Pituitary-Adrenocortical and Lymphocyte Responses to Bromocriptine-induced Hypoprolactinemia, Adrenocorticotropic Hormone, and Restraint in Swine (43125)

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Abstract. **A study was conducted with castrated male pigs (barrows) to evaluate effects of bromocriptine-induced hypoprolactinemia (6 days) on basal and adrenocorticotropic hormone (ACTH)-altered (single injection) pituitary-adrenocortical function, on lymphocyte proliferative responses, and on interleukin 2 production. In addition, the study was designed to measure the short time course of pituitary-adrenocortical and lymphocyte responses to ACTH and to a 30-min restraint stressor. Blood samples were taken via indwelling jugular catheters at −0.5, +0.5, +2, and +5 hr (with reference to time of acute treatment exposure) on Day 6 of the study. Lymphocyte responses were measured only at the 2-hr interval. Exposure (6 days) to bromocriptine (CB154) was associated with 53% reductions ($P < 0.05$) in plasma prolactin (1.37 ± 0.13 vs 0.60 ± 0.04 vs 0.68 ± 0.08 ng/ml) when averaged across all time intervals in control, CB154-treated, and CB154 + ACTH-treated pigs, respectively. The reductions in plasma prolactin were associated with a reduction ($P < 0.05$) in basal plasma cortisol at only one time interval (+0.5 hr) when CB154-treated pigs were compared with controls (17.7 ± 4.2 vs 26.9 ± 3.2 ng/ml). CB154 had no effect on plasma ACTH or growth hormone concentrations for the time periods at which they were measured. CB154 treatment produced numerical, but not statistically significant, 38% reductions in interleukin 2 production (6.31 ± 1.8 vs 3.91 ± 1.47 units/ml). Lymphocyte proliferative responses to the mitogen concanavalin A and interleukin 2 production decreased 65 and 75% ($P < 0.05$), respectively, 2 hr subsequent to ACTH administration when compared with control animals. Hence, under the conditions of this study, only a modest association between lowered plasma prolactin concentrations and basal cortisol concentrations was evident. The data suggest the absence of dopamine regulation of basal plasma ACTH in pigs and provide evidence for a rapidly occurring inhibitory effect of ACTH administration on specific lymphocyte activities.** [P.S.E.B.M. 1990, Vol 195]

Adrenocorticotropic hormone (ACTH) is the primary regulator of glucocorticoid steroidogenesis in fasciculata/reticularis cells of the adrenal cortex (1, 2). However, other modulators of adrenocortical function have been measured. One such is the hormone prolactin (PRL) which is known to modulate adrenocortical steroidogenesis in rodents (3, 4) and in fetuses of several species (5, 6).

In addition to their mutual, albeit unequal, effects on the adrenal cortex, ACTH and PRL also appear to share another target site, lymphoid cells. Effects of ACTH on lymphoid cells may be primarily indirect via glucocorticoids (7, 8); however, suggestive evidence exists for ACTH receptors on mouse splenocytes (9) and human mononuclear leukocytes (10). Additionally, ACTH *in vitro* suppressed the antibody response of mouse spleen cells (9) as well as the production of γ-interferon (11). Similarly, PRL receptors have been detected on human lymphocytes (12), and primarily stimulatory *in vivo* and *in vitro* effects of PRL on certain activities of the immune system have been documented for rats (13–15) and mice (16–18).

How rapidly lymphocytes respond to alterations in plasma ACTH and cortisol has been only partially

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documented for pigs (19). Additionally, although plasma PRL increases rapidly subsequent to a restraint stressor (20) and specific, high affinity PRL receptors are present on porcine fasciculata/reticularis cells (21), the actual effect of PRL on porcine adrenocortical steroidogenesis has not been determined. Also, no experiments have been reported which evaluate a role for PRL in the modulation of lymphocyte activities in swine. Hence, the current study was conducted with swine to determine if (i) PRL modulates basal or ACTH-altered plasma cortisol concentrations, (ii) PRL influences mitogen-stimulated lymphocyte blastogenesis or interleukin 2 production (IL-2, a lymphokine produced by a subset of T-lymphocytes (22)), and (iii) a response of these latter two lymphocyte activities subsequent to a single ACTH injection or to a single 30-min restraint stressor occurs within 2 hr.

