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Plasma Prolactin, Growth Hormone, Luteinizing Hormone and Glucocorticoids After Prostaglandin $F_{2\alpha}$ in Heifers[1] (38295)

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Prostaglandins (PG) of the E series stimulate release of growth hormone (GH), prolactin (PRL), and adrenocorticotropin (ACTH) in several species (1–6). For example, Hertelendy *et al.* (7) recently demonstrated that plasma GH of sheep increased approximately sevenfold within 20 min of injection of PGE_1, and these changes were independent of changes in plasma-free fatty acids, glucose or insulin. To our knowledge reports on the ability of the F series of prostaglandins to cause release of pituitary hormones in ruminants have been limited to studies (8) of luteinizing hormone (LH). Our studies suggested that an LH surge occurred in heifers about 3 days after $PGF_{2\alpha}$ administration (9). The objective of the present investigation was to determine whether concentrations of plasma PRL, GH, glucocorticoids, insulin, free fatty acids, glucose, and LH concentrations were altered in heifers within 18 hr of $PGF_{2\alpha}$ administration.

Materials and Methods. In a preliminary experiment four cows received im 1 ml of 0.85% sodium chloride. Serum prolactin, growth hormone, and glucocorticoid concentrations did not change significantly ($P > 0.05$) during the 6 hr postinjection interval. For this reasor. saline controls were not included in the subsequent experiments.

In the first experiment, three concentrations of $PGF_{2\alpha}$ Tham salt[2] were injected im to determine their effects on release of PRL, GH, LH, and glucocorticoids. Holstein heifers in diestrus were given: (a) an im injection of 30 mg $PGF_{2\alpha}$ dissolved in 2 ml sterile saline (6 heifers); (b) an im injection of 15 mg $PGF_{2\alpha}$ in 2 ml saline followed 6 hr later by a second im injection of 15 mg (4 heifers); or (c) an im injection of 60 mg $PGF_{2\alpha}$ in 2 ml saline (6 heifers). Blood was collected from jugular cannulae into heparinized tubes immediately before (0 hr) $PGF_{2\alpha}$ injections. After $PGF_{2\alpha}$ injections it was collected at 10-min intervals for 1 hr, and then at 1.5, 2, 4, 6, 12, and 18 hr. After centrifugation the plasma was frozen. Bovine PRL (10) and GH (11) and LH (12) were determined by double antibody radioimmunoassays[3]. Glucocorticoids were assayed using the protein binding assay described by Smith *et al.* (13). PRL and GH determinations were made in all plasma samples, whereas glucocorticoids and LH were measured at 0, 0.5, 1, 1.5, 2, 4, 6, and 12 hr after $PGF_{2\alpha}$. Heifers after receiving the second injection of 15 mg $PGF_{2\alpha}$ were sampled at the same frequency as when they received the first injection of $PGF_{2\alpha}$.

In a second experiment, we examined the effect of iv administration of $PGF_{2\alpha}$ to establish the time-course response of plasma free fatty acids, glucose, and insulin in addition to the release of PRL, GH, glucocorticoids and LH. Eight heifers were divided into two groups; four were given a single rapid iv injection of 5 mg $PGF_{2\alpha}$ and four received 15 mg $PGF_{2\alpha}$ infused iv by means of a peristaltic pump at a rate of 0.5 mg/min over 30

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[2] $PGF_{2\alpha}$ Tham (trimethamine) salt (MW=475.6) was kindly provided by Dr. J. W. Lauderdale of the Upjohn Company, Kalamazoo, MI.

[3] Luteinizing hormone (B8), (prolactin B1) and growth hormone (B12) standards were supplied by the National Institute of Arthritis and Metabolic Diseases, National Institute of Health, Bethesda, MD. Dr. L. E. Reichert (Emory University) generously supplied highly purified LH (LER-1072-2) for iodination in the LH radioimmunoassay.

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min. Insulin was determined by double antibody radioimmunoassay (14). Plasma free fatty acids were measured according to the method of Ko and Royer (15) and glucose was assayed using a Hycel Mark 10 Autoanalyser.

Blood samples were collected from jugular cannulae at 3, 2, 1, and 0 hr before $PGF_{2\alpha}$ was administered. After $PGF_{2\alpha}$ was injected or the $PGF_{2\alpha}$ infusion was started samples were collected at 5-min intervals through 60 min; at 70, 80, and 90 min; at hourly intervals through 12 hr; and at 18 hr. PRL and GH were measured in all plasma samples. Glucocorticoids, LH, insulin, glucose and fatty acids were measured in all samples before $PGF_{2\alpha}$ administration and after $PGF_{2\alpha}$ at 15, 30, 45, 60, 90 min; and at 2, 4, 6 hr. LH was also determined in samples collected at 12 and 18 hr.

