EFFECT OF DOPAMINE AGONISTS OR ANTAGONISTS, TRH, STRESS AND PIGLET REMOVAL ON PLASMA PROLACTIN CONCENTRATIONS IN LACTATING GILTS[a,b]

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ABSTRACT

The effect of dopamine agonists (ergocryptine), antagonists (chlorpromazine, haloperidol, reserpine, pimozide), thyrotropin releasing hormone or stress (restraint, piglet removal) on prolactin release was studied in primiparous lactating gilts. All animals were fitted with surgically implanted jugular catheters before farrowing. The only drug treatments which resulted in a significant change in PRL concentrations in blood were thyrotropin releasing hormone (increase) and ergocryptine (decrease). The results suggest that dopamine may not be the only regulator of prolactin in lactating pigs. Further studies are needed to identify drugs which would be useful in clinical situations for treatment of lactation failure due to low prolactin secretion. In the two stress-exposed groups, there was a gradual, steady decline in the plasma concentration of prolactin which resulted from loss of suckling contact with the piglets. Thus, snare restraint does not increase prolactin secretion in lactating sows confirming the results of other studies on pigs in different physiologic states.

Keywords: Prolactin, Dopamine, Stress, Lactation, Pig

INTRODUCTION

Adenohypophyseal and plasma concentrations of PRL may be low in periparturient sows with insufficient milk production (1,2). Exogenous administration of E. coli endotoxins suppressed circulating PRL concentrations (3,4) and produced clinical changes indistinguishable from those previously reported (5) and very similar to those frequently reported in association with field cases of insufficient milk production (6,7).

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Unlike other adenohypophyseal hormones, prolactin (PRL) is believed to be predominantly under inhibitory control by hypothalamic prolactin inhibitory factor(s) (PIF). Studies using dopamine (DA) and DA agonist infusions in rats, sheep, dogs, rabbits, monkeys, pigs, and cattle have demonstrated that DA inhibited PRL release from the adenohypophysis and is likely the physiologic PIF (8-10). Similar studies using DA-antagonists have demonstrated that it is possible to stimulate PRL release in the rat, primate, and ruminant (9,11). Recently, haloperidol was demonstrated to cause prolactin release in nonpregnant, nonlactating sows (12).

In general, prolactin is considered to be a stress-sensitive hormone. Increased PRL concentrations in blood have been measured following a variety of imposed stresses in rats (13), sheep and goats (14). However, Hoagland et al. (15) reported that prepuberal gilts or young boars did not have similar increases in PRL following restraint with venipuncture.

These studies were conducted to (1) test the hypothesis that PRL is predominantly under inhibitory dopaminergic control in the pig and that release can be stimulated in lactating sows by dopamine antagonists and (2) since PRL release is influenced by other hormones such as estrogens, studies were designed to determine what effect, if any, stress might have on PRL in lactating sows. The drugs examined were selected from various classes of pharmaceuticals (phenothiazine, butyrophenone, and piperidine tranquilizers) and administered at only a single dosage, since the primary focus was identification of a class(es) of drugs that significantly stimulated PRL release for a prolonged period of time as a prelude to multi-dosage studies.

The dopamine antagonists selected have their action at different sites. Reserpine depletes presynaptic storage vesicles, reducing concentrations of DA as well as epinephrine and norepinephrine (16). Chlorpromazine, a phenothiazine derivative, blocks postsynaptic DA receptors (16). Haloperidol (butyrophenone) and pimozide (diphenylbutylpiperidine) also block the post-synaptic DA receptors (16). Dosages were selected based upon the limited information available in the pig and extrapolation on a weight basis from dosages used in the rat, dog, and sheep. Two types of stress (restraint with venipuncture and piglet removal) were examined to determine how these might alter prolactin concentrations and potential response to drugs.

MATERIALS AND METHODS

Animals

This study was conducted on lactating gilts (Duroc x Landrace x Large White, 145 to 170 kg at day 104 to 108 of gestation) 5 to 20 days postpartum (PP+5 to +20). Blood samples were collected via surgically implanted jugular catheters permitting repeated blood collection with minimum disturbance of the animals. The sows were housed indoors in individual farrowing crates (Exp. I) or in farrowing crates in a barn with open southern exposure (Exp. II).

