

Further Characterization of the Pituitary-Adrenocortical Responses to Stress in Chiroptera

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ABSTRACT Previous studies on bats from this laboratory have revealed the presence of exceptionally high circulating levels of glucocorticoids in two species of the sub-order Megachiroptera. In the present study, the following questions were asked: (1) what effect does the routine handling and examination of captive bats have on the activity of their hypothalamic-pituitary-adrenocortical (HPA) axis?; (2) are the unusually high plasma levels of cortisol and corticosterone found in *Pteropus hypomelanus* associated with high levels of circulating adrenocorticotrophic hormone (ACTH)?; (3) are there diurnal changes in stress responsivity in this species?; and (4) how do levels of glucocorticoids in *P. hypomelanus* compare with those found in other species of Chiroptera (both micro and megachiropteran species)? Of five species examined, *P. hypomelanus* had slightly higher total glucocorticoid levels than *P. pumulis*, but ~8-fold higher levels than in three species of Microchiroptera (*Artibeus jamaicensis*, *Carollia perspicillata*, and *Myotis lucifigus*). There was a pronounced diurnal rhythm in glucocorticoid levels in one species (*M. lucifigus*) for which this was determined. A 1-h period of restraint stress increased glucose and glucocorticoid levels in *P. pumulis*, and also increased ACTH and glucocorticoids in *P. hypomelanus*. Fifteen minutes of routine handling (weighing, measuring, etc.) elicited a significant rise in plasma glucocorticoids in *P. hypomelanus* to combined peak (cortisol plus corticosterone) levels of over 1,000 ng/ml (100 µg%). There was no significant difference in the response to handling in bats tested in the morning or evening. Basal ACTH levels as detected by radioimmunoassay were low in *P. hypomelanus*, in spite of high steroid levels. Displacement of labeled ACTH by plasma of *P. hypomelanus* was roughly parallel to that produced by synthetic human ACTH. The identity of immunoreactive ACTH was confirmed by subjecting separate aliquots of plasma to an immunoradiometric assay (IRMA), which operates on a different principle from the RIA and necessitates an intact molecule closely resembling human ACTH. We conclude that bats possess a "stress response" similar in some ways to that seen in other mammals. The extremely high constitutive and stress-induced levels of glucocorticoids in the Megachiroptera are nearly unique among mammals. Although high circulating levels of ACTH were detected by IRMA, but not by RIA, it is possible that the Chiropteran adrenal gland is hypersensitive to ACTH or possesses exceptionally active intracellular steroidogenic mechanisms. Finally, the routine daily procedures associated with measuring, weighing, and monitoring bats in captivity constitutes a significant stress to the animal as revealed by pronounced activation of the HPA axis. © 1994 Wiley-Liss, Inc.

Stress may be defined as a real or perceived threat to homeostasis, and encompasses stimuli ranging from internal insults such as hypoglycemia, hypovolemia, and hypoxia to emotional stimuli such as exposure to a novel environment. Mammals possess several mechanisms to combat such threats, or to restore homeostasis (e.g., blood glucose levels) after the insult has occurred. An important response to stress in all mammals, thus far studied, is the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Afferent signals arising from sensory receptors converge in the hypothalamus and stimulate the release of corticotropin-releasing hormone and other factors into the hypophyseal portal circulation (Plotsky,

'85; Plotsky et al., '89). These factors activate the corticotropes of the anterior pituitary to secrete ACTH. ACTH, in turn, activates the adrenal cortex to synthesize glucocorticoids. Fish and mammals primarily synthesize the steroid hormone cortisol, but birds and other vertebrates make almost exclusively corticosterone.

The characteristics of the HPA axis in Chiroptera have yet to be clearly elucidated. We (Widmaier and Kunz, '93) and others (Gustafson and Belt, '81) have reported that certain species of bats have exceptionally high circulating levels of cortisol and corticosterone that are matched in ver-

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tebrates only by squirrel monkeys (Brown et al., '70; Cassorla et al., '82). In the squirrel monkey, it has been shown that a defect in the glucocorticoid receptor is partly responsible for chronically elevated glucocorticoid levels (i.e., inadequate negative feedback of the steroid on ACTH due to a mutated receptor) (Cassorla et al., '82). The basis for the unusually high levels of both glucocorticoids in bats is presently unknown. Moreover, it is not known if the steroid levels are correlated with equally high concentrations of ACTH, since this hormone has yet to be measured in Chiroptera.