All data were analyzed by the split plot method (16) to account for repetitive measurements on individual heifers, and selected contrasts were compared using Scheffe's procedure (17).

Results. Since there were no significant differences ($P > 0.05$) among the responses of PRL within the first 6 hr after 15, 30, or 60 mg im of $PGF_{2\alpha}$, PRL data in the text are expressed as averages of all three treatments. Prior to im administration of $PGF_{2\alpha}$ plasma PRL averaged 26 ng/ml (Fig. 1a). PRL increased within 10 min of $PGF_{2\alpha}$ injection to 81 ng/ml, and remained above pretreatment values ($P < 0.01$) for at least 2 hr before returning to preinjection values at 4 hr. There was a similar surge of PRL after the second 15 mg injection of $PGF_{2\alpha}$ at 6 hr.

Plasma PRL averaged 11 ng/ml during the 3-hr interval before iv administration of $PGF_{2\alpha}$ (Fig. 2a). After the 5-mg injection, PRL increased to 160 ng/ml at 15 min and then declined gradually toward basal values at 2 hr. Within 15 min after initiation of infusion (0.5 mg/min) of $PGF_{2\alpha}$, PRL concentration rose to 237 ng/ml and remained between 200 and 317 ng/ml until 20 min after the infusion was stopped, before declining toward basal values at 2 hr.

Before $PGF_{2\alpha}$ was injected, plasma GH averaged 3 ng/ml (Fig. 1b). Although GH increased to 9 ng/ml within 10 min after 15 mg $PGF_{2\alpha}$ was injected im, this average was not significantly different from pretreatment values ($P > 0.05$). Similarly, plasma GH concentrations after the second 15 mg injection of $PGF_{2\alpha}$ were not different ($P > 0.05$) from basal values. Within 30 min of the 30– and 60–mg $PGF_{2\alpha}$, GH increased 7– and 26-fold, respectively. Considering all three doses of $PGF_{2\alpha}$, peak plasma GH concentrations were linearly related to log of the dose ($P < 0.05$). Plasma GH began to decline within 1 hr of $PGF_{2\alpha}$ injections.

In the second experiment plasma GH averaged 10 ng/ml during the 3-hr interval before iv administration of $PGF_{2\alpha}$ (Fig. 2b), but it peaked at 45 ng/ml ($P < 0.01$) within 25 min after injection of 5 mg $PGF_{2\alpha}$. Plasma GH then declined to basal values at 45 min. Ten minutes after $PGF_{2\alpha}$ infusion was stopped, GH peaked at 76 ng/ml ($P < 0.01$) before returning to basal concentrations at 2 hr.

Total glucocorticoids averaged 11.8 ng/ml (Fig. 1c) prior to im injection of $PGF_{2\alpha}$, but at 30 min after each dose of $PGF_{2\alpha}$, they increased more than 6-fold returning to slightly less than preinjection values by 4 hr. Although there was no significant difference ($P > 0.05$) between the peak glucocorticoid responses after 15-mg or 30-mg injections, the 60-mg injection caused a significantly larger release ($P < 0.05$). Furthermore, the peak glucocorticoid values at 30 min after $PGF_{2\alpha}$ were linearly related to the log of the dose of $PGF_{2\alpha}$. When $PGF_{2\alpha}$ was given as a 5-mg iv injection, glucocorticoids peaked at 69 ng/ml ($P < 0.01$) within 15 min, and constant iv infusion of $PGF_{2\alpha}$ caused an even greater increase in plasma glucocorticoids (Fig. 2c).

Plasma glucose averaged 82 mg/100 ml plasma before iv injection of 5 mg $PGF_{2\alpha}$, and glucose did not change significantly until 60 min after $PGF_{2\alpha}$ when it averaged 130 mg/100 ml (Table I). Before the iv infusion of $PGF_{2\alpha}$ commenced glucose averaged 88 mg/100 ml plasma, and it increased ($P < 0.01$) to 147 mg/100 ml at 30 min after the infusion started (Table I).

Although plasma insulin appeared to increase 45 min after $PGF_{2\alpha}$ (Table I), these responses only approached statistical significance ($P \simeq 0.10$).