Experiment I

The following drugs were evaluated for their ability to alter PRL release:

a. Dopamine antagonists

Chlorpromazine (Thorazine-(TM), Pitman Moore, Washington Crossing, NJ) - was administered to three sows on PP+6 at a dosage of 0.5 mg/kg in a single injection deep into the superficial and middle gluteal muscle masses.

Reserpine (Serpasil-(TM), Ciba Pharmaceutical, Summit, NJ) - was given to three sows on PP+6 at a dosage of 0.025 mg/kg in the same manner outlined for chlorpromazine.

Haloperidol (Haldol (TM), McNeil Pharmaceutical, Spring House, PA), was administered intravenously to six sows on PP+5 at a dosage of 10 mg/sow.

Pimozide (McNeil Pharmaceutical, Spring House, PA) - Intravenous injections of 0, 0.5, or 1.0 mg/kg in the tartaric acid vehicle were given on PP+20 (two animals/dosage).

b. Dopamine agonist

Ergocryptine (CB-154, Sandoz Pharmaceuticals, E. Hanover, NJ). Two pigs received an intravenous infusion of ergocryptine at a dose rate of either 0.025 or 0.050 mg/kg/24 hr in 0.9% NaCl between days PP+7 and PP+10.

c. Thyrotropin releasing hormone (TRH, Abbott Laboratories, Chicago, IL) was given to five sows on PP+5 as a single intramuscular injection of 500 ug/sow.

In animals receiving chlorpromazine, reserpine, TRH, and pimozide challenges, piglets were permitted to nurse between 0800 and 1000 hr and then were separated from the sow and penned adjacent to her head behind a wire mesh barrier until 1300 hr when they were again permitted to nurse. In the haloperidol treated pigs, the piglets had continuous access to the sow between 0800 and 1300 hr. The test drugs were administered at either 1000 or 1100 hr.

Initially, the magnitude of the suckling induced increase in PRL was unknown. Subsequent work in our laboratory demonstrated that the suckling induced increase in PRL is approximately double the presuckling concentrations, substantially less than the anticipated drug stimulated increase in PRL observed in other species (17), minimizing the probability of suckling induced changes significantly altering any drug induced changes. Thus, the piglets were allowed to remain with the sows during the haloperidol challenge.

Blood samples were collected at 15-min intervals before and after drug administration except in the TRH treated pigs where samples were collected at 10-min intervals immediately after administration of the TRH.

Experiment II

The investigations were conducted on sequential days and the order was randomized among the six sows. Both trials were performed between 17 and 20 days postpartum with sample collection starting at 1000 hr.

Snare Stress--Throughout the entire experiment (3 hr) the piglets were penned adjacent to the sow's head and not permitted to nurse. Blood collection was started approximately 5 minutes following separation of the piglets behind the mesh barrier. Blood samples were collected at 10-min intervals for 1 hr preceding the start of the snare test at which time the animals were restrained with a standard hog snare, a constricting band placed around the base of the ear, and blood collected by venipuncture from an ear vein. A blood sample was then collected from the anterior vena cava by venipuncture before the animals were returned to the crates and the snare removed. Blood samples were collected via the cannula at 5-min intervals during the time of snare application.

Piglet Removal--As a control on the effect of the piglet removal in the snare restraint stress situation, piglets were completely removed from the sow's vicinity. Blood samples were collected every 20 min during a 2-hr period when the piglets were allowed to nurse at will and then at 1-hr intervals for 6 hr after the piglets were moved to a separate building. The sows did not receive any olfactory, visual or auditory stimuli from the piglets.

Blood Collection and Handling

In both experiments, blood samples were mixed with 0.1 ml saturated sodium oxalate to prevent coagulation, centrifuged within 10 minutes of collection, and the plasma frozen on dry ice. The samples were stored at -20°C until assayed. The cannulas were flushed with a volume of heparinized saline (100 I.U./ml) sufficient to fill the cannula between samples.

Hormone Analysis

PRL concentrations were determined according to the method of Mulloy and Malven (18) with minor modifications., Iodine-125 was used in place of Iodine-131, and the radioactivity added to each assay tube reduced to 10,000 dpm. The PRL antibody was obtained from Research Products International, Illinois and was used at a final dilution of 1:64000. The sensitivity of the assay was 0.1 ng per assay tube. The interassay and intraassay coefficients of variations were 11% and 13%, respectively. PRL concentrations were expressed in terms of the NIH reference preparation of porcine PRL (NIH LER-2073).

