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Surgery Potentiates Adrenocortical Responses to Hypoxia in Dogs (41578)

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Abstract. We studied the effect of prior surgery on the ACTH and corticosteroid responses to acute hypoxia. Five conditioned, pentobarbital-anesthetized, gallamine-paralyzed mongrel dogs were exposed to 24 min of isocapnic hypoxia (11% O_2/89% N_2) 2 hr (Expt I) and approximately 1 week (Expt II) after implantation of femoral arterial and venous catheters. ACTH and corticosteroid responses were assessed by RIA of arterial plasma samples. Arterial PO_2 fell similarly in both experiments from 82 to 26 Torr. This caused significant increases in ACTH of similar magnitude in both experiments. Corticosteroid levels increased more in Expt I than Expt II indicating an apparent potentiation by surgery of the adrenocortical response to hypoxia. Two additional dogs were studied in reverse order under lighter anesthesia such that ACTH and corticosteroid levels after surgery were higher than in the first set of experiments. Under these conditions, hypoxia still produced a large increase in ACTH and corticosteroids after acute surgery. Correlation of log ACTH with corticosteroid levels (adrenal dose response) revealed a significant increase in slope in dogs with acute surgery suggesting that surgery interacted with hypoxia either to change the metabolic clearance rate of corticosteroid or to increase adrenal sensitivity to ACTH.

Surgery is a potent stimulus to the pituitary–adrenocortical system (1). Many prior studies of adrenocortical activity during hypoxia have utilized acute-animal models consisting of surgical preparation of the animal followed by a brief recovery period and subsequent administration of hypoxia with measurement of corticosteroid secretion (2) and ACTH (3). Injections of ACTH or corticosteroids have been shown to inhibit the ACTH and corticosteroid responses to subsequently administered stress (4). However, stress in rats which elevates endogenous ACTH and corticosterone levels does not affect the magnitude of ACTH and corticosterone responses to subsequent stimuli (4) and thus a facilitatory effect of stress is inferred. In dogs, prior stress has been reported to facilitate or inhibit corticosteroid responses to subsequent stimuli (5). It is likely, then, that previous assessments of adrenocortical activity during hypoxia using acute animals models have made biased estimates of the true sensitivity of the adrenocortical system to decreases in arterial oxygen tension. Another confounding factor often ignored that has been shown to alter the ACTH response to hypoxia is hyperventilation secondary to decreases in arterial PO_2 (3).

Therefore, to determine the effects of hypoxia per se and to compare these effects to those of surgery plus hypoxia, ACTH and corticosteroid levels were measured in paralyzed, artificially ventilated dogs with and without surgical implantation of catheters 1 to 2 hr before administration of hypoxia.

Materials and Methods. Seven (16–29 kg) conditioned mongrel dogs used in this study were maintained on a 12:12 light–dark cycle (lights on 0600 hr) with food and water provided *ad lib.* Food was removed from the cage at approximately 1500 hr on the afternoon prior to experimentation. At approximately 0730 hr the following day, dogs were weighed and brought to the laboratory where they were anesthetized with either 28 mg/kg iv (Expts I and II; $N = 5$) or 26 mg/kg iv (Expts III and IV; $N = 2$) pentobarbital sodium and intubated with a Rusch–Foregger endotracheal tube. Additional anesthetic was administered as needed. Rectal temperature was monitored and maintained above 38°C.

Experiment I—hypoxia after surgical procedure. Using aseptic techniques, indwelling femoral arterial and venous Tygon catheters (polyvinyl chloride, i.d. 0.05 in, o.d. 0.09 in) were implanted, exteriorized at the back using a trochar and taped into a zippered jacket (Alice King Catham). This procedure was complete by 0930 hr at which time a sample for determination of ACTH and corticosteroids was drawn. At 1030 hr, dogs were placed on a Harvard positive pressure respirator and

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paralyzed with gallamine triethiodide (3 mg/kg iv; courtesy of Peter Mullen of Davis and Geck). End tidal CO_2 was monitored (Beckman LB1) and maintained between 5.0 and 6.0%. Mean arterial blood pressure (MABP) was measured and recorded using a Statham P23AC pressure transducer and a Grass model 5 polygraph. After two control samples (15 min apart), animals were exposed to 11% O_2/89% N_2 for 24 min administered via the inflow of the respirator. Arterial samples were drawn every 6 min for the measurement of arterial blood gases and pH, hematocrit, ACTH, and corticosteroids (methods described below).