Materials and Methods

Animals, Surgery, and Blood-Sampling Techniques. Seventy-five cross-bred castrated male pigs (barrows, one-fourth Yorkshire, one-fourth Landrace, one-fourth Large White, one-fourth Chester White) were used in this study. The average weight at sacrifice was 54.7 ± 0.4 kg. All barrows were housed in environmental chambers measuring 4.9 × 5.2 m and were maintained at a constant 20 ± 5°C dry bulb temperature. Within each chamber each barrow was housed in an individual pen measuring 1.2 × 0.6 m, and each animal had visual, olfactory, auditory, and tactile contact with another animal in an adjacent pen. Animals were provided a corn and soybean meal-based ration and water *ad libitum* and were maintained on a photoperiod consisting of 12-hr light (lights on 0600–1800 hr).

A microrenathane catheter (2.03 mm o.d. × 1.02 mm i.d.; Braintree Scientific Inc, Braintree, MA) coated on its internal and external surfaces with 7% TDMAC-heparin (Polysciences Inc., Warrington, PA) was surgically implanted in the external jugular vein. For this procedure, pigs were anesthetized initially with 400 mg of ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, NY) and were maintained subsequently on 2% halothane (Halocarbon Laboratories Inc., Hackensack, NJ). The external jugular vein was then exposed and catheterized using previously described techniques (23). Surgery was conducted 6 days before use in the experiment. Patency of these catheters was maintained, and blood samples were obtained as described previously (24). Blood samples once obtained were placed immediately on ice, and subsequently centrifuged at 1500 g_{av} for 15 min at 2°C. The supernatant from this centrifugation was then recentrifuged at 3584g_{av} for 20 min at 2°C. Plasma for PRL and cortisol was then stored at −20°C, whereas an additional aliquot for ACTH determination was frozen rapidly in liquid nitrogen and stored at −80°C.

To sacrifice the pigs, the pigs were anesthetized initially via intravascular injection of 700 mg of sodium thiopental (Abbott Laboratories, North Chicago, IL) and subsequently killed via exsanquination. Tissue samples were then removed for additional aspects of the study not reported herein.

Administration of Bromocriptine and ACTH. Endogenous secretion of PRL was inhibited by use of the drug bromocriptine mesylate (25) (CB154; Innovative Research of America, Toledo, OH). Tablets (50 mg of CB154 each) were implanted subcutaneously in the throat region, using the same incision used to implant the catheter. Each pellet released CB154 at a rate of 2.4 mg/day based on the manufacturer's specifications, or a total dosage of 9.6 mg of CB154 was administered per animal per day when four tablets were used. Plasma PRL was compared in experimental barrows having none, one, two, or four placebos (cholesterol:methyl cellulose:α-lactose; i.e., the carrier-binder for the CB154 tablets). Plasma PRL concentrations did not differ ($P > 0.05$) among any of these treatments (1.76 ± 0.25, 1.56 ± 0.16, 1.24 ± 0.06, and 1.77 ± 0.14 ng/ml, respectively; $n = 6$ per treatment). Hence, it was deemed unnecessary to use placebos in animals not implanted with CB154 tablets. However, all animals were subjected to the same amount of surgical trauma by having subcutaneous "pockets" made which contained tablets in CB154-treated animals. ACTH(1–24) (Cortrosyn, 25 units/250 μg, Organon, Oakforest, IL) was solubilized at a concentration of 250 μg/ml of 0.9% NaCl containing 0.1% bovine serum albumin (pH 2.6). Aliquots were frozen rapidly in liquid nitrogen and stored frozen at −80°C until used. Before use the ACTH preparation was diluted to 20 μg of ACTH/500 μl of 0.9% NaCl (pH 6.6). This ACTH preparation was then injected through the catheter at a dosage of 0.4 μg/kg body wt (approximately 20 μg or 2 units/animal). The ACTH was then flushed through the catheter with 10 ml of heparinized saline. Animals other than those injected with ACTH received 500 μl of vehicle followed by 10 ml of heparinized saline.

Radioimmunoassay Procedures. Double antibody radioimmunoassay procedures were used for porcine PRL, porcine ACTH, cortisol, and growth hormone (GH) and have been described previously in detail (20, 24, 26). For the current experiment, the within-assay variability (as determined by the average coefficient of variability (CV) of duplicate analyses of experimental samples) for porcine PRL was 3.25%. Two porcine PRL assays were conducted with a between-assay CV of 17.8%. The assay sensitivity was 40 pg/tube as determined by the lowest standard in the linear range of the standard curve. For cortisol all samples were analyzed on the same assay with a within-

assay CV of 2.6% and a sensitivity of 10 pg. For ACTH all samples were analyzed on a single assay with a within-assay CV of 3.6% and a sensitivity of 0.52 pg/tube. For GH all samples were analyzed on a single assay with a within-assay CV of 4.9% and a sensitivity of 49 pg/tube.