Cortisol concentrations were determined using an RIA procedure previously validated in this laboratory (19). The interassay coefficient of variation was 11%.

Statistical Analysis

Alterations in plasma PRL concentrations in Experiment I were evaluated by fitting regression lines to the PRL values during the intervals before and following drug administration and compared within each drug treatment group. Slopes were compared using a Student's t-test (20).

Changes in cortisol (GC) concentrations following the stresses in Experiment II were compared with pre-stress concentrations using Student's t-test. Linear regressions were computed for the PRL data according to the method of least squares and the slopes compared using Student's t-test.

RESULTS

Experiment I

The dosages of pimozide selected for the pig were inappropriate since all animals showed signs of toxicosis, including disorientation, vomition, and inappetence. There were no increases in concentrations of plasma prolactin in animals receiving either pimozide or the tartaric acid vehicle.

The PRL concentrations following ergocryptine infusion are shown in Figure 1. No behavioral changes were observed in any ergocryptine treated animal. Concomitant with the decline in PRL concentrations was a decline in the rate of piglet growth. Piglets attempted to nurse more frequently during the treatment period and it is assumed that the gilts had a reduced milk secretion as reflected by the decline of piglet growth.

The change in PRL concentrations following chlorpromazine administration is presented in Figure 2. The dosage of chlorpromazine was sufficient to produce moderate sedation for 2 to 5 hr following drug challenge. The sows were subjectively evaluated to be nursing normally during the latter part of this interval. The slopes of regression lines fitted to the intervals 1100 to 1300 hr and 1300 to 2300 hr did not differ from zero or from a regression line fitted to the interval 0800 to 1100 hr.

PRL concentrations following reserpine administration (group 2) are presented in Figure 3. The sows were moderately sedated for 3 to 10 hr following reserpine challenge. The drug did not appear to adversely affect nursing behavior. Comparison of slopes during the same intervals examined for chlorpromazine demonstrated that the slopes were not different from zero nor significantly different between intervals.

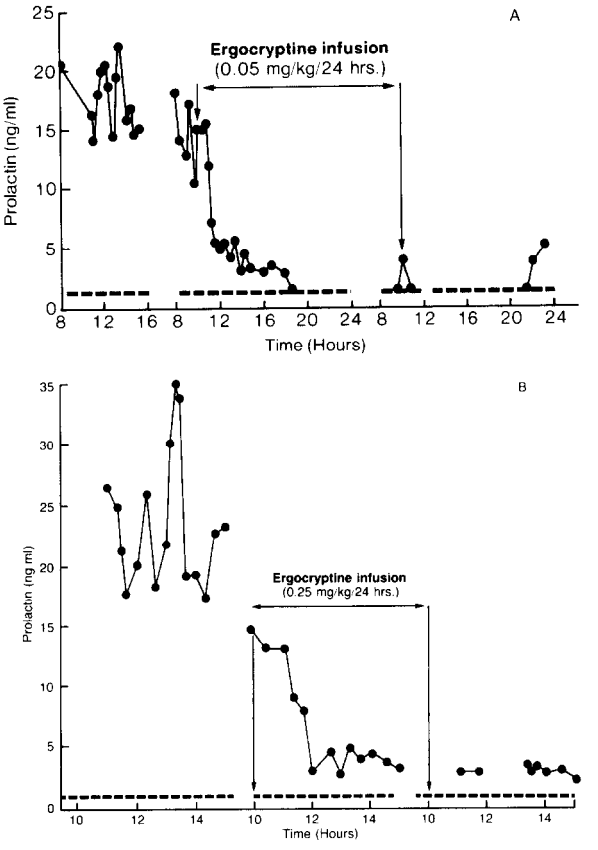

Figure 1. PRL concentrations in plasma of sows treated with ergocryptine at a dosage rate of 0.05 mg/kg/24 hr on days 8 to 9 (A) or 0.025 mg/kg/24 hr on days 9 to 10 (B). The sensitivity limit of the prolactin assay (2 ng/ml) is indicated by the broken line. The abscissa is discontinuous with hour of day indicated for the three consecutive sampling days.

The results following haloperidol administration are shown in Figure 4. The slopes determined for the intervals 0800 to 1000 hr and 1000 to 1300 hr did not differ significantly from one another or from zero.