After 24 min of hypoxia, animals were allowed to recover from the gallamine injection (30–60 min) after which catheters were filled with heparin (1000 U/ml), 10^6 U of penstrep im (Combiotic) were administered, and the animals were placed in a heated recovery room until the following morning when they were returned to their cages. Catheters were flushed every day and rectal temperature was monitored. Only normothermic dogs [<39.0°C; (6)] were used.

Experiment II—no surgery on day of experiment. After at least 5 days recovery, the same animals as in Expt I were brought to the laboratory and anesthetized. After at least 1 hr, dogs were exposed to hypoxia following the same regimen as described above. In one dog with nonfunctional catheters, percutaneous catheters were placed in the contralateral saphenous vein (Deseret Angiocath) for induction of anesthesia and a femoral artery (Becton Dickinson White pediatric arterial needle and a Cook wire guide and catheter) just after induction of anesthesia. Percutaneous arterial catheter placement did not stimulate ACTH or corticosteroid levels.

Experiments III (no surgery) and IV (surgery). The procedures were essentially the same as Expts I and II except that (i) the dogs were more lightly anesthetized (26 mg/kg, iv) so that the surgical procedure would elevate ACTH and corticosteroids to much higher levels and (ii) the order of experimentation was reversed such that Expt III (no surgery) was performed with percutaneous catheters (described above) several weeks before Expt IV (surgery).

Measurements. (i) Arterial PO_2 (P_aO_2), CO_2 (P_aCO_2), and pH (pH_a) were measured using a Radiometer (Copenhagen) BMS 3MK2 blood gas analyzer. (ii) Hematocrit (HCT) was estimated using an Autocrit II (Clay–Adams). (iii) Plasma ACTH was measured by radioimmunoassay (6, 7). (iv) Plasma corticosteroid was measured by radioimmunoassay using a commercially obtained antiserum (Radioassay Systems antiserum No. 147) and tritiated cortisol (New England Nuclear). Intraassay variability was 11% (4.7 ± 0.51 μg/dl; $n = 10$); interassay variability was 13% (2.25 ± 0.30 μg/dl, $n = 22$). The relative cross-reactivity of the antiserum with corticosterone was 200% as compared to cortisol (100%). Therefore, the results of this assay are called "corticosteroids." Other cross-reactivities relative to cortisol (100%) as stated by the supplier are progesterone—4.5%, desoxycorticosterone—4.5%, all others each ≤3%. This RIA correlates well with a transcortin competitive protein binding assay (8) previously used in our laboratory ($r^2 = 0.866$; transcortin assay = 0.95RIA + 0.93).

Statistical analyses. A two-way analysis of variance (ANOVA) corrected for repeated measures on both dimensions (9) was used to evaluate the effects of time (response to hypoxia) and surgery on each of the variables measured. In addition, analysis of covariance (ANOCOVA) corrected for repeated measures (9) was performed where indicated using the SAS-BMDP2V program. If the interaction among factors was significant ($P < 0.05$ or 0.01), a multiple range test using Newman–Keuls distribution of a Studentized range statistic (9) was employed to evaluate differences among individual cell means. If the interaction between response to hypoxia and surgery was not significant but the overall response was, the multiple range test was used to evaluate differences among pooled means for each time point. In addition, regression, comparison of slopes and intercepts, and correlation techniques were used where indicated (10). Statistical analysis of pH_a was performed after conversion to hydrogen ion concentration.

Results. Table I shows mean values of certain measured variables in Expts I and II. P_aCO_2 and pH_a were unaltered by hypoxia indicating that the strict criteria of isocapnia and normal acid base balance were met. The significant increases in HCT and MABP which

occurred during hypoxia were statistically similar in both experiments. Hypertension due to hypoxia was exhibited at 6, 12, and 18 min. At 24 min, multiple range comparisons of pooled means revealed that MABP was no longer distinguishable from control. HCT was significantly elevated at all time points during hypoxia.

Surgery in Expt I caused significant elevations of ACTH (154.2 ± 49.0 pg/ml) and corticosteroids (8.5 ± 3.0 μg/dl) as compared to nonoperated animals (Expt II). Approximately 30 min later, ACTH and corticosteroid levels had fallen significantly to 69.6 ± 4.2 pg/ml and 5.7 ± 1.3 μg/dl, respectively, at which time experimentation began. Control P_aO_2 values were 80 ± 4 Torr in both groups. Control ACTH and corticosteroid levels at time 0 in Expt I were 69.4 ± 5.3 pg/ml and 5.6 ± 0.9 μg/dl, respectively, while in Expt II they were 25.4 ± 4.6 pg/ml and 1.3 ± 0.5 μg/dl, respectively. Both ACTH and corticosteroid control levels were significantly higher in Expt I by paired t test ($P < 0.05$).