Immunologic Assay Procedures. Mitogen-stimulated lymphocyte proliferative responses and the IL-2 assay were performed as described previously (27). In brief, heparinized whole blood was centrifuged (10 min at $700g_{max}$). The buffy coat was diluted with an equal volume of medium (Dulbecco's modified Eagle's medium; Gibco Laboratories, Grand Island, NY; 25 m*M* hepes, Sigma; 100 units/ml penicillin, 100 μg/ml streptomycin, 250 μg/liter amphotericin-B, Gibco; 50 μg/ml gentamicin, Schering Laboratories, Maplewood, NJ; essential and nonessential amino acids and vitamins), layered onto 4 ml of a density gradient (Histopaque 1077; Sigma), and subsequently centrifuged for 40 min at $950g_{max}$. The cells at the medium-Histopaque interface were collected, mixed with medium, again layered over Histopaque, and centrifuged for 30 min at $700g_{max}$. The cells were collected from the interface and washed three times in medium, with the final wash in medium plus 10% (v/v) fetal bovine serum (FBS, Hybri-sure; Hazelton Dutchland, Denver, PA). Triplicate assays, with three mitogens and medium controls, were performed in U-bottom microtiter plates (no. 76-042-05; Flow Labs, McLean, VA) using 2×10^5 cells/well in a 200-μl final volume of medium + 10% FBS. Mitogens were used at the following concentrations: concanavalin A (Con A), 20 μg/ml (Pharmacia Fine Chemicals, Piscataway, NJ); phytohemagglutin, 100 μl/ml (Gibco) and pokeweed mitogen, 100 μgl/ml (Gibco). Cultures were incubated for 48 hr in a 93% air, 7% CO_2 humidified atmosphere at 37°C, then pulsed with 1 μCi/well [^{3}H]thymidine, and incubated for an additional 18 hr before being harvested onto glass fiber discs. After liquid scintillation counting, mean counts per min (cpm) for test samples were computed for triplicate assays, and data were reported as cpm of [^{3}H]thymidine incorporation in mitogen-stimulated cells, minus cpm of unstimulated cells.

Interleukin 2 Assay. Interleukin 2 determinations were performed in conjunction with the lymphocyte blastogenesis assays. Isolated lymphocytes (2×10^6 cells) were incubated in a 93% air, 7% CO_2, humidified atmosphere at 37°C in 2 ml of medium ± 10% FBS containing 10 μg/ml Con A. After 24 hr, supernatants were harvested and frozen (−20°C) until assayed. Interleukin 2 was measured using the IL-2-dependent cell line, CTL-FD. Briefly, 2×10^5 washed CTL-FD cells were added to a series of five 2-fold serial dilutions of supernatant in medium plus a medium control (RPMI 1640, Gibco; 5×10^{-5} *M* 2-mercaptoethanol, Sigma; 5% v/v FBS; 100 units/ml penicillin; 100 μg/ml streptomycin). Cultures were incubated for 48 hr, pulsed with 1 μCi/well [^{3}H]thymidine, incubated for an additional 12 hr, and harvested onto glass fiber discs for liquid scintillation counting. Data were analyzed using a logit data analysis program with all samples being compared with an in-house standard.

Experimental Design and Procedures. Barrows were catheterized and implanted with CB154 (where applicable) on Day 0, were bled serially, and killed on Day 6. Animals were randomly assigned to one of five treatment groups: Group 1, control barrows; Group 2, barrows subjected to a 30-min restraint on Day 6; Group 3, barrows given an injection of ACTH on Day 6; Group 4, barrows implanted with CB154 tablets on Day 0; and Group 5, barrows implanted with CB154 tablets on Day 0 and given an injection of ACTH on Day 6. Each of these five main treatment groups was divided further into barrows which were killed at 0.5, 2, or 5 hr after injection of ACTH or vehicle and/or after initiation of restraint. Blood samples were taken on Day 6 and, with reference to the injection of ACTH or vehicle or initiation of restraint stressor, at −0.5, +0.5, +2, and +5 hr. These time intervals closely approximated 0730, 0830, 1000, and 1300 hr. Hence, some animals had only two blood samples removed (−0.5 and +0.5 hr), whereas other animals may have had as many as four blood samples removed. This design accounts for the fewer replicates at 2 and 5 hr. The study was designed to have five complete replicates with five barrows killed at each of the above-noted time intervals, and for each treatment. However, in the first two replicates of this study the doses of CB154 were being adjusted for optimal effects on plasma PRL. Additionally, in the first replicate the ACTH dose used was lower than in subsequent replicates. Hence, hormonal data for these CB154-treated animals, and data from control animals of replicate 1 are presented separately in Table I. Hormonal data associated with the 15 pigs of replicate 1 and for the 6 pigs of replicate 2 which received CB154 or CB154 + ACTH were not presented elsewhere, and the immunologic data associated with these two lower doses of CB154 were not used.