There was no change ($P > 0.05$) in plasma free fatty acids after iv injection or infusion of $PGF_{2\alpha}$ (Table I).

Average plasma LH concentrations at 1.5–6 hr after im administration of 15–60 mg $PGF_{2\alpha}$ increased twofold or greater ($P < 0.05$) over baseline estimates (Fig. 1d), but the interval to LH increase and the amounts of LH released were not consistent (ranging from 2.7 to 17.0

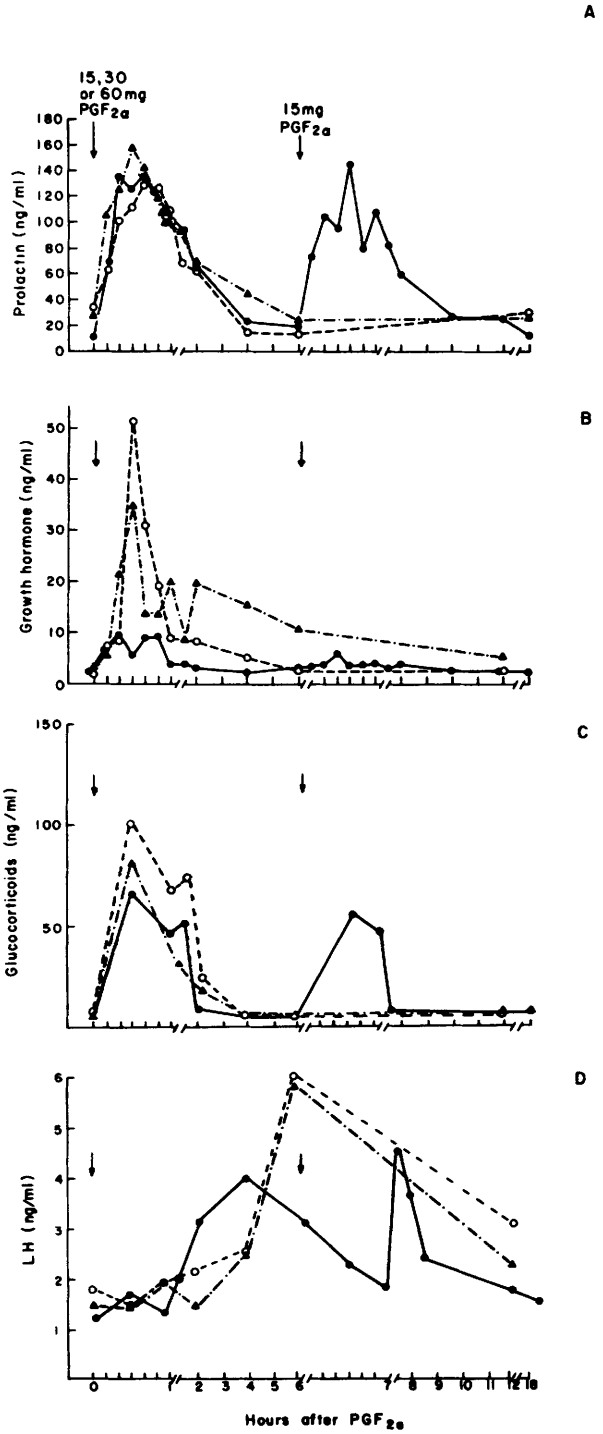

FIG. 1. PRL, GH, glucocorticoids, LH after 15 (2X) (●—●) 30 (▲–·–·▲) or 60 (0---0) mg $PGF_{2\alpha}$. Standard errors of means ranged from 1 to 27, 1 to 14, 1 to 19 and 0.1 to 2.1 ng/ml, respectively, and were generally proportional to the mean.