Figure 1 shows the mean changes from control of P_aO_2, ACTH and corticosteroid levels in Expts I and II. The 11% O_2 significantly decreased P_aO_2 by 6 min of hypoxia; P_aO_2 plateaued by 18 min. The changes in P_aO_2 in Expt I vs Expt II were statistically indistinguishable indicating that both experiments resulted in similar levels of hypoxia. The ACTH responses were highly variable as indicated by the large mean-squared error for interaction. Neither ANOVA nor ANOCOVA (before or after log normalization) revealed significant interaction between surgery and hypoxia with respect to ACTH. However, there was a significant overall ACTH response to hypoxia. Pooled mean Newman–Keuls comparison revealed significant increases in ACTH at 18 and 24 min of hypoxia. The interaction of corticosteroids with time and surgery assessed by ANOVA or ANOCOVA was highly significant indicating that surgery did alter the plasma corticosteroid response to hypoxia. Comparison of individual cell mean values indicated that in Expt I, corticosteroid levels had risen significantly by 12 min whereas in Expt II, corticosteroid levels had increased significantly by 18 min of hypoxia. Corticosteroid levels at all time points in Expt I were greater than in Expt II. When

TABLE I. P_aCO_2, pH_a, BLOOD PRESSURE, AND HEMATOCRIT RESPONSES TO HYPOXIA BEGUN AFTER 0-min SAMPLE

	Time (min)												
	Expt I[a]						Expt II[b]						
	−15	0	6	12	18	24	−15	0	6	12	18	24	MSe[c,d]
P_aCO_2 (Torr)	33	32	33	32	34	34	36	37	35	35	36	34	6.7
pH_a	7.43	7.42	7.45	7.44	7.42	7.40	7.38	7.38	7.40	7.41	7.40	7.41	(5.6)[c]
MABP (mm Hg)	102	99	128	129	126	123	90	91	115	112	106	100	57.7
HCT (%)	39	39	44	44	45	47	35	35	36	38	39	38	4.0

[a] Surgery 2 hr before.
[b] Surgery at least 5 days before.
[c] MSe (mean squared error for interaction) from ANOVA for pH_a listed in units of H^+ concentration (nmole).
[d] MSe degrees of freedom = 20. $F_{interaction}$ NS for all variables listed. $F_{response}$ NS for P_aCO_2 and pH_a; $F_{response}$ $P < 0.05$ for MABP (mean arterial blood pressure) and HCT. Multiple range test ($P < 0.01$; pooled analysis): HCT greater than control at 6, 12, 18, and 24 min; MABP greater than control at 6, 12, and 18 min.

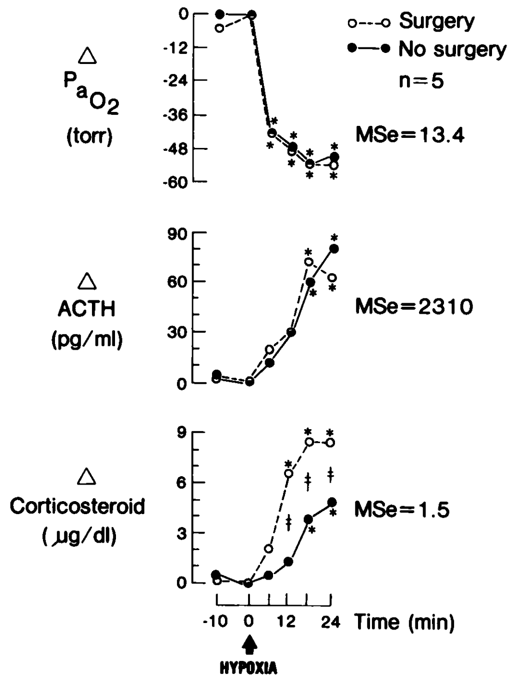

FIG. 1. P_aO_2, ACTH, and corticosteroid changes from control during hypoxia ($N = 5$). Open circles represent values from Expt I (surgery) and shaded circles represent values from Expt II (no surgery). (*) different from 0-min sample; (‡) Expt I different from Expt II. (MSe) = mean squared error for interaction from ANOVA on nontransformed data (20 degrees of freedom). P_aO_2, ACTH: $F_{interaction}$ = NS; $F_{response} = P < 0.01$; Corticosteroid: $F_{interaction} = P < 0.01$; $F_{response} = P < 0.01$.

analyzed as changes from own control, the increase in corticosteroids was greater in Expt I than Expt II at 12, 18, and 24 min of hypoxia.