In the present study, we sought to determine whether immunoreactive ACTH was present in chiropteran plasma and at what concentrations. Also, in a continuing attempt to characterize the hormones of the HPA axis in the highly diverse order Chiroptera (~950 species), glucocorticoid levels were determined in two or three species each in both the Micro- and Megachiroptera. Finally, the effects of routine daily maintenance procedures (handling, weighing, etc.) on HPA axis hormones was investigated to determine if such procedures constituted a repeated stress to these animals. This was prompted in part by our earlier observations that levels of glucocorticoids in three species of bats were very high after subjecting the animals to such procedures (Widmaier and Kunz, '93). However, no baseline (pre-handling) samples were available in that study and thus it was unclear to what extent the high steroid levels were attributable to the effects of the handling procedures themselves.

MATERIALS AND METHODS

Animals

Pteropus hypomelanus, *P. pumulis* (Megachiroptera: Pteropodidae) and *Carollia perspicillata* (Microchiroptera: Phyllostomidae) are housed at the Lube Foundation, Inc., a captive breeding facility in Gainesville, Florida. The colony of *P. hypomelanus* was established in 1990 from 24 wild-caught animals taken in Borneo. The colony of *P. pumulis* was established in 1992 from 12 wild-caught animals taken in the Philippines. The colony of *C. perspicillata* was established from captive animals that were formerly housed in the Cincinnati Zoo. Each species is housed as breeding or non-breeding groups in hexagonal, double-wire enclosures, approximately 9 m in diameter and 2 m high. Each of the outdoor portions of these enclosures surrounds a smaller, centrally-located,

hexagonally shaped (3 m diameter) roost building located in each of the enclosures. The enclosures are designed so that the bats can feed, rest, and fly freely. Except during cold months (December and January) bats are allowed to move freely between the roost enclosure and the surrounding flight cage. Animals selected for each experiment were temporarily removed from larger groups and housed in large rectangular cages (~4 × 3 × 6 m) to facilitate capture and reduce disturbance to other members of the colony during experimental procedures. Each bat was marked with metal or plastic thumb or wing bands and uniquely-coded transponders for identification. A small colony of *Artibeus jamaicensis* (Microchiroptera: Phyllostomidae), housed at Silver Springs Park, Silver Springs, Florida, also provided blood samples for glucocorticoids and was housed in a small wood and wire enclosure (1 × 1.5 × 2 m). Finally, wild *Myotis lucifugus* (Microchiroptera: Vespertilionidae) were captured individually at a maternity colony in Hillsborough, New Hampshire.

Water and food

At the Lube Foundation, bats were fed daily at approximately 1700 h by placing stainless-steel feeding and drinking cups randomly throughout the enclosures. These cups were removed and cleaned each day between 1000 h and 1400 h. Water and food, including freshly cut fruits and vegetables were supplemented with monkey chow and Vionate (multivitamin). *Myotis lucifugus* is insectivorous and typically feeds nightly beginning at or around dusk (Anthony and Kunz, '77).

Protocols for bleeding and plasma collection

Unless otherwise specified, blood was collected from each bat by venipuncture of a small wing vein using a 22-gauge needle. These animals were not anesthetized and were restrained by hand during bleeding. Five to six aliquots of blood (45–50 µl each) were taken in heparinized microcapillary tubes and centrifuged to separate plasma and cells. In one case, animals were first anesthetized and then bled into EDTA-containing plastic tubes for analysis of ACTH by IRMA (below). In all cases, plasma was stored at –20°C, transported to Boston in liquid nitrogen or on dry ice, and later stored at –20°C until assays were conducted.

Experiments

Routine handling stress (*Pteropus hypomelanus*)

Fully grown but immature male and female *P. hypomelanus* (411–597 g) were tested in May 1993 for sensitivity to the effects of routine monitoring procedures. A single blood sample was collected within 3 min after removing a bat from its roost. Each animal was then weighed on an Ohaus pan balance and subjected to routine morphological measurements. These included taking measurements of the forearm, third metacarpal, thumb, first phalanx, second phalanx, half wingspan, tibia and zygomatic arch for growth analysis. The entire procedure for each bat required approximately 10–15 min. A second blood sample was collected immediately following these measurements. The second sample was obtained at this timepoint in order to replicate the handling/sampling protocol in our earlier study. Blood was centrifuged and the plasma was collected and frozen for subsequent hormone radioimmunoassays. One group of five bats was tested in the morning (1000–1100 h), and a second group of 14 bats was tested in the late afternoon (1500–1800 h).

Restraint stress (*Pteropus hypomelanus* and *Pteropus pumulis*)

Six mature (non-breeding) female *P. hypomelanus* (426–569 g) and *P. pumulis* (165–180 g) bats were removed from their roosts between 0830 h and 1030 h in March (*P. pumilis*) or May (*P. hypomelanus*) 1993, and bled via venipuncture. In the case of *P. hypomelanus*, the bleeding was performed using plastic syringes and collection tubes. This was done to ensure that blood that was to be assayed for ACTH did not come in contact with glass, which non-specifically binds ACTH. Samples were collected into a solution of EDTA (final concentration 2 mM) to inhibit proteolysis and coagulation. Bats were then placed singly into restraint cages as previously described (Widmaier and Kunz, '93) for 1 h. Briefly, the cages consisted of a plastic mesh cylinder (9 cm diameter, 25 cm length) with wood bases. The cylinders were lined with rubber mesh to prevent abrasions to the restrained animals. After 1 h, the bats were removed from the restraint cages and a second blood sample was taken. The animals were then returned to their roost/flight enclosure.