Pigs subjected to restraint were transferred from their pens to a mobile restraining cage as reported previously (20). After the 30-min restraint, depending on the assigned time of sacrifice, animals were either sacrificed immediately or returned to their home pens until time of sacrifice. Hence, the restraint stressor also included the process of transfer to the restraining device and, for some, from the restraining device back to the home pen.

Statistical Analyses. Data were analyzed using a multiway analysis of variance for repeated measures to test for main effects and interactions and using the General Linear Models of the Statistical Analysis System (28). There were two main factors, treatment and

Table I. Effects of Bromocriptine (CB154) for 6 Days on Plasma Prolactin and Cortisol Concentrations[a]

	−0.5 hr		+0.5 hr		+2 hr	
	PRL (ng/ml)	Cortisol (ng/ml)	PRL (ng/ml)	Cortisol (ng/ml)	PRL (ng/ml)	Cortisol (ng/ml)
Control (n = 3)	1.46 ± 0.22b	22.63 ± 8.26a	1.44 ± 0.25b	20.59 ± 4.68b	0.93 ± 0.15b	4.78 ± 1.49a
CB154 (2.4 mg/day) (n = 3)	0.98 ± 0.10a, b	14.54 ± 2.91a	0.99 ± 0.20b, c	17.93 ± 2.85b	0.81 ± 0.08b	12.56 ± 1.12a
CB154 (4.8 mg/day) (n = 3)	0.65 ± 0.05a	24.11 ± 3.04a	0.69 ± 0.03c	11.99 ± 3.50b	0.81 ± 0.04b	10.90 ± 5.70a

[a] Values represent mean ± SE. Means with different letters are significantly different ($P < 0.05$) when different treatment groups are compared within a given time interval. The time intervals pertain primarily to additional treatment groups whose data are not presented (i.e., restraint, ACTH injection). The actual times correspond closely to 0730, 0830, and 1000 hr.

time, with repeated measures being made over time. In this analysis the appropriate error term used to test for treatment effects is that associated with pigs (treatment). To test for differences among hours (the repeated measure term), the residual mean square is the appropriate error term (29). Subsequent tests to determine significant differences among individual means used procedures detailed by Winer (29) and involved use of the conservative Student-Newman-Keuls test. Data were tested for normality of distribution via the Shapiro Wilk Statistic, and for homogeneity of variance via the F_{max} test. Data were log or square root transformed to fulfill assumptions of analysis of variance. A probability level of <0.05 was considered significant.

Results

Administration of 2.4 mg of CB154/day for 6 days was associated with an apparent 27% reduction ($P > 0.05$) in plasma PRL (Table I). Administration of 4.8 mg of CB154 daily for 6 days was associated with a 44% reduction ($P < 0.05$) in plasma PRL during the first two time intervals. In subsequent replicates, to reduce plasma PRL further, four CB154 tablets (9.6 mg/day) were used. Plasma cortisol was not affected by these lower two concentrations of CB154.

Body and Adrenal Weights. There were no effects of treatment on body weight, weight gain, or adrenal gland weights during the 6-day experimental period (Table II).

Plasma PRL. The multifactorial analysis of variance indicated very significant effects ($P < 0.0001$) of treatment, time, and treatment × time interaction on plasma PRL concentrations. Additional analyses indicated that plasma PRL was reduced significantly in CB154-treated barrows when compared with those in control barrows at all time periods measured (Fig. 1). A 30-min restraint was associated with a 108% increase ($P < 0.05$) when compared with prestressor concentrations or a 169% increase ($P < 0.05$) when compared with control barrows at the same time period (Fig. 1). ACTH injection and associated changes in plasma cortisol were without effect on plasma PRL. There was an effect of time of observation on plasma PRL in control and ACTH-injected animals as evidenced by significantly decreased plasma concentrations at +0.5 and +2 hr when compared with −0.5 hr.

Table II. Effects of Treatments on Final Body Weights, Weight Gain, and Adrenal Weights

Treatment	Final body weight (kg)	Weight gain (kg)	Adrenal weight (g)
Control (n = 12)	55.7 ± 1.7[a]	5.8 ± 0.5	3.00 ± 0.12
ACTH (n = 12)	52.9 ± 2.4	4.5 ± 0.5	3.16 ± 0.18
Restraint (n = 12)	53.6 ± 2.1	4.9 ± 0.4	3.10 ± 0.11
CB154 (n = 9)	53.9 ± 0.9	5.0 ± 0.4	3.09 ± 0.19
CB154 + ACTH (n = 9)	55.4 ± 2.2	5.9 ± 0.4	3.29 ± 0.15

[a] Each value represents the mean ± SE. There were no significant treatment effects ($P > 0.05$) for any factors measured.

