_B$ agonists and a $GABA_A$ antagonist into the VMH altered PRL secretion. These results suggest that GABAergic neurons in both regions participate in regulating PRL secretion, but by different receptor systems. The neurochemical pathways between activation of GABA receptors and changes in PRL release are yet to be determined.

## Materials and Methods

Suffolk and Hampshire rams that had been castrated for at least 2 mo were maintained outdoors at the Veterinary Research Farm, Urbana, IL (latitude 40°N) until undergoing surgery for the placement of guide-cannulae into the brain. After surgery, they were housed indoors, fed alfalfa pellets and sheep chow (Ralston-Purina Co., St. Louis, MO), and given free access to water. Experiment series 1 was carried out from March through August 1995 on animals exposed to a natural long-day photoperiod. Experiment series 2 was carried out during January–February 1996 on animals that had been kept under a controlled long-day (16L:8D) photoperiod starting October 1, 1995. Experiment 3 was carried out during the breeding season (October–November, 1996) on animals kept under a controlled short-day (11L:13D) photoperiod initiated at the start of the experiment. The experiments were reviewed by our institutional committee on laboratory animal care and conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

### Surgery

Neurosurgery for bilateral placement of the guide-cannulae was carried out under aseptic conditions previously described *(22)*. Briefly, after anesthesia was induced, the head was secured firmly in a stereotaxic instrument (Kopf Instruments, Tujunga, CA). Following a skin incision and removal of a circular piece of skull (2.5 cm in diameter), the sagittal sinus was doubly ligated and retracted. Radiopaque dye (Renografin-76; E.R. Squibb and Sons, Princeton, NJ) was injected into the third ventricle, and lateral radiographs were taken to aid in the placement of guide-cannulae in the mPOA or VMH. Stainless-steel guide-cannulae with stylets extending 2 mm then were placed bilaterally into either the mPOA or VMH areas (*see* 22 for coordinate details). The cannulae and a protective cap were anchored to the skull with dental acrylic and screws, and the incision then was closed. The animals were given at least 3 wk to recover. Twenty-gage, stainless-steel guide-cannulae were used in experiments 1 and 3. However, the guide-cannulae in experiment 2 were made from 18-gage, thin-wall, stainless-steel tubing, since these animals had been used previously in a steroid implant study where this was a prerequisite.

#### *Experimental Protocol and Design*

Starting at least 3 wk after implantation of the guide-cannulae, and 1 d before drug dialysis, groups of three animals had a catheter inserted into the jugular vein for the purpose of blood collection. Each animal was placed into an individual “metabolism” pen and allowed 12–14 h to acclimate. On the day of drug dialysis, the stylets were removed from the bilateral guide-cannulae and replaced by microdialysis probes. The probes then were connected to an infusion pump (Sage™ Instruments, Boston, MA) by means of a microline (Cole-Parmer, Chicago, IL). Each animal then received a 4-h period control dialysis of aCSF at a flow rate of 2 μL/min. During the subsequent 4 h, either the dialysis of aCSF was continued, or the solution was switched to the designated drug treatment with the same probes left in place. During the entire 8-h period, jugular blood samples were collected at 10-min intervals. Blood samples were collected in 100 μL of heparin (125 U/mL) and centrifuged within an hour of collection, and plasma was stored at –20°C until assayed for PRL. After each dialysis session, the microdialysis probes were replaced by the stylet, and the animals were returned to group pens after receiving Liquamycin LA-200 (Pfizer, New York, NY) antibiotic at 6.2 mL/100 kg im dose. Subsequent dialysis sessions on the same group of animals were conducted at weekly intervals. Thus, each animal received a total of two (experiment 2) or three dialysis sessions.