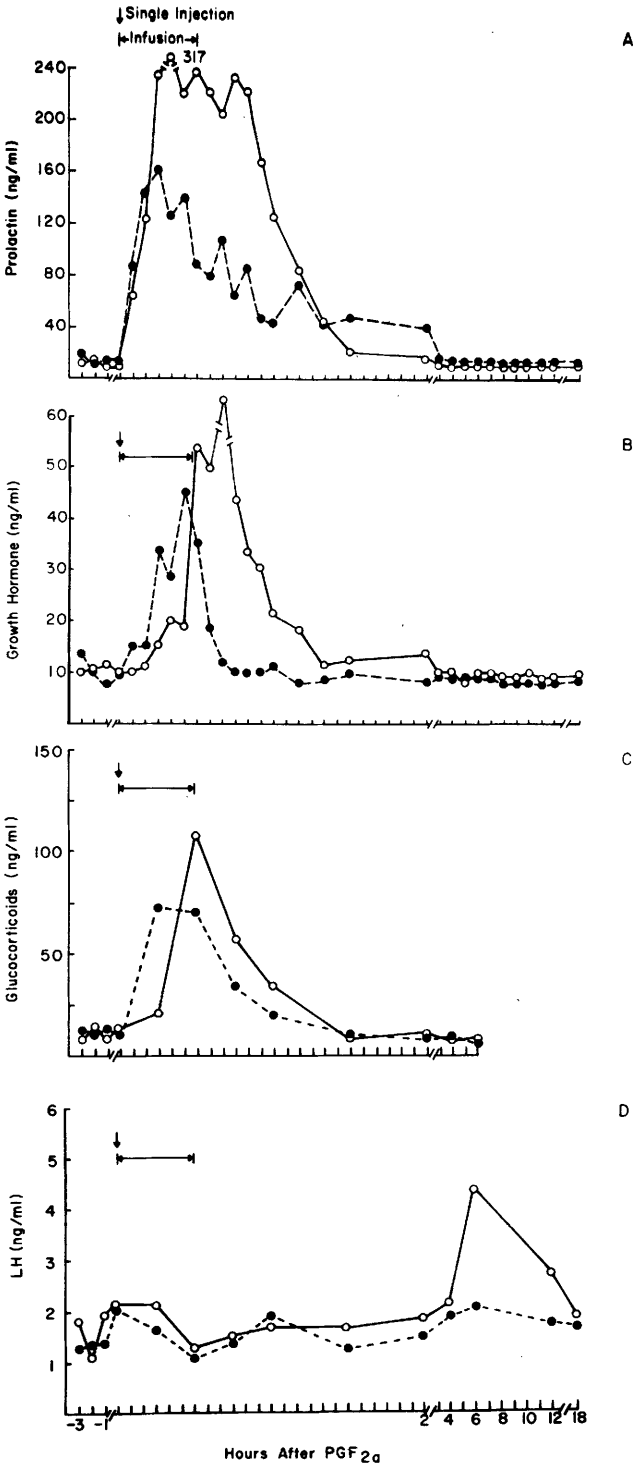

FIG. 2. PRL, GH, glucocorticoids, LH after a single 5 mg iv injection (●---●) or a 0.5 mg/min infusion for 30 min (0—0) of $PGF_{2\alpha}$. Standard errors of means ranged from 1 to 59, 1 to 28, 1 to 17 and 0.1 to 2.6 ng/ml, respectively, and were generally proportional to the mean.

TABLE I. Effect of Exogenous $PGF_{2\alpha}$ on Plasma Insulin, Free Fatty Acids and Glucose.

Treatment	Insulin (ng/ml)	Fatty acid (μg/l)	Glucose (mg/100 ml)
5 mg iv injection			
Pretreatment baseline	34 ± 12[a]	680 ± 126	82 ± 9
Posttreatment peak	82 ± 49[b] (45 min)	685 ± 106	130 ± 27[c] (60 min)
0.5 mg/min infused iv for 30 min			
Pretreatment baseline	23 ± 9	891 ± 147	88 ± 5
Posttreatment peak	50 ± 11 (45 min)	891 ± 86	147 ± 14[c] (30 min)

[a] Values are means ± SE of four heifers in each group.
[b] Values in parenthesis are times after initial $PGF_{2\alpha}$ administration when peak responses occurred.
[c] $P < 0.01$.

ng/ml) among heifers. There was a similar release of LH 6 hr after the start of iv infusion of $PGF_{2\alpha}$ (Fig. 2d), but the 5-mg iv injection did not cause a significant release of LH ($P > 0.05$).

Discussion. The present experiments show that there is a dramatic increase in plasma PRL and GH within 5–15 min after im or iv administration of $PGF_{2\alpha}$ in heifers. The surges in PRL were greater than that normally induced by milking (10), and the PRL peak resembled that after iv injection of thyrotropin-releasing hormone (TRH) in cows (18). The GH response after $PGF_{2\alpha}$ in our heifers was greater than that reported for lactating cows given TRH (18). But the magnitude and duration of the PRL and GH surges were considerably less than those which normally occur at parturition (19). Perhaps continuous infusion of $PGF_{2\alpha}$ for longer periods would result in more prolonged elevations in plasma PRL and GH concentrations. The somewhat delayed response of GH to $PGF_{2\alpha}$ infusion (Fig. 2b) as compared with the 5 mg iv injection may be associated with the relatively low concentrations of $PGF_{2\alpha}$ infused at any discrete interval and the rapid (approximately 2 min) clearance of $PGF_{2\alpha}$ from plasma (Louis, Stellflug and Hafs, unpublished data).