The high variability in the ACTH values results from the fact that in two animals, a smaller increase in ACTH occurred in Expt I (+30 pg/ml average) than in Expt II (+179 pg/ml average) whereas in three animals, a larger increase in ACTH occurred in Expt I (+84 pg/ml average) than in Expt II (+6 pg/ml average) even though the corticosteroid responses were consistent from animal to animal.

This dichotomy is illustrated in Fig. 2 which shows individual ACTH and corticosteroid responses in two dogs. Hypoxia caused a larger increase in ACTH in the surgery experiment in the dog, Ares, which was paralleled by corticosteroids in both experiments. In the dog, Oscar, however, the prior surgery experiment elicited a smaller ACTH response to hypoxia yet a dramatically larger corticosteroid response. This would seem to indicate that something was potentiating the plasma steroid response to ACTH.

Figure 3 shows the results of the two dogs in Expts III and IV. Surgery (Expt IV) elevated ACTH to levels greater than 1000 pg/ml. ACTH and corticosteroid levels were decreasing when hypoxia (P_aO_2 28–30 Torr) was applied. Both ACTH and corticosteroid levels continued to fall until between 6 and 12 min of hypoxia. Then stimulation of both ACTH and corticosteroids occurred. The change in ACTH from its nadir to peak was numerically larger in Expt IV than Expt III.

The correlations of log ACTH and corticosteroid levels (adrenocortical "gain") in samples withdrawn before and at the end of 24 min of hypoxia are shown in Fig. 4. Surgery significantly altered this relationship by increasing the slope and decreasing the Y intercept ($P < 0.05$). One dog which showed an indeterminate gain in Expt II (no change in ACTH) was excluded from this analysis. The individual numbers from Expts II and III (no surgery) fit with data describing a log ACTH–corticosteroid dose response curve in conscious dogs (11). The slope of the regression line of Expts II and III (no surgery) was not statistically different from slopes of ACTH–corticosteroid dose-response relationships either from angiotensin II infusions in conscious dogs derived from data of Ramsay *et al.* (12) or from the ACTH infusion data of Wood *et al.* in conscious dogs (11). However, the regression line from Expts II and III had a significantly lower elevation than the regression line derived from Ramsay *et al.* (12) or Wood *et al.* (11).

Discussion. Prior stress may either inhibit (5) subsequent adrenocortical responses to stress by elevating corticosteroid levels ("negative feedback") or may facilitate (4, 5) responses to a subsequent stress. Surgery has been considered a facilitatory influence on the ACTH response to subsequent stress (4). The ACTH responses to hypoxia in the present experiments were highly variable. Even so, Expt I showed a similar mean ACTH response to Expt II even though it had been preceded by a large corticosteroid "negative

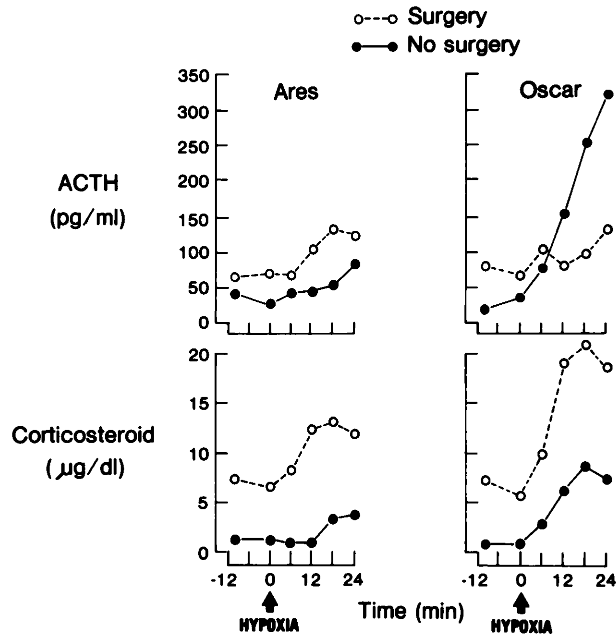

FIG. 2. Individual responses of two dogs which illustrate variability of the ACTH response to hypoxia with or without surgery. Notice that the corticosteroid response to hypoxia in surgery experiment is larger in both dogs.

feedback" signal. This is similar to observations by Dallman and Jones (4) in the rat. They inferred that prior stress not only produced a corticosteroid negative feedback signal but also "facilitated" the ACTH response to subsequent stress. This is consistent with the results of the present experiments. One animal actually exhibited a large increase in corticosteroids (+6.4 μg/dl) with no detectable change in ACTH. Despite this, prior surgery clearly potentiated the corticosteroid response to hypoxia. This effect could not be explained by differences in either hematocrit (which is an estimate of the oxygen carrying capacity of the blood) or blood pressure. This suggests that prior estimates of changes in corticosteroids during hypoxia using acute animal models (2, 3) have probably overestimated the sensitivity of anesthetized dogs to increase steroid secretion. Even in two dogs with very high control corticosteroid values (Expt IV), an increase in ACTH (above the decay in ACTH from high levels) occurred and was numerically larger than in Expt III. This suggests that even with a very large corticosteroid feedback signal produced by surgery, the ACTH response was not suppressed.