### *Drugs*

The $GABA_A$ and $GABA_B$ agonists, muscimol and baclofen, respectively (Sigma, St. Louis, MO) the $GABA_A$ antagonist, BMI (Sigma), and the $GABA_B$ antagonists, SAC (Research Biochemical International, Natick, MA), and CGP (55845A), a gift from W. Fröstl of Novartis, Basel, Switzerland, were dissolved in aCSF. SAC was first dissolved in 0.1 *N* NaOH and neutralized with 0.1 *N* HCl before dilution in aCSF.

### *Dialysis Probe and Dialysis Buffer*

The microdialysis probe designed for use in sheep was of the concentric design with a membrane length of 2 mm. The dialysis buffer (aCSF) was composed of 127.6 m*M* NaCl, 2.5 m*M* KCl, 0.69 m*M* $CaCl_2$, 1 m*M* $MgSO_4$, 2.3 m*M* $NaH_2PO_4$, and 9.7 m*M* $Na_2HPO_4$ (pH 7.4) *(22)*. Briefly, the probe was constructed from a nitrocellulose hollow-fiber dialysis membrane with a molecular mass cutoff of 13 kDa (Spectrum Microgon, Laguna Hills, CA) fitted to 24-gage stainless-steel tubing through which a fused silica tubing passed (Polymicro Technologies Inc., Phoenix, AZ) and exited from the microline inlet. Since experiment 2 used 18-gage, thin-wall, guide-cannulae, the probes were adapted with 20-gage collars for a good fit.

### *Preliminary Experiments*

Concentration of both the GABA agonists and antagonists were chosen based on their effects on behavior and ability to alter circulating LH concentrations *(22,23)*. The initial concentration of $GABA_B$ receptor antagonist was chosen to approximate, without considering delivery efficiency and pattern, an equivalent concentration of phaclofen used by Scott and Clarke *(27)*, i.e., 10 μg/μl or approx 40 m*M*. Because phaclofen is of relatively low potency ($IC_{50}$ of 118 μ*M*), we chose the more potent SAC ($IC_{50}$ of 5.1 μ*M*) *(41)*. Assuming that 2 m*M* of SAC was approximately equivalent to 40 m*M* phaclofen, we used 2 m*M* SAC for a first trial. This dose did not change LH secretion. Thus, we then increased the dose to 5 m*M* for the next trial. This dose also did not affect LH secretion.

The dose of $GABA_A$ receptor antagonist, BMI, was chosen by a bracketing approach of testing the effects of perceived low and high doses on LH release and behavior. The object was to find a dose that consistently changed LH secretion without causing changes in behavior. Initially, BMI was tested at 50 and 250 μ*M* in the mPOA (*n* = 2–3) and VMH (*n* = 2–3) of testosterone-treated animals. At 50 μ*M* there was no observable effect on LH or behavior at either site, whereas 250 μ*M* caused a small suppression of LH and no observed effect on behavior. Subsequently, BMI was tested at 500 μ*M* in the mPOA only of castrated animals. This dose caused marked suppression of LH accompanied with hyperexcitable behavior. Accordingly, for experiment 2, we reduced the BMI dose to 375 μ*M* for infusion into the mPOA, but used 1 mM for infusion into the VMH on the expectation, based on previous observations with muscimol *(22)*, that at this site this dose of BMI would have relatively little effect on behavior—an expectation that proved incorrect.

Assuming an approx 8% probe delivery efficiency *(22)*, it was estimated that a 4-h infusion delivered approx 51 μg/site of SAC when infused at a concentration of 5 m*M*. Although it was not possible actually to measure the achieved drug concentration at the site, a calculation of 0.08 times 5 m*M* suggests a concentration of approx 400 μ*M* was delivered immediately adjacent to the dialysis probe.

Using the same assumptions for BMI delivery, it was calculated that a 4-h infusion of 375 μ*M* delivered 7.3 μg/site, whereas a 2.5-h infusion of 1 m*M* delivered 12.2 μg/site. The concentrations of BMI delivered were estimated at 30 and 80 μ*M*, respectively. For comparison, it is noted that the $IC_{50}$ of bicuculline is reported as 5 μ*M* *(42)*.