Myotis lucifugus

Blood was obtained as described above from adult females and one male (6.9–8.1 g after cor-

recting for food in gut) within 2–3 min of handling the animals in late July 1993. Of the females, most were post-lactational, and three were non-breeding. Blood was collected into glass capillary tubes and processed for hormone assays. Samples were collected either just before the bats left the roost to feed (beginning about 1900 h), at midday (~1200 h), or immediately following their return from feeding (~0500–0600 h). The midday samples and a group of five to six morning and evening samples were obtained via venipuncture. Because of the small blood volume of these animals, subsequent morning and evening samples were obtained via decapitation immediately upon removing the animal from its roost. Morning and evening venipuncture samples were each pooled to provide sufficient plasma for one additional steroid determination.

Assay procedures and data analyses

ACTH was determined in 25 µl plasma by direct RIA using primary polyclonal antibodies from IgG Corporation (Nashville, TN). Human ACTH₍₁₋₃₉₎ was used as standard and was provided as a courtesy by Dr. Jean Rivier, The Salk Institute, San Diego, CA. Labeled ACTH was obtained from Amersham (Arlington Heights, IL). Since this is the first report of chiropteran ACTH, a pool of plasma from several *P. hypomelanus* was obtained and used to test for parallelism using the ACTH standard curve.

In a separate sample of blood collected from 6 anesthetized non-breeding female *P. hypomelanus* (collected into EDTA, only), plasma was subjected to an immunoradiometric assay (IRMA) for human ACTH (Nichols Institute, San Juan Capistrano, CA). We are indebted to Dr. Hershel Raff, Medical College of Wisconsin, for performing this assay and supplying the necessary materials. The assay employs a monoclonal antibody directed against one epitope of the human ACTH molecule and polyclonal antibodies directed against a different site. Details of the assay have been published elsewhere (Raff and Findling, '89; Zahradnik et al., '89). Cortisol and corticosterone were determined using commercial RIA kits (ICN Biochemicals, Inc., Irvine, CA) as previously described (Widmaier and Kunz, '93). Glucose was measured using the glucose oxidase method (Trinder kit; Sigma Chemical Co., St. Louis, MO). Paired (before and after stress) data were analyzed by paired t-test.

RESULTS

A summary of glucocorticoid levels in several species of Chiroptera is shown in Table 1. The

TABLE 1. Resting glucocorticoid levels in Chiroptera¹

Species	Cortisol (ng/ml)	Corticosterone (ng/ml)
Microchiroptera		
<i>A. jamaicensis</i>	58 ± 3 (3)	26 ± 1 (3)
<i>C. perspicillata</i>	N.D.	34 ± 10 (2) ²
<i>M. lucifigus</i> (morning)	14 ± 4 (4) ²	85 ± 37 (5) ⁴
<i>M. lucifigus</i> (midday)	49 ± 18 (4)	52 ± 16 (4) ⁴
<i>M. lucifigus</i> (evening)	170 ± 53 (4) ^{2,3}	Undetectable (5)
Megachiroptera		
<i>P. pumulis</i> (basal)	394 ± 112 (5)	55 ± 9 (4)
<i>P. pumulis</i> (stressed)	502 ± 60 (6)	72 ± 17 (5)
<i>P. hypomelanus</i>	483 ± 68 (26)	121 ± 40 (5)

¹For *M. lucifigus*, blood was obtained by decapitation or venipuncture immediately after removing the animal from its roost either just after returning from feeding (morning), in the midday, or just prior to leaving the roost for feeding (evening). The number of animals is given in parentheses. N.D., not determined.

²One sample was a pool from either four or five bats.

³t = 3.02, *P* < 0.02 vs. morning value.

⁴Two (midday) and three (morning) bats had undetectable corticosterone levels and were assigned a value of 25 ng/ml (detection limit). All evening samples were below the assay detection limit. Three morning samples were also below the limit of detection in the cortisol assay, and were assigned a value of 10 ng/ml.

highest values for both cortisol and corticosterone were observed in each of the megachiropteran species. Cortisol and corticosterone were both present in blood of each species if sufficient plasma was available to conduct the two tests. There was a significant daily rhythm in circulating cortisol in *M. lucifigus*, but peak levels (evening) were only ~35% of those observed in the Megachiroptera. Corticosterone followed an inverse daily rhythm compared to cortisol, with highest levels in the morning and undetectable levels in the evening.

