PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **186**, 333–343 (1987)

Stressor-Associated Alterations in Porcine Plasma Prolactin[1] (42622)

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Abstract. Experiments were conducted to determine effects of restraint and thermal stressors on plasma prolactin (PRL) in castrated male pigs. A single 20-min restraining period in a restraining cage which prevented both movement and injury increased ($P < 0.05$) plasma PRL when applied at either 0800 or 1600 hr. Exposure to 32°C at 0800–1000 hr or at 1600–1800 hr produced more moderate increases ($P<0.05$). A combination of 20 min restraint and 2 hr at 32°C produced a response similar to restraint alone. Twenty minutes after stressor application plasma PRL concentrations in pigs exposed to restraint or restraint +32°C at 1600 h were greater ($P<0.05$) than concentrations measured in all other treatment groups at that time interval. However, there were no statistically significant differences in additional quantitative indices of the plasma PRL responses (maximal level, maximal change, or integrated response above basal levels) among restraint, 32°C, or restraint +32°C, nor between morning and afternoon applications of treatment. Such data do not provide, therefore, any strong evidence for stressor-dependent or circadian differences in plasma PRL response. A second study subjected castrated male pigs to 20°C (controls), 20±12°C (cyclic temperature, sine wave variation), 5°C constant, and 5 ±12°C cyclic for 20 days. After 6 days exposure to 5°C constant or 5 ± 12°C cyclic there were decreases ($P<0.05$) of 59 and 67% respectively in plasma PRL when compared either with pretreatment levels or with levels in pigs at 20 or 20±12°C. There were no differences in PRL responses between cyclic vs constant temperatures. These results are the first to indicate that plasma PRL in pigs is affected by acute restraint and thermal stressors.

Early studies with rodents provided indirect evidence for stressor-related increases in plasma PRL (1–3). Subsequently, it was shown that a variety of animal species respond to various acute stressors—immobilization, handling, heat, cold, or ether exposure—by an increase in plasma PRL concentrations. Such results have been reported for rats (3–7), hamsters (8, 9), cattle (10, 11), goats (12), sheep (13), turkeys (14), and also for humans (15). Swine, however, have heretofore been unique in that plasma PRL levels were not affected by short-term stressors. Neither physical restraint for 1 min nor generalized stress of vascular catheterization was associated with increased plasma PRL in nulliparous female swine (gilts) (16). Snaring and venipuncture of postpartum sows were without effect on plasma PRL, as was a 1-min electrical stimulation (17, 18). Gilts had elevated plasma PRL concentrations during surgery (19, 20), which could reflect effects of either the anesthetics (two of which were thiobarbiturates sodium pentothal and sodium thiamylal) or the surgical trauma. Indeed, in rats sodium pentobarbital (an oxybarbiturate) dramatically increased plasma PRL (5, 21). A longer term stressor (32°C for 9 days) was associated with elevated plasma PRL concentrations in pregnant gilts. Such elevations were most marked after 5 days of exposure (22). In a more recent study exposure to 10 or 30°C for 1, 2, or 7 days was without effect on basal serum PRL concentrations in ovariectomized gilts, but 7 days exposure to 10°C reduced, and to 30°C enhanced, the PRL response to exogenous thyrotropin-releasing hormone (TRH) (23).

The current studies were conducted to evaluate effects of acute and chronic stressors on plasma PRL in castrated male pigs (bar-

[1] This work was supported in part by USDA Competitive Research Grant 86-CRCR-1-195 to H.G.K. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

0037-9727/87 $1.50

rows). To ascertain if the PRL response is stressor dependent, two different putative acute stressors were employed—restraint and elevated ambient temperature—both of which are known to stimulate rapid and dramatic increases in plasma PRL in rodent species (24, 25). Further, since in rodents there is evidence that the PRL response is dependent upon the time of day at which the stressor is applied (26, 27), stressors were applied at two different times of the day. An experiment was also conducted to determine effects of long-term (6 and 20 days) heat and cold exposure on porcine plasma PRL.