Plasma GH. To assist in our interpretation of any potential CB154-induced effects on either adrenocortical function or lymphocyte activity, plasma GH concentrations were also measured in nine control and nine CB154-treated barrows at the −0.5-hr time interval. There were no differences ($P > 0.05$) between GH concentrations in control (1.20 ± 0.05 ng/ml) and CB154-treated barrows (1.42 ± 0.30 ng/ml).

Plasma ACTH. During the 5.5 hr in which blood samples were taken, there were no changes in plasma ACTH in control barrows (Fig. 2), nor did CB154 administration for 6 days alter plasma ACTH, when compared with controls, at any time period measured ($P > 0.05$). As anticipated, restraint produced a dramatic 5-fold increase in plasma ACTH which returned rapidly to prestressor concentrations. Administration of cortrosyn (ACTH(1–24)) resulted in supraphysiologic plasma ACTH concentrations (20.39 ± 4.86 ng/ml) within 30 min. Apparent plasma ACTH concentrations remained very high at 2 hr (6.41 ± 2.11 ng/ml) and 5 hr (5.24 ± 2.28 ng/ml) postinjection. These latter data are not included in Figure 2 because the exceedingly high concentrations would necessitate a distorted scale.

Plasma Cortisol. The multifactorial analysis of variance indicated a trend toward treatment effects ($P = 0.062$), and a significant ($P < 0.0001$) time effect, and a treatment × time interaction were measured.

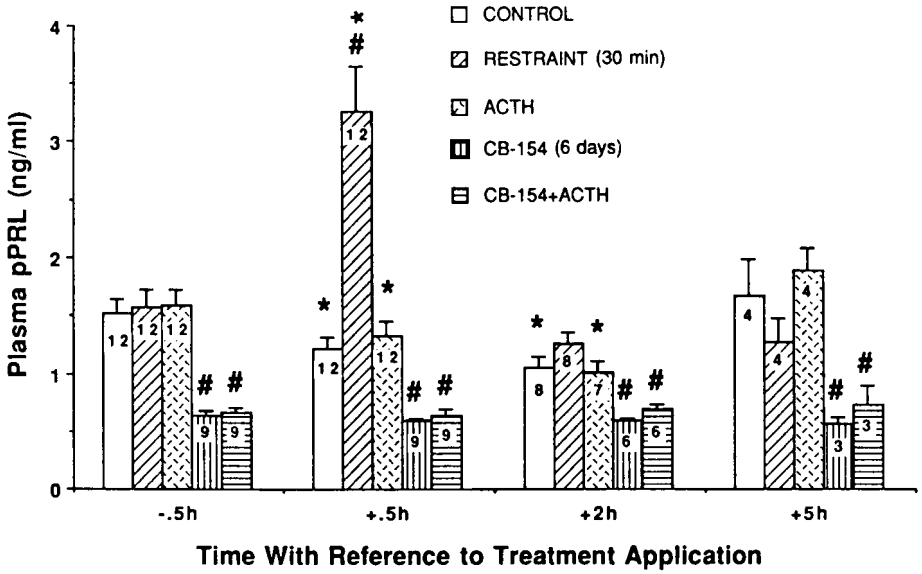

Figure 1. Plasma porcine prolactin (pPRL) concentrations measured at various time intervals, with reference to injection of ACTH or vehicle or initiation of a 30-min restraint stressor. Each bar represents the mean ± SE. The number of barrows per treatment is given within each bar. Means at +0.5, 2, and 5 hr which are marked with an * are significantly different ($P < 0.05$) from their respective pretreatment mean at −0.5 hr. Means with a # are significantly different from the mean of the control pigs at that time. Statistical analyses conducted on log-transformed data.