### *Experiment Series 1*

The objective was to compare the effects on PRL secretion of aCSF, baclofen and muscimol. In separate experiments, drugs were dialyzed into either the mPOA (*n* = 9) or VMH (*n* = 8). The treatments were dialysis of aCSF for 4 h followed by aCSF, baclofen (1 m*M*), or muscimol (1 m*M* in the VMH, 250 μ*M* in the mPOA) for an additional 4 h. This procedure delivered 7.9 μg of baclofen and 4.5 or 1.1 μg, muscimol, repectively, to each infusion site *(22)*. All animals received each of the three treatments in random order. Two animals with mPOA cannulae were eliminated from the experiment owing either to damaged cannulae or health problems leaving seven animals with cannulae in the mPOA.

### *Experiment Series 2*

The objective was to compare the effects on PRL secretion of SAC and BMI. In separate experiments, drugs were dialyzed into either the mPOA (*n* = 6) or VMH (*n* = 6). The treatments were microdialysis of aCSF for 4 h followed by either BMI (375 μ*M* in the mPOA for 4 h, 1 m*M* in the VMH for 2.5 h) or SAC (5 m*M* in both sites for 4 h). Each animal was treated with both drugs, and the order of drug treatments was randomized. Data from two animals with guide-cannulae in the VMH and one with guide-cannulae in the mPOA were excluded from the statistical analysis owing to improper probe placements or cannulae damage.

### *Experiment 3*

Experiment 3 was designed to test the effectiveness of GABA antagonists applied to the VMH on PRL secretion. Six animals with guide-cannulae in the VMH were used in this study. The 4-h drug treatments were aCSF-aCSF, aCSF-CGP 55845A (500 μ*M*), and aCSF-BMI (375 μ*M*). In experiment 2, SAC (5 m*M*) was ineffective in changing PRL or LH release. Therefore, in this experiment, a more potent $GABA_B$ antagonist, CGP 55845A *(43)*, was used. In experiment 2, BMI dialyzed into the mPOA caused overt aggressive behavior and agitation. Thus, in experiment 3, the drugs were dialyzed only in the VMH, and the concentration of BMI also was reduced.

### *Hormone Assays*

Every third plasma sample was assayed in duplicate for PRL using a radioimmunoassay previously described *(44)*. Sensitivity was 0.5 ng/mL NIH-PRL-S17 at 90% binding. The intra-assay coefficients of variation were 5.49 and 4.13%, and the interassay coefficients of variation were 15.11 and 22.96% for agonists and antagonists experiments, respectively.

### *Histology*

At the end of each experiment, the animals were euthanized. In some cases, the brains were removed after perfusion via carotid artery with saline followed by 4% formalin fixative after which the hypothalami were isolated and immersed in fixative. In others, no fixative was used, and the hypothalami were frozen. Sections collected were histologically processed and stained with Luxol fast blue to localize probe placement. Evaluation of probe placement was made with the aid of diagrams from Lehman et al. *(45)*.

### *Statistical Analysis*

Across experiments, concentrations of drug in some cases varied with site of infusion. Thus, effects of drugs within sites rather than between sites were analyzed. In all experiments, the mean PRL concentration during the first 4 h and last 4 h of each dialysis session was calculated. For experiment series 1 and experiment 3, the differences in concentrations between the first 4 h (aCSF) of infusion and the last 4 h of infusion then were determined. Subsequently, the mean differences induced by each treatment were compared using analysis of variance for repeated measures followed by Dunnett's test to compare each drug with aCSF. For experiment series 2, comparisons of mean PRL levels during the first 4 h vs the last 4 h were made using the two-tailed Student *t*-test for paired observations. All analyses were performed with the aid of a computer program (GB-STAT, Dynamic Microsystems, Silver Spring, MD). Data are presented both in Figures 2–4 to illustrate temporal patterns of response, and in Tables 1–3 to facilitate statistical comparison of responses.

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