Materials and Methods. *Animals, surgery, and blood-sampling techniques.* Thirty-nine crossbred barrows ($\frac{1}{4}$ Yorkshire:$\frac{1}{4}$ Landrace:$\frac{1}{4}$ Large White:$\frac{1}{4}$ Chester White) weighing 70–102 kg were used in the acute stressor study, while 16 crossbred barrows initially weighing 34–50 kg were used in the chronic stressor experiment. Barrows were housed in environmental chambers measuring 4.9 × 5.2 m, capable of maintaining a temperature range of −20 to 40°C. Within these chambers, each barrow was housed in an individual pen of dimensions 1.2 × 0.6 m. 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*. Throughout both studies animals were maintained in 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 jugular vein of Halothane-anesthetized pigs using previously described techniques (28). Surgery was conducted either 6–7 days (acute stressor study) or 3 weeks (chronic stressor study) prior to treatment initiation. Patency of these catheters was maintained by periodic flushing with 10 ml of sterile 0.15 *M* NaCl solution which contained 50 IU/ml of heparin and 1% benzyl alcohol, with pH adjusted to 7.4. Prior to blood sampling a 1.5- to 1.8-m catheter extension (Silastic Medical Grade, 3.18 mm o.d. × 1.57 mm i.d.) was attached to each animal to allow blood to be obtained from outside the pen and without disturbing the pig. In both studies blood samples were obtained using 10-ml syringes containing 10 mg EDTA in 200 μl 0.15 *M* NaCl solution. Blood samples were immediately placed on ice, and subsequently centrifuged at $1500g_{av}$ for 20 min at 2°C. The supernatant from this centrifugation was subsequently recentrifuged at $3584g_{av}$ for 20 min at 2°C. Plasma was then stored frozen at −20°C.

Prolactin radioimmunoassay. PRL was measured using a homologous double-antibody radioimmunoassay procedure. Porcine PRL (USDA-pPRL-I-1, 103 IU/mg) was used for iodination and as a standard, and was dissolved in 0.01 *M* $NaHCO_3$ (1 μg/10 μl). Aliquots (20 μl) were placed into 1.7-ml microcentrifuge tubes, rapidly frozen in liquid nitrogen, and stored at −80 to −100°C until used. Prior to iodination this 2 μg/20 μl of pPRL was thawed, and to it were added 60 μl 0.5 *M* sodium phosphate buffer, pH 7.5, 50 μl enzymobead preparation (Bio-Rad Laboratories, Richmond, CA), 1 mCi ^{125}I in 10 μl NaOH (Amersham Corp., Arlington Heights, IL), and 25 μl 1% β-D-glucose. After an incubation period of 6 min, the incubation mixture was transferred to a 14 × 0.8-cm column of Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) to separate ^{125}I-pPRL from free ^{125}I. Iodinated pPRL was then rapidly frozen in liquid nitrogen and stored at −80 to −100°C. Before use ^{125}I-pPRL was repurified on a 1 × 50-cm AcA 54 Ultrogel column (LKB Industries, Rockville, MD) to separate monomeric ^{125}I-pPRL from oligomers and free iodide. In the RIA pPRL was measured in duplicate 200-μl plasma aliquots in a final incubation volume of 600 μl which consisted of plasma, 200 μl buffer (0.01 *M* sodium phosphate, 0.015 *M* EDTA, 0.15 *M* NaCl, 0.1% N_aN_3, 1% BSA, pH 7.6; Buffer A), 100 μl first antibody (goat anti-porcine prolactin, 1:180,000 dilution in assay tube, Research Products International Corporation, Mount Prospect, IL) in Buffer A containing 0.2% normal goat serum in lieu of BSA, and 100 μl (10,000 cpm) ^{125}I-pPRL in Buffer A. First antibody and sample or standards were preincubated for 24 hr at 4°C. Iodinated pPRL was then added, and after a second 24-hr incubation at 4°C, 100 μl of second antibody (antiserum to goat

gamma globulin, dilution of 1:120 in RIA tube, Calbiochem–Behring, San Diego, CA) was then added. After a third incubation at 4°C for 16 hr, 2 ml Buffer A containing 3% BSA was added, tubes were centrifuged at $3584g_{av}$ for 30 min at 4°C, and supernatants were decanted. Radioactivity within precipitates was counted on a gamma counter. The normal operating range for the standard curves was 0.02–5 ng. Serial dilutions of three porcine plasma pools (20–200 μl) produced competition curves with an average slope ($b = -0.97$) which did not differ ($P > 0.05$) from that of the standard curve ($b = -0.96$). An estimate of accuracy for the pPRL RIA was obtained by adding five quantities of pPRL (0.2–1.6 ng) to two pools of porcine plasma. The average accuracy of estimates was 101.74%. Plots of expected vs measured ng pPRL had an average slope ($b = 1.01$) which did not differ significantly from 1, and a y intercept (0.005) which did not differ from 0 ($P > 0.05$). Sensitivity of the assay, as determined by calculating the lower 95% confidence limit for the y intercept (100% tubes) of the nine standard curves reported in this paper was 8.6 pg pPRL, and as indicated by the lowest standard in the linear range of the standard curve, it was 20 pg. The interassay coefficient of variability (CV) was 13.7%, and the intraassay CV measured over the 1115 samples assayed in duplicate was 3.1%. Specificity of the antisera used for the PRL RIA has been previously reported (29) with no interference by porcine luteinizing hormone (pLH), porcine follicle-stimulating hormone (pFSH), or porcine growth hormone (pGH) being noted.