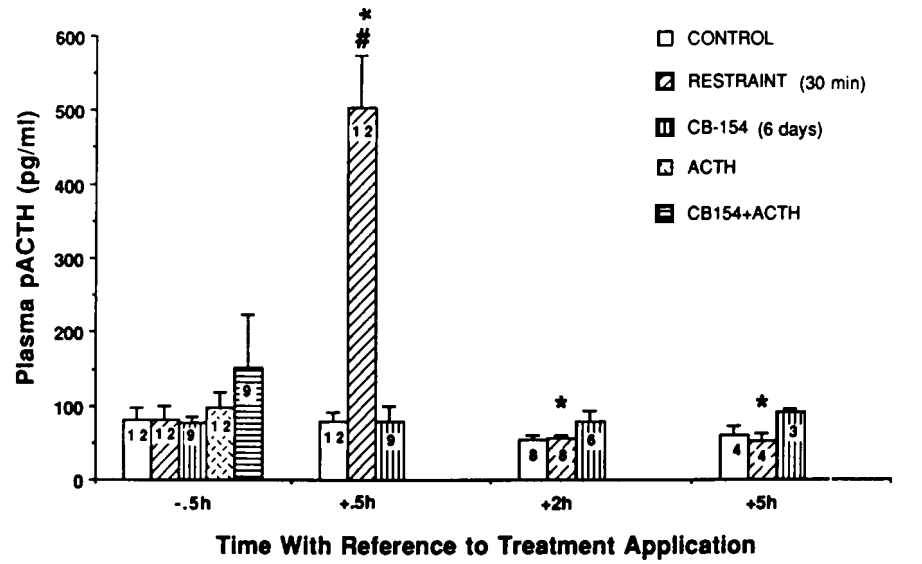

Figure 2. Plasma immunoreactive porcine adrenocorticotropic hormone (pACTH) measured at various time intervals, with reference to injection of ACTH or vehicle or initiation of a 30-min restraint stressor. Each bar represents the mean ± SE. The number of barrows per treatment is given within each bar. Means at +0.5, 2, and 5 hr which are marked with an * are significantly different ($P < 0.05$) from their respective pretreatment mean at −0.5 hr. Means with a # are significantly different from the mean of the control pigs at that time. Statistical analyses conducted on log-transformed data.

Basal cortisol concentrations at −0.5 hr before any acute treatments (Fig. 3), did not differ among treatments, indicating an absence of effects of CB154 and plasma PRL reductions on plasma cortisol at this time period. However, 1 hr later plasma cortisol concentrations were reduced ($P < 0.05$) by CB154 administration when compared with controls. Additionally, cortisol in control barrows demonstrated time-related changes ($P < 0.05$) during the sampling period, which were absent in CB154-treated barrows. Plasma cortisol was increased within 30 min by ACTH injections into barrows in either the absence or presence of CB154 treatment (Fig. 3). Restraint produced a plasma cortisol response almost identical to that associated with ACTH injections. In ACTH-injected animals, plasma cortisol at 2 hr postinjection was below ($P < 0.05$) preinjection levels

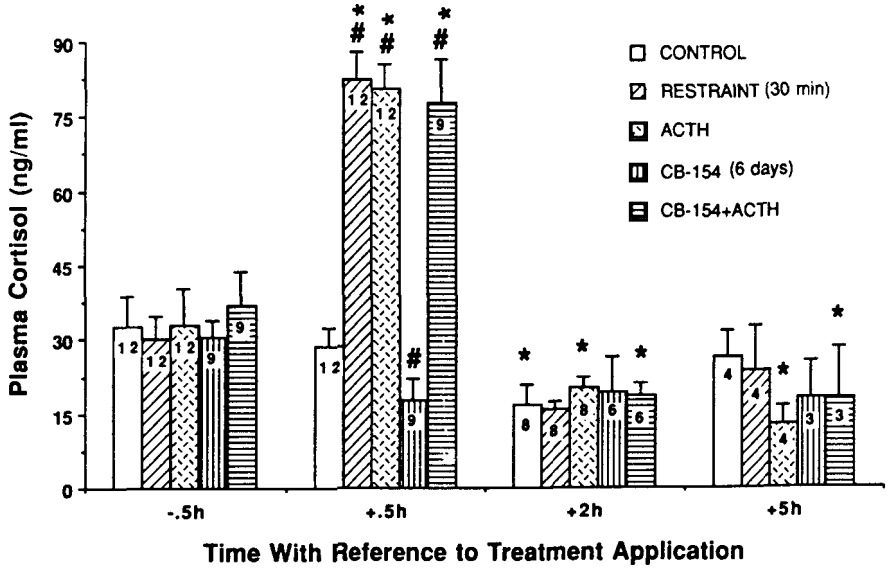

Figure 3. Plasma cortisol measured at various time intervals, with reference to injection of ACTH or vehicle or initiation of a 30-min restraint stressor. Each bar represents the mean ± SE. The number of barrows per treatment is given within each bar. Means at +0.5, 2, and 5 hr which are marked with an * are significantly different ($P < 0.05$) from their respective pretreatment mean at −0.5 hr. Means with a # are significantly different from the mean of the control pigs at that time. Statistical analyses conducted on square root-transformed data.

irrespective of apparently elevated plasma ACTH concentrations.