Change in PRL concentrations following TRH administration are presented in Figure 5. The slope of the regression line between 1100 and 1300 hr was different from zero ($P < 0.05$) and from the interval 0800 to 1100 hr ($P < 0.05$).

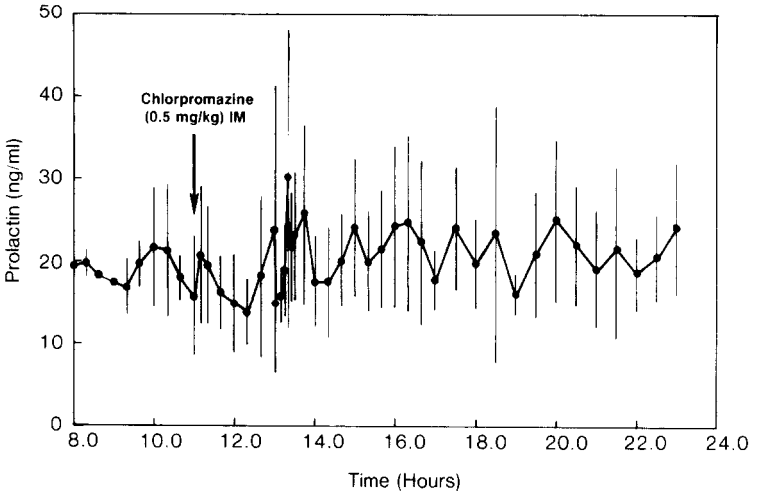

Figure 2. PRL concentration following administration of chlorpromazine at a dosage rate of 0.5 mg/kg i.m. to three sows on day PP+6. Data presented as mean ± SD.

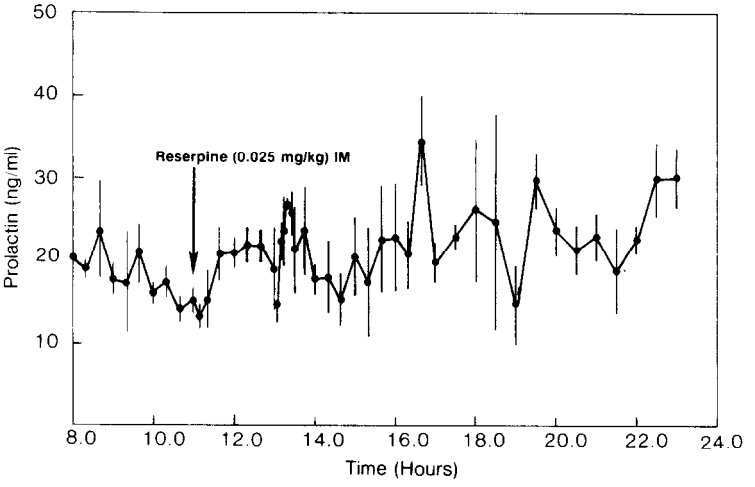

Figure 3. Changes in PRL concentration following administration of reserpine at a dosage of 0.025 mg/kg intramuscularly to three sows on day PP+6. Data presented as mean ± SD.

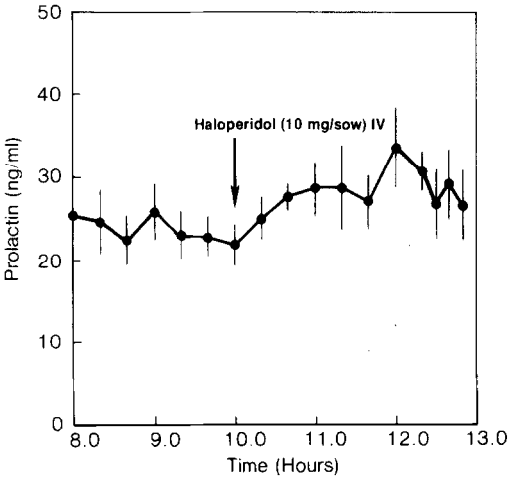

Figure 4. Changes in PRL concentration following administration of 10 mg haloperidol per sow to six sows on day PP+5. Data presented as mean ± SD.