Experimental design and procedures: Acute stressor study. This experiment involved a randomized complete block design. Each block used eight barrows, was conducted over a 2-week time period, and included the following eight treatments to which pigs were randomly assigned: (i) control animals sampled during the morning and afternoon hours (0700–1600 hr, $n = 5$), (ii) control animals sampled during the afternoon and nighttime hours (1500–2400 hr, $n = 5$), (iii) pigs subjected to a 20-min restraint (0800–0820 hr, $n = 5$), (iv) pigs subjected to a 20-min restraint (1600–1620 hr, $n = 5$), (v) pigs subjected to 32°C for approximately a 2-hr period (0800–1000 hr, $n = 5$), (vi) pigs subjected to 32°C for approximately a 2-hr period (1600–1800 hr, $n = 5$), (vii) pigs subjected to a 20-min restraint (0800–0820 hr, $n = 5$) and to 32°C for 2 hr (0800–1000 hr), and (viii) pigs restrained for 20 min (1600–1620 hr, $n = 4$) and subjected to 32°C for 2 hr (1600–1800 hr). For morning treatments (i, iii, v, vii) blood samples were obtained at 20-min intervals initially (0700–1100 hr), and at hourly intervals thereafter (1200–1600 hr). For afternoon treatments (ii, iv, vi, viii), blood samples were obtained at 20-min intervals initially (1500–1900 hr), and at hourly intervals thereafter (2000–2400 hr).

Pigs subjected to restraint were transferred from their pens to a mobile restraining cage constructed of steel bars padded with foam rubber with cage dimensions of 1.22 m long × 0.25 m wide × 0.7 m high. One side of this cage was adjustable, and hence the pig could be firmly immobilized without causing it physical pain. Transfer time was 2–4 min, and blood samples were taken immediately after transfer (0800 or 1600 hr). A subsequent blood sample was obtained just prior to returning the pig to its home pen (0820 or 1620 hr). Pigs subjected to 32°C were transferred from their home pen to a similar pen in a second environmental chamber 24 hr prior to treatment. On the day of treatment at either 0800 or 1600 hr, the temperature in the environmental chamber was increased from 20 to 32°C at an average rate of 60°C/hr. There was an average temperature overshoot of 4.2°C which lasted 20 min before a stable 32°C was achieved. Two hours after the initial temperature change, ambient temperature was returned to 20°C at an average rate of 54.4°C/hr. After an average temperature undershoot of 6.4°C lasting 24 min, and a rebound to 24°C lasting 26 min, temperatures stabilized at 20°C. The average exposure time to 32°C or higher was 1.8 hr. In this study pigs were maintained at 20°C (drybulb temperature) and an average dewpoint temperature of 2.8°C (average relative humidity of 33%), except—as indicated above—for those animals subjected to a transient 32°C (average dewpoint temperature of 5.3°C and relative humidity of 20%).

Chronic stressor study. In this study four

barrows were placed in each of four different environmental chambers 3 weeks prior to initiation of the study, and maintained at a constant 20°C. At the initiation of the study one group of four barrows remained at 20°C constant and a relative humidity which ranged from 22 to 58% (dewpoint temperatures of −2 to 11°C). A second group of four barrows was subjected to 20 ± 12°C cyclic temperature (sine wave) with maximum temperature at 1300 hr (32°C, dewpoint 0.5°C), and minimum temperatures at 0100 hr (8°C, dewpoint −0.2°C). Coincident with temperature maximum and minimum, relative humidities of 11 and 60% were measured. A third group of four barrows was subjected to a constant 5°C and a relative humidity of 50–70% (dewpoint −5 to 0°C) throughout the study. A fourth group of four barrows was subjected to 5 ± 12°C cyclic temperature (sine wave) with maximum temperature (17°C, dewpoint −5.3°C) at 1300 hr, and minimum temperture (−7°C, dewpoint −7°C) at 0100 hr. Relative humidities of 24% at temperature maximum and 100% at temperature minimum were measured. At 6 days prior to temperature changes, and after 6 and 20 days of treatment animals were bled via the indwelling jugular catheter at 6-hr intervals for a 24-hr period (0700, 1300, 1900, 0100, and 0700 hr). During the conduct of the study two animals died due to endocarditis, possibly resulting from contaminating microorganisms gaining access through the catheter. Data from these animals are not reported for any of the bleedings.