Lymphocyte Responses. Lymphocyte measures were conducted on cells obtained 2 hr subsequent to injection of ACTH or vehicle or initiation of the 30-min restraint and only in pigs killed at this 2-hr period. Administration of ACTH within 2 hr induced 65% reductions ($P < 0.05$) in lymphocyte proliferative response to ConA (Table III) and 75% reductions ($P < 0.05$) in IL-2 production (Fig. 4). Restraint was associated with more modest numerical reductions ($P > 0.05$) in these measures of lymphocyte activity. Data associated with CB154 administration are not presented in Table III because of the small sample size ($n = 2$ for each treatment) which resulted from the partial clotting of some blood samples. However, CB154 did produce modest 15–22% numerical decreases in lymphocyte proliferative responses.

Discussion

Although it is known that plasma PRL in pigs increases in response to stressors (20) and has adrenocortical receptors (21), the question remains as to the physiologic role of this increased PRL. The hypothesis addressed by the current study is that the role of PRL in swine is to modulate adrenocortical function, mitogen-stimulated lymphocyte blastogenesis, and IL-2 production during the acute stress response. With reference to this hypothesis, there are several salient observations to be made from the data reported herein: (i) CB154-induced reductions in plasma PRL for 6 days were associated with a slight but significant reduction in basal, but not ACTH-stimulated, plasma cortisol; (ii) CB154 administration had no effect on basal plasma ACTH concentrations under conditions in which basal PRL was reduced by 53%; and (iii) no statistically significant effects of reductions in plasma PRL on

Table III. Effects of Bromocriptine (CB154), ACTH Injection, and a 30-Min Restraint on Mitogen-Stimulated Lymphocyte Proliferative Responses

Treatment	Phytohemagglutinin stimulation (Δcpm × 10^{-3})[a]	Con A stimulation (Δcpm × 10^{-3})	Pokeweed stimulation (Δcpm × 10^{-3})
Control ($n = 4$)	362 ± 63a	168 ± 23a	130 ± 17a
ACTH[b] ($n = 3$)	228 ± 10a	58 ± 11b	89 ± 25a
Restraint ($n = 4$)	240 ± 28a	108 ± 40a, b	99 ± 18a

[a] Δcpm = counts per min of [^{3}H]thymidine incorporation in mitogen-stimulated cells minus cpm of nonstimulated cells. Values represent mean ± SE. Means for a given mitogen with different letters are significantly different ($P < 0.05$).
[b] ACTH was injected at a dose of 0.4 μg/kg body weight. All measures were taken 2 hr after injection of ACTH or vehicle or after initiation of the 30-min restraint.

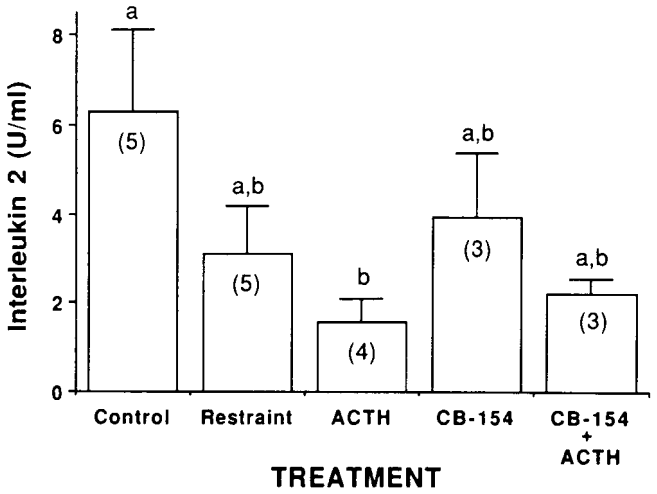

Figure 4. *In vitro* interleukin 2 production by cultured lymphocytes obtained from peripheral blood 2 hr after injection of ACTH or vehicle or initiation of a 30-min restraint stressor. Each bar represents the mean ± SE. The number of barrows per treatment is given within each bar. Means which are significantly different ($P < 0.05$) have different superscripts. Statistical analyses conducted on log-transformed data.

lymphocyte proliferation or IL-2 production responses were measured, although numerical differences were apparent.

Hence, effects of CB154-associated PRL reductions on adrenocortical function in the present studies were suggestive but inconclusive. Plasma cortisol reductions were apparent at only one time. This decrease occurs at a time when the diurnal rhythm of plasma cortisol concentrations in pigs are decreasing from their maximal levels (24), which may suggest that reductions of PRL alter this diurnal rhythm. However, the current results provide only modest support for the hypothesis that PRL, or dopamine (30, 31), modulates porcine adrenocortical function. Similarly, conclusive effects of CB154 and associated reductions in plasma PRL on the aspects of lymphocyte activity measured were absent. However, the small sample sizes available for these particular measures undoubtedly contributed to this lack of statistical significance. Therefore, for both adrenal function and lymphocyte activity of swine, if indeed PRL is a modulator, than either alternative measures or greater more prolonged reductions in PRL are needed to reveal its apparently subtle modulatory role.