The protocols associated with daily handling and maintenance in *P. hypomelanus* elicited a significant increase in plasma levels of cortisol (Fig. 1A). This was true whether the collections were made in the morning (*P* < 0.04) or late afternoon (*P* < 0.001). Likewise, corticosterone levels were also significantly elevated (*P* < 0.02) after handling (Fig. 1B). Although evening steroid values (both basal and stressed) tended to be higher than those in the morning, this difference was not statistically significant.

Immunoreactive ACTH was detected in pooled plasma of *P. hypomelanus*, and diluted in parallel with a standard curve using human ACTH₁₋₃₉ as standard (Fig. 2). The coefficient of determination (*r*²) between the standard curve and the immunoreactive ACTH in *P. hypomelanus* was 0.97.

Restraint stress for 1 h increased plasma ACTH levels by over 4-fold in *P. hypomelanus* (Fig. 3). However, this difference was not significant because of one bat whose post-stress levels of ACTH did not change. Sufficient plasma was not available for complete determinations of cortisol and

corticosterone in addition to the ACTH measurements. For cortisol, three bats had a mean (± SE) baseline value of 466 ± 104 ng/ml. Plasma was only available for a single cortisol determination following stress (1,154 ng/ml). No plasma was available for baseline corticosterone measurements in this experiment, but the post-stress level of this hormone was 212 ± 106 ng/ml (*n* = 5).

In six anesthetized female bats (*P. hypomelanus*), plasma was assayed for ACTH using an immunoradiometric assay (IRMA). In those animals, ACTH levels were 782 ± 301 pg/ml (range 194–2,250 pg/ml). Cortisol and Corticosterone levels in the same animals were 717 ± 111 ng/ml and 482 ± 105 ng/ml, respectively. As with the RIA, there was little or no matrix effect of bat plasma in the IRMA (i.e., serial dilutions of plasma samples resulted in approximately equal levels of irACTH, after correction for dilution) (not shown).

The response to restraint stress was tested in one other megachiropteran. Restraint produced a significant hyperglycemia in *P. pumilis* (baseline: 75 ± 10 mg/dl; post-stress: 139 ± 20 mg/dl; *t* = 3.83, *P* < 0.01), and also increased levels of both cortisol and corticosterone (Table 1).

DISCUSSION

This report demonstrates that the HPA axis of two species of Megachiroptera (Pteropodidae) *Pteropus pumilis* and *P. hypomelanus* responds to stress with increases in glucocorticoids and glucose. Although the latter was only measured in *P. pumilis*, stress-hyperglycemia was observed in *P. hypomelanus* in a previous study (Widmaier and

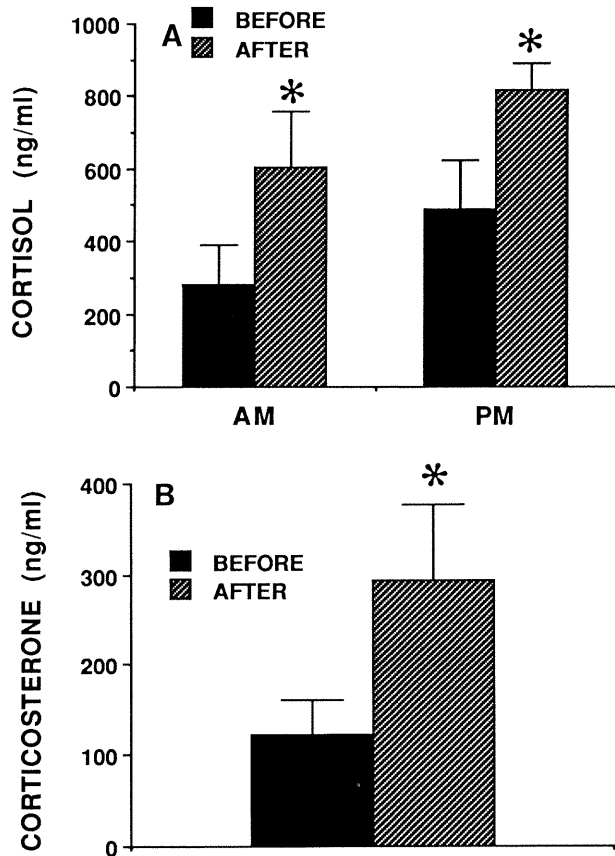


Fig. 1. Effect of handling on glucocorticoid levels in plasma of *P. hypomelanus*. Groups of bats were removed individually from their roosts either in the AM (1000–1100 h, $n = 2$ males and 3 females) or PM (1500–1800 h, $n = 14$ females). A blood sample was collected from a wing vein within 3 min (“Before” sample), and the animals were then subjected to