Statistical analysis. Both studies involved at least two main factors, treatment and time, with multiple blood samples taken over time from the same animal. Hence, data were analyzed initially using multiway analysis of variance for repeated measures to test for main effects and interaction, and used the General Linear Models of the Stastical Analysis System (30). Subsequent tests to determine significant differences among individual means followed the procedures detailed by Winer (31), and involved use of the conservative Student–Newman–Keuls a posteriori test for comparison of all means, or use of the more robust a priori linear orthoganal combinations for preplanned comparisons of specific means (32, 33). Comparisons among parameters obtained from the original data, that is, basal levels, maximal levels, maximal changes, and integrated responses, involved a multiway analysis of variance, and the above-indicated comparisons of means. Data were tested for normality of distribution via the Shapiro Wilk Statistic (34), and for homogeneity of variance via the F_{max} test. Data were log transformed where necessary to fulfill assumptions of analysis of variance. A probability level of <0.05 was considered significant. The integrated responses above baseline values were computed using the Trapezoidal Rule (35), which calculates the area under the response curve.

Results. *Acute stressor study.* For both the morning (AM) and afternoon (PM) controls, there were no significant differences among hours in plasma PRL concentrations (Figs. 1, 2). Rather, the levels remained relatively constant about an overall mean of 1.34 ng/ml. With both the AM and PM treatment groups, restraint for 20 min and the associated activities of moving the pigs into the restraining cage induced within 20 min very dramatic increases ($P < 0.05$) in plasma PRL (Figs. 1, 2). The PRL concentrations achieved at 1620 hr in animals subjected to restraint or restraint +32°C were greater ($P < 0.05$) than concentrations measured in all other treatment groups (AM and PM) at that time interval, that is, 20 min after treatment initiation. Such increases were transient, and plasma PRL levels returned to levels not significantly different from prestressor baseline levels within 40 min (AM) or 60 min (PM) after removing animals from the restraining cage. There was considerable variability in the magnitude of individual responses to restraint as indicated by the large standard error bars. Indeed, in terms of maximal increase of plasma PRL above basal levels (maximal minus basal PRL concentration, ng/ml), the responses varied over a 40-fold range (0.3–12.9 ng/ml). Such dramatic variability in PRL response could not be readily correlated with behavioral response to restraint, that is, the duration or magnitude of struggling, panting, or vocalizations.

Subjecting pigs to an ambient temperature of 32°C for 2 hr produced a more moderate, but still significant, increase in plasma PRL

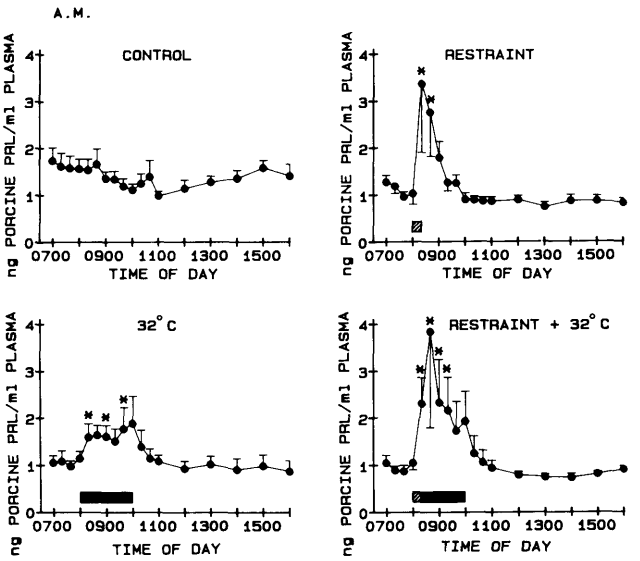

FIG. 1. Plasma porcine PRL concentrations in control or morning stressor-treated barrows. Control and restraint animals were maintained at 20°C throughout the study. One group of five barrows was placed in a restraining cage for a 20-min period (0800–0820 hr) as indicated by the hatched bar. A second group of five barrows was subjected to 32°C for 2 hr (0800–1000 hr) as indicated by the solid bar. A third group of five barrows was concomitantly subjected to restraint (0800–0820 hr) and to 32°C (0800–1000 hr). Blood samples were obtained via jugular catheter and at 20 min (0700–1100 hr) or hourly (1200–1600 hr) intervals. Each datum point represents the mean±SE of five observations. *Means significantly greater than pretreatment basal levels as determined by the Student–Newman–Keuls test or by preplanned linear orthogonal combinations ($P<0.05$). Statistics were computed on log-transformed data.