Potential effects of GH on both adrenocortical (32) and lymphocyte (33) activities existed in the current study because CB154 in humans can increase GH secretion (34, 35). However, at the doses used, there were no apparent effects of CB154 on basal plasma GH concentrations when measured at a single time point.

Data reported herein are the first for pigs which suggest an absence of dopaminergic regulation of basal plasma ACTH concentrations, although the frequency of blood sampling was not sufficient to allow conclusions concerning effects on putative porcine ACTH ultradian secretion characteristics. In sheep, however, bromocriptine administration (2.5 mg daily for 10 days) was also without effect, not only on basal plasma ACTH concentrations but also on ultradian rhythms (36). In humans bromocriptine therapy reduces plasma ACTH within 4 hr in patients with pituitary-dependent Cushing's syndrome (37), but there is no evidence that dopamine is involved in regulation of normal human ACTH secretion (38). In rats dopamine receptors are present in the pars intermedia (39), and dopamine tonically inhibits secretion of propiomelanocortin-derived peptides from that lobe (40). In dogs dopamine may also inhibit release of ACTH from the pars distalis (41). Hence, the current data with pigs suggest that ACTH derived from the pars intermedia does not contribute significantly to basal levels of ACTH; these data may also indicate that, unlike the dog and similar to the rat and sheep, dopamine is not involved in regulation of release of ACTH from the pars distalis. Further experiments, however, which include more frequent measurements and perhaps higher, more prolonged doses of CB154 are needed to verify these tentative conclusions.

In ACTH(1–24)-injected barrows, plasma ACTH concentrations attained supraphysiologic concentrations for up to 5 hr, whereas in restrained barrows plasma ACTH was 40-fold less and decreased quite rapidly. In both treatment groups, however, the plasma cortisol responses were similar. This suggests two things. First, the adrenal may have become refractory to further stimulation in ACTH-injected animals because plasma cortisol was diminished in spite of sustained plasma ACTH concentrations (42, 43). Second, the dose of ACTH used (approximately 2 units/animal), even though 100-fold lower than those used previously with swine (44, 45), is clearly more than is needed to produce maximal adrenal stimulation. Similar conclusions were proposed previously with reference to tests concerning human adrenal secretory capacity (46).

Glucocorticoids such as cortisol are generally suppressive of immune system activity (7). Specifically with pigs, it has been reported previously that daily 2-hr restraint for 3 days was associated with increased plasma cortisol concentrations, as well as a reduced delayed-type hypersensitivity reaction by Day 2 (19). It was postulated that these *in vivo* effects were in part mediated by increased cortisol, because *in vitro* studies with porcine lymphoid cells demonstrated direct inhibitory effects of physiologic concentrations of cortisol (19). Furthermore, adrenalectomized mice, or those injected with metyrapone to inhibit corticosterone production, did not exhibit the stress-associated suppression of immune responses of intact controls (47). The presently reported results with pigs substantiate previ-

ously reported data. They extend our knowledge by indicating that a single injection of ACTH and the associated increases in plasma cortisol are followed very rapidly by a reduction in the functioning of the immune system as measured by significantly decreased IL-2 production and mitogen-stimulated lymphocyte blastogenic responses. *In vitro* glucocorticoid inhibition of lectin-induced IL-2 production by rat spleen cells and human lymphocytes (48) as well as bovine lymphocytes (49) has been reported previously.

When interpreting the immune system response in the current study, one cannot overlook the potential direct involvement of ACTH on lymphocytes. However, it should also be emphasized that although ACTH(1–24) is steroidogenically active, it has not in other species produced the immunologic effects observed with ACTH(1–39) (50).

The results suggest that CB154 administration for 6 days and concomitant significant 0.69 ng/ml reductions in plasma PRL were associated with a reduced basal plasma cortisol concentrations at one time point, but were without significant effects on either mitogen-stimulated lymphocyte proliferative responses or IL-2 production. CB154 did not alter basal ACTH plasma concentrations in swine, thereby suggesting that dopamine may not be involved in the regulation of basal secretion of ACTH in this species. ACTH injection, and to a lesser extent a 30-min restraint, produced very rapid suppressive effects on IL-2 production and Con A-stimulated lymphocyte proliferative responses, which are presumably mediated in part by increases in plasma cortisol.

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