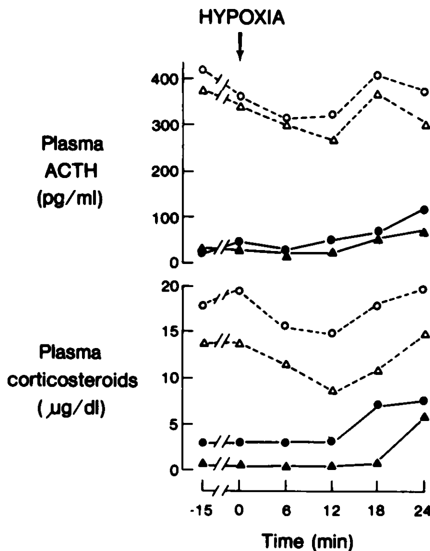

FIG. 3. ACTH and corticosteroid levels during hypoxia in Expt III (closed symbols, solid lines) and Expt IV (open symbols, dashed lines) (N = 2).

It has been reported that pentobarbital exerts a minimal effect on the responsivity of the adrenocortical system to hypoxia in dogs

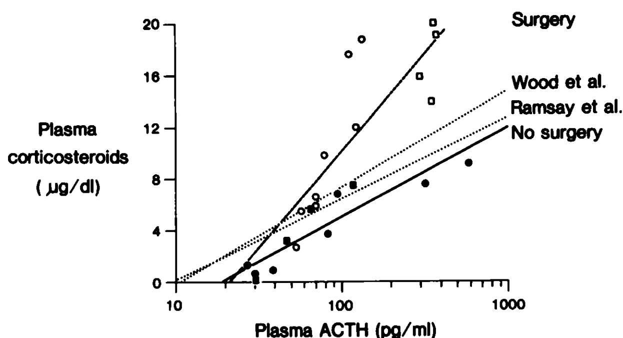

FIG. 4. Correlation of ACTH and corticosteroid levels (adrenal dose response = "gain"). *Prior surgery (dashed line):* open circles (Expt I); open squares (Expt IV). Corticosteroids = 15.7 [log ACTH] − 21.8 {$r = 0.87$, $P < 0.05$} *No surgery (continuous line):* closed circles (Expt II); closed squares (Expt III). Corticosteroids = 7.0 [log ACTH] − 9.0 {$r = 0.92$, $P < 0.05$}. Dotted lines from the data of Wood *et al.* (11) and Ramsay *et al.* (12) in conscious dogs.

allowed to recover for 24 hr after surgical implantation of adrenal venous catheters (13). Therefore, the advantages of the narcotized, paralyzed dog model may outweigh the advantages of a conscious dog model when evaluating the effects of hypoxia per se without the confounding influences of hyperpnea and, when not prevented, respiratory alkalosis.

The correlation of log ACTH and corticosteroid levels suggests several conclusions: Anesthesia with pentobarbital and paralyzation with gallamine did not acutely alter the adrenocortical sensitivity to ACTH (slope of the log ACTH–corticosteroid dose-response curve) when the results of these studies are compared with those of others using conscious dogs (11, 12). It also indicates that hypoxia without surgery may not significantly alter the clearance or volume of distribution of corticosteroids. Prior surgery increased the apparent adrenocortical gain during hypoxia. Since this correlation was performed using measurements of peripheral steroid levels and not steroid secretion rates, there are several confounding processes that are possible. Surgery may have interacted with hypoxia to cause a large decrease in the metabolic clearance rate and/or volume of distribution of corticosteroids such that peripheral corticosteroid levels were elevated to a greater extent by similar increases in ACTH. It is also possible that surgery resulted in increased secretion of a large amount of unidentified steroid which cross-reacts with the corticosteroid antibody used. Finally, the increase in adrenal gain may be real and caused by some nonimmunoassayable corticotrophic factor in light of the fact that changes in adrenal sensitivity to ACTH have been reported to occur in rats (14) and dogs (15) (increase in adrenal gain assessed by administration of exogenous ACTH to dogs 6 hr after hemorrhage).

The main point of this study to be reiterated is that surgery does indeed alter the plasma corticosteroid response to hypoxia in a manner that bears further investigation.

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