($P < 0.05$). Such increases were more readily apparent in the AM treatment (Figs. 1, 2), and in both treatment groups plasma PRL rapidly returned to baseline levels after treatment termination. Combined application of both stressors initiated the dramatic increase in PRL previously observed with restraint. Additionally, in the AM group there tended to be a prolongation ($P > 0.05$) of the elevated PRL response when both restraint and 32°C were applied (Figs. 1, 2).

As indicated in Fig. 3a, and as previously alluded to, there were no significant differences in baseline PRL levels among any of the treatment groups. For each animal the baseline level was designated to be the average of the three pretreatment plasma PRL concentrations. In order to more thoroughly compare the PRL response among the various treatment groups, several parameters were considered: the maximal PRL concentration for each animal, the maximal change above baseline levels, and the integrated plasma PRL response above baseline levels. Only pigs subjected to PM restraint had maximal PRL levels above those in control pigs ($P < 0.05$; Fig. 3b). The maximal change—a parameter which considers variations in baseline levels of individual pigs—was elevated ($P < 0.05$) for PM restraint (when compared with controls), and both AM and PM pigs subjected to restraint plus 32°C (Fig. 3c). Finally, the integrated plasma PRL response—the area above baseline values under the response curve—for a 4-hr period (0800–1200 hr for AM groups or 1600–2000 hr for PM groups), which constitutes the complete hormonal response during the above time intervals, was significantly elevated above control values for PM restraint, and for both AM and PM restraint plus 32°C (Fig. 3d). Although in terms of restraint the maximal changes and integrated response of plasma PRL appeared greater for PM compared with AM pigs, there were no significant differences between AM and PM for any response variable considered.

Chronic stressor study. In this study at 6

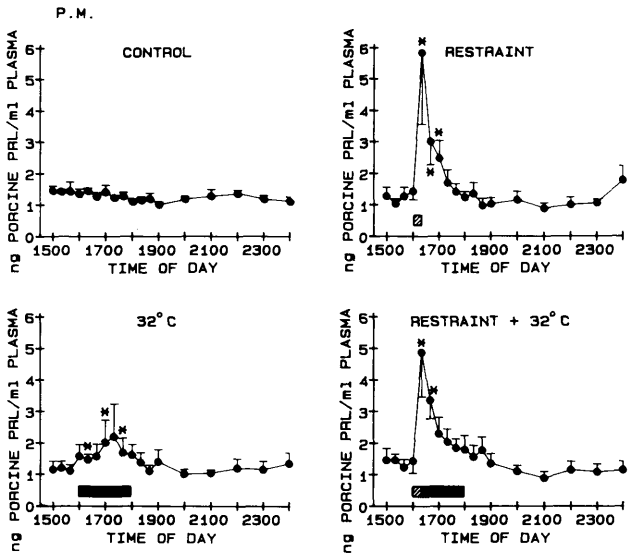

FIG. 2. Plasma porcine PRL concentrations in control or afternoon stressor-treated barrows. Control and restraint animals were maintained at 20°C throughout the study. One group of five barrows was placed in a restraining cage for a 20-min period (1600–1620 hr) as indicated by the hatched bar. A second group of five barrows was subjected to 32°C for 2 hr (1600–1800 hr) as indicated by the solid bar. A third group of four barrows was concomitantly subjected to restraint (1600–1620 hr) and to 32°C (1600–1800 hr). Blood samples were obtained via jugular catheter and at 20-min (1500–1900 hr) or hourly (2000–2400 hr) intervals. Each datum point represents the mean ± SE of four-five observations. *Means significantly greater than pretreatment basal levels as determined by the Student–Newman–Keuls test or by preplanned linear orthogonal combinations ($P < 0.05$). Statistics were computed on log-transformed data.

days prior to treatment initiation preexposure plasma PRL levels were not significantly different among the various experimental groups (Fig. 4). However, after 6 days exposure, there were dramatic 67 and 59% decreases ($P < 0.05$) in plasma PRL in pigs housed at 5 ± 12 and 5°C constant, respectively. These reduced plasma PRL levels were also below those measured in pigs housed at either 20 or 20 ± 12°C ($P < 0.05$). After 20 days exposure plasma PRL remained low in cold-exposed pigs, but these levels were no longer significant when compared with those of pigs housed at 20 or 20 ± 12°C, due to the modest reductions in plasma PRL in the latter treatment groups. After 20 days of temperature exposure, there was no evidence for any circadian changes in plasma PRL in pigs associated with any treatment groups (Fig. 5).