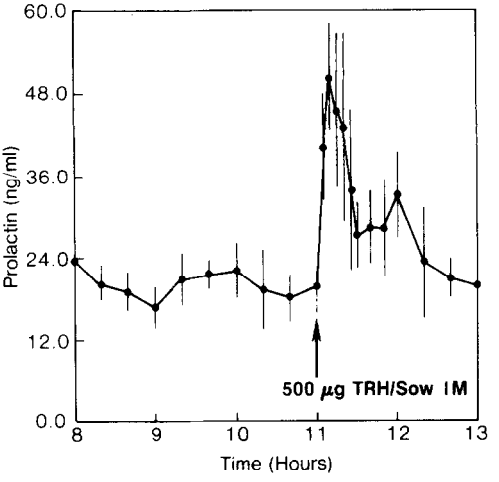

Figure 5. Change in PRL concentration following administration of 500 ug TRH/sow on day PP+5. Data presented as mean ± SD for five sows.

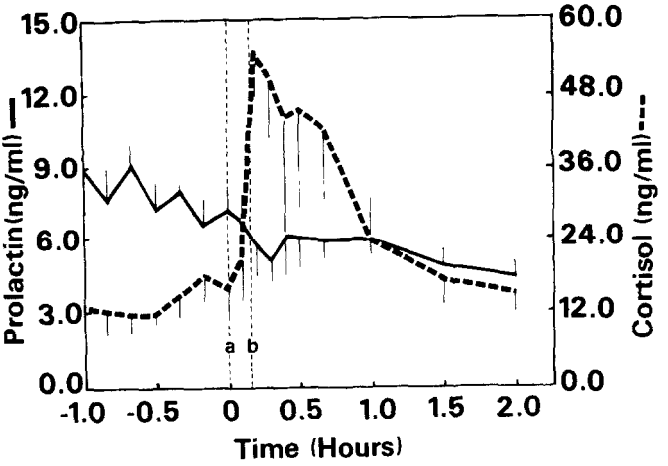

Figure 6. Effect of snaring and venipuncture (ear vein and anterior vena cava) on cortisol and prolactin concentrations. Piglets were separated from sows by a wire mesh during sample collection (n=6). Pigs were backed from their crates starting at time "a", sampling was completed and the animals returned to their crates by time "b." Vertical bars represent SD.

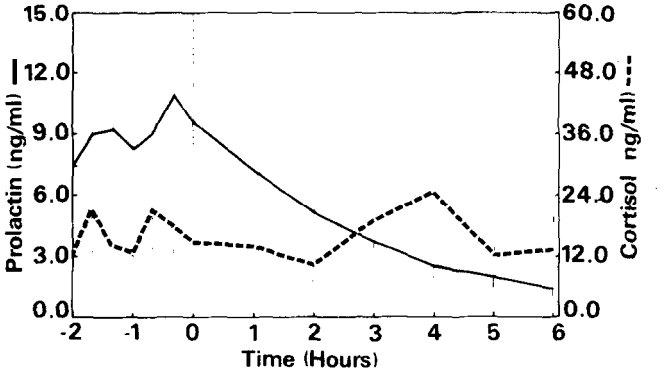

Figure 7. Effect of piglet removal on cortisol and prolactin concentrations in five sows. Piglets were removed at time 0. Vertical bars represent SD.

Experiment II

Due to failure of a cannula only five sows were used in the piglet removal study. The plasma PRL and GC concentrations obtained following snare restraint and venipuncture are presented in Figure 6. The PRL concentrations did not increase after snaring the sows and declined steadily throughout the sampling period. The slope of the regression line fitted to the PRL concentration is less than zero ($P < 0.05$). The GC concentrations increased between the start of the experiment and the return of the animals to their crates. The 10 and 15 minute sample concentrations were greater ($P < 0.01$) than the pre-stress concentrations. Mean GC concentrations gradually decreased reaching pre-stress concentrations by 2 hr post-snaring.

Removal of the piglets from the sow simulated weaning and the decline in PRL concentrations was expected (Fig. 7). The slope of the regression line was significantly less than zero ($P < 0.05$). Glucocorticoid concentrations in plasma were not affected by piglet removal. The slopes of the regression lines for PRL concentrations following initiation of the restraint or piglet removal did not differ. The slope following time 0 was significantly different than the slope of the regression line for the 2 hr preceding piglet removal only (Fig. 7, $P < 0.05$).

DISCUSSION

The decline in PRL concentrations observed following ergocryptine infusions confirms results obtained by Kraeling et al. following subcutaneous administration of 120 mg ergocryptine to postpartum sows (10). However, these data also highlight the sensitivity of the pig to ergocryptine since the dosage used was 0.025 or 0.05 mg/kg/24 hr or a total dose of approximately 4 to 8 mg/sow/24 hr. A suppressive effect of ergocryptine on PRL release has previously been reported in rodents (9), the cow (21,22), the sheep (23), and human (24). Since ergocryptine is a relatively specific DA-antagonist, these results are consistent with the hypothesis that DA is a physiologically active PIF in the pig.

The increase in PRL concentrations observed following TRH administration was similar to that previously reported in the pig (25), rat (9), and ruminant (26) and indicates that there were significant amounts of prolactin available to be released from the pituitary glands of the lactating gilts in these experiments. Due to the relatively short duration of effect (less than 1 hr), TRH is not likely to be of therapeutic value in stimulating PRL release in animals with low serum PRL concentrations. Previous work (27) has also indicated that the ability of TRH to stimulate PRL release is inhibited in the presence of endotoxins, further restricting its potential clinical value.

No changes in PRL concentrations in plasma were observed after chlorpromazine or pimozide. Although a slight increase in PRL was observed in the 2 hr following reserpine and haloperidol administration, the increase was substantially less than the increases observed in other species. A naturally occurring change in PRL of similar magnitude was observed between 2130 and 2300 hr in the reserpine treated animals suggesting that the change in PRL concentrations observed following drug administration could be attributed to normal fluctuations in basal PRL release. If DA is playing a dominant role in the regulation of PRL release in the pig, a substantial increase in PRL

concentration would have been anticipated following administration of these DA-antagonists. Kendall et al. (12) have reported increases of 6 to 32-fold in PRL after haloperidol in non-lactating, cyclic female minipigs. Their dosage of 5 mg of haloperidol per animal used in their study is approximately five times the dosage on a per weight basis used in this study. This dosage (>0.1 mg/kg) will produce profound sedation in full size gilts.

The lack of significant increases in serum PRL concentrations following haloperidol, pimozide, reserpine, and chlorpromazine challenge may be attributable to (1) inappropriate drug dosages to stimulate PRL release in lactating sows or (2) DA playing a relatively insignificant role in the regulation of PRL release. Since the PRL response to drug stimulation is frequently biphasic, it is possible that the dosages being investigated were sufficient that the agonistic and antagonistic characteristics of the drugs were approximately balanced resulting in no net stimulation of PRL release. Although this possibility cannot be discounted, it seems unlikely that the dosages would have been inappropriate for all classes of drugs investigated. This limitation could, however, be rectified by conducting a multiple dosage study.

Another interpretation is that, unlike other species, PRL release in the pig is not predominantly under inhibitory control by dopamine. PRL increased following pituitary stalk transection (28) suggesting that the hypothalamus does secrete a PIF. Schally et al. (29) proposed that gamma amino butyric acid may be the physiologic PIF in the pig. This possibility has not been extensively investigated. However, a response to haloperidol has been reported in nonlactating pigs (12).

Possible explanations for the discrepancy between the decline in PRL observed following ergocryptine administration and lack of stimulation following DA-antagonist administration are:

1. DA receptors are present on the lactotrophs and when stimulated are capable of suppressing PRL release and synthesis.

2. Relatively little DA is normally released into the hypothalamo-hypophyseal portal system.

3. DA-antagonists actively complex with DA-2 receptors on the lactotrophs.

4. The number of DA-2 receptors is large relative to the number of DA and DA-antagonist molecules.

5. Although many of the DA receptors are blocked, there are sufficient numbers of receptors vacant that relatively few DA molecules are prevented from binding with the DA receptors by the DA-antagonists.

Although the snare restraint stress imposed in Experiment II failed to cause a PRL rise, cortisol increased significantly. At least one report shows a cortisol rise but no prolactin increase after stress in a strain of rats (30). One might have anticipated that the different endocrine milieu found in lactating sows as compared to prepubertal gilts would have caused a different response to the stress. The modest but steady decrease in plasma prolactin during the period when piglets were removed is comparable to the rate of

decrease seen in sows at weaning (25). Since the decline was seen in the group with only piglets removed as well as in the restraint stress group, this decrease was due to the piglet removal and not the restraint.

This study suggests that DA-antagonists are unlikely to be effective in stimulating PRL release in the periparturient sow. Since it is possible to stimulate PRL release with lower dosages of DA-antagonists in nonlactating female pigs (12), these results may be due to the lactating sow having an altered prolactin regulatory system.

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