Hedge (20) presented evidence that $PGF_{2\alpha}$ indirectly stimulated ACTH release from the rat pituitary by acting at the median eminence. The dramatic increases in plasma glucocorticoids we observed within 15–30 min after administration of $PGF_{2\alpha}$ in cattle may involve release of corticotropin releasing factor from the hypothalamus, ACTH from the anterior pituitary, or a direct effect of $PGF_{2\alpha}$ on the adrenal cortex, but which of these applies cannot be ascertained from our experiment.

Carlson *et al.* (8) recently reported an increase in LH in sheep during diestrus within 30 min of an intracarotid injection of $PGF_{2\alpha}$, but in the present experiment LH release occurred 1.5–6 hr after $PGF_{2\alpha}$. In plasma samples collected before and after im injection of $PGF_{2\alpha}$, progesterone began to decrease within 10 min and estradiol commenced to rise within 1 hr of the injections (21). These data suggested that steroid hormone changes precede the plasma LH changes in response to $PGF_{2\alpha}$. In our previous studies (9) we observed an ovulatory release of LH to occur about 3 days after $PGF_{2\alpha}$, and these releases of LH were also preceded by decreases in progesterone and increases in estradiol concentrations typical of cows in proestrus. Our previous failure to observe these 1.5–6 hr post-$PGF_{2\alpha}$ alterations in LH concentrations is probably due to the fact that the animals were not sampled with sufficient frequency. While we cannot definitively explain the mechanism of the 1.5–6 hr release of LH in the present study, it seems likely that the time-course and mechanism for release of LH are considerably different from those for PRL, GH or glucocorticoids.

Plasma glucose concentrations increased at 45 min after $PGF_{2\alpha}$ administration whereas insulin increases only approached statistical significance. The marginally significant insulin peaks occurred 60 min after 5 mg iv of $PGF_{2\alpha}$ and 30 min after the start of the iv infusion. The asynchrony between glucose and insulin responses precludes speculation on cause-effect relationships.

The multiplicity of hormonal responses we observed after $PGF_{2\alpha}$ may be related to the

hypothesis that prostaglandins are a common intermediate in the intracellular mechanism of action for several hormones (22). Recent evidence indicates that prostaglandins may be mediators in control of release of several hypothalamic releasing hormones (6). The finding that inhibitors of PG biosynthesis also inhibit the effects of hypothalamic releasing hormones on TSH and LH secretion supports the concept that PGs may affect several hormones (23). Other evidence, however, suggests that PG injected directly into the anterior pituitary (6) or incubated with anterior pituitaries *in vitro* (24) have no effect on LH or FSH release. Thus, the responses of several hormones to $PGF_{2\alpha}$ as we observed in the present study may simply reflect a nonspecific reaction similar to that observed with a stimulus such as stress. It should be noted, however, that the heifers showed no visible signs of stress after $PGF_{2\alpha}$ treatment. Furthermore serum PRL, GH and glucocorticoids did not change after im administration of saline in control cows.

Summary. Blood plasma PRL, GH and glucocorticoids in heifers increased severalfold within 5–15 min after im injections of 15, 30, or 60 mg $PGF_{2\alpha}$ or after a single 5 mg iv injection of $PGF_{2\alpha}$. Plasma LH increased at least twofold over basal estimates but not until 1.5–6 hr after im $PGF_{2\alpha}$. Constant iv infusion of $PGF_{2\alpha}$ at the rate of 0.5 mg/min for 30 min produced greater plasma concentrations of PRL, GH, glucocorticoids and LH than those found after im injections. Plasma glucose increased 59–67% above pretreatment values between 30 and 60 min after iv administration of $PGF_{2\alpha}$. Plasma insulin increased more than twofold over basal estimates at 45 min after $PGF_{2\alpha}$ administration, but these increases only approached significance ($P \simeq 0.10$). Free fatty acids did not change significantly ($P > 0.05$) after $PGF_{2\alpha}$ administration. While exogenous $PGF_{2\alpha}$ administration causes marked increases in several plasma hormones and glucose, the mechanism of action is unclear.

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