Discussion. Data presented in this paper represent the first demonstration that acute restraint or thermal stressors are capable of eliciting an increase in plasma PRL in pigs. A 20-min restraint produced a rapid, transient increase, whereas 2 hr at 32°C produced a more moderate effect. Combinations of the two stressors produced a response most similar to restraint alone. In terms of the magnitude of PRL response, there was no statistically significant additive effect of the combination of stressors for any of the quantitative parameters considered (maximal concentration, maximal change, integrated response). Previously reported studies with rodents provided evidence for circadian variations in PRL response (26, 27); however, in the current study there was no strong statistical support in barrows either for a circadian sensitivity in PRL responsiveness to the stressors or for an enhanced efficacy of either stressor. The considerable individual variability in the presence or absence of a response, or more noticeably in the magnitude of the PRL response, is consistent with observations in other species (9, 15).

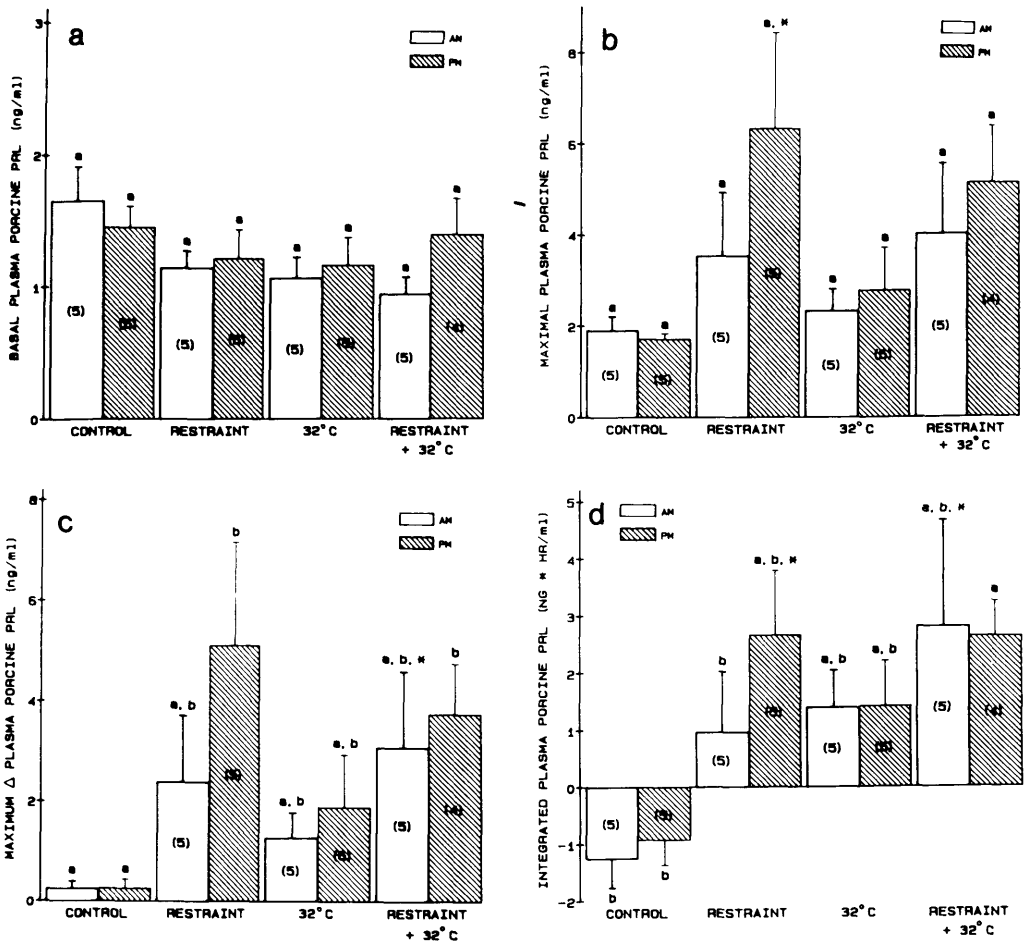

FIG. 3. (a) Basal plasma porcine PRL concentrations in control or stressor-treated barrows. For each animal the basal concentration was represented by the average of the three pretreatment concentrations measured at either 0700, 0720, and 0740 hr, or at 1500, 1520, and 1540 hr. (b) Maximal plasma porcine PRL concentrations, for which the highest plasma PRL concentration measured at any time point was used. (c) Maximum change (Δ) in plasma porcine PRL was computed as the maximal PRL concentration minus basal PRL concentration. (d) Integrated plasma porcine PRL response was computed using the Trapezoidal rule (35) which computes the area under the response curve above basal concentrations for the 4-hr intervals (0800–1200 or 1600–2000 hr). Each bar represents the mean± SE. Numbers in parentheses indicate the number of barrows per treatment. Means with different superscripts are significantly different ($P<0.05$). Statistics involved use of log-transformed data, analysis of variance, the a posteriori Student–Newman–Keuls (SNK) test, the results of which are represented by letter superscripts, and the more robust a priori linear orthogonal combinations, the results of which are indicated by asterisks and which take precedence over SNK test results. Hence, maximal PRL concentrations for PM-Restraint barrows are greater than those for PM-Controls (*$P<0.05$), PRL maximal changes for AM-Restraint + 32°C are greater than those of AM-Controls (*$P<0.05$), and the integrated PRL responses for PM-Restraint and AM-Restraint + 32°C are greater than those of their respective controls (*$P < 0.05$).

Previously reported studies concerned with acute stressors applied to pigs were unable to produce such PRL elevations (16–19). Differences in response among this and previous studies might be ascribed to the nature or duration of the stressor, or perhaps sex and/or strain differences. The restraint stressor employed in the current study was

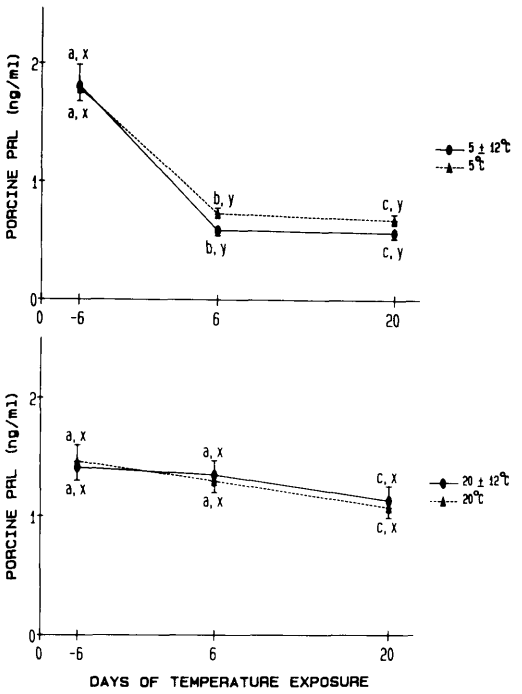

FIG. 4. Plasma PRL concentrations in barrows maintained in constant (5 or 20°C) or cyclic (sine wave, 5 ± 12 or 20 ± 12°C) temperatures. Blood samples were obtained via indwelling jugular catheters at 6 days prior to treatment initiation (represented by −6, and at which time all temperatures were a constant 20°C) and 6 and 20 days after treatment initiation. On each of those days, PRL was measured in blood samples obtained at 0700, 1300, 1900, 0100, and 0700 hr. Hence, each datum point represents the mean ± SE of 14–20 observations from three to four barrows. Means with different super/subscripts (a, b, c) are significantly different ($P < 0.05$) when compared on any given treatment day. Means with different super/subscripts (x, y) are significantly different ($P < 0.05$) when compared among treatment days but at the same temperature. All statistics were computed on log-transformed data.

longer than those previously used: 1 min (16) and 10 min (18). The restraining procedure also differed: immobilization cage vs snout snare. Previous experiments involved gilts (16, 19) or sows (17, 18). Hence, the possibility exists that in pigs, sexual differences exist for PRL response to stressors. Some studies with other species have noted such sexual differences in either the presence of a PRL response (9), or in the magnitude or duration of the PRL response (36). However, most studies indicate that both sexes are capable of responding to stressors with an elevation in plasma PRL (5, 15, 27, 36). Potential strain differences in PRL response must remain a matter for speculation.

The concept that alterations in ambient temperature can modify plasma PRL is not new. Rats maintained at 40°C for 1 hr exhibited a 10-fold increase in plasma PRL, whereas 1 hr at 4°C produced a moderate and inconsistent decrease (24). Elevated environmental temperatures increase, and reduced temperatures decrease plasma PRL in cattle (37, 38). Elevated temperatures are also associated with increased plasma PRL in men (39). Data presented herein, however,

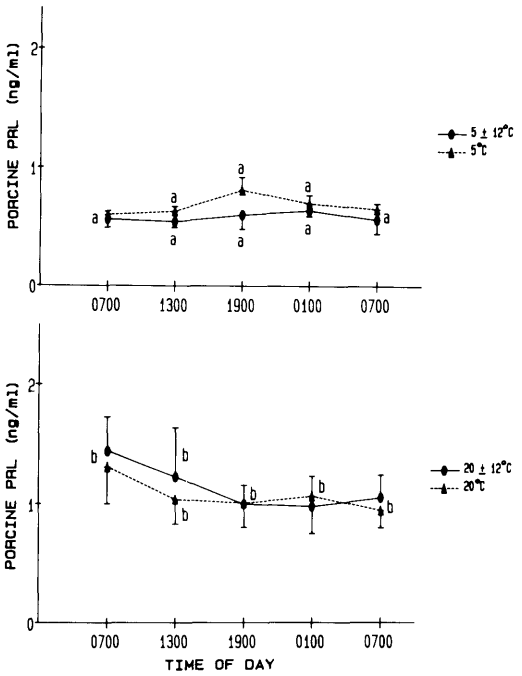

FIG. 5. Plasma PRL concentrations in barrows maintained in constant (5 or 20°C) or cyclic (sine wave, 5 ± 12 or 20 ± 12°C) temperatures for 20 days. Blood samples were obtained via indwelling jugular catheters at the time periods indicated. Each datum point represents the mean ± SE of three to four barrows. Statistical comparisons were made among hours of the day for each temperature, but not among temperatures at any given hour. Hence, super/subscripts (a, b) indicate that plasma PRL levels did not differ with time of day at any of the temperatures ($P > 0.05$). All statistics were conducted on log-transformed data.

are the first indication that a brief exposure to 32°C is also capable of stimulating an increase in plasma PRL in pigs, although recent reports demonstrated that 9 days exposure of pregnant gilts to 32°C increased plasma PRL (22), while 7 days exposure of ovariectomized gilts to 10°C reduced, and to 30°C enhanced, plasma PRL response to TRH (23). In the current study the response measured was, however, modest compared with that observed in rats and cattle, but might be enhanced with greater temperature elevations.

In the chronic stressor study, environmental temperatures also briefly reached 32°C at 1300 hr for pigs subjected to 20 ± 12°C. Blood samples were obtained at the time of this temperature acrophase, but there was no measurable effect on plasma PRL at that time. This observation might suggest that the animals had adapted to the increased temperature prior to the first sampling period which occurred 6 days after the initial exposure, or that the rate of change of environmental temperature is important in determining the response elicited. The rate of change in the acute stressor study (60°C/hr) was 30-fold greater than in the chronic stressor study (2°C/hr). Precedents exist for both possibilities (36, 40).

The reduction in plasma PRL after 6 days exposure to 5 or 5 ± 12°C is also a new finding in terms of the porcine PRL response to environmental temperature, although such effects have been reported for other species. Indeed in rats (24, 36) and in cattle (36, 37), the response is quite rapid and occurs after as little as 1 hr of cold exposure. For humans a 1.5-hr exposure to 13°C produced a significant decrease in plasma PRL (41).

The current studies were not designed to elucidate mechanisms by which porcine PRL might be altered during restraint or temperature stressors (42), but either pituitary secretion or metabolic clearance rates could be involved. Similarly, the physiological function of altered levels of PRL in pigs subjected to thermal or restraint stressors is unknown. However, one may speculate from results obtained in other species that modulation of the adrenal gland (43–48), kidney (44, 49), or immune system (50, 51) may be involved. Hence, changes in plasma PRL during thermal or restraint stressors may be assisting at several levels with the adaptation of the animal to its altered environment.

In summary the data indicate that barrows respond to two different environmental changes which are putative stressors with significant increases in plasma PRL in a manner qualitatively similar to other species.

The authors thank the National Institute of Diabetes, Digestive, and Kidney Diseases, as well as the National Hormone and Pituitary Program for the porcine prolactin used in the RIA. We are also very grateful to Mr. David Sypherd and Mrs. Moira Wilhelm for the excellent technical assistance they provided during the conduct of these studies. We also thank Mrs. Lynn Sterkel for the artwork, and Mrs. Elda Peterson for her assistance in the preparation of the manuscript.

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Received April 27, 1987. P.S.E.B.M. 1987, Vol. 186.
Accepted July 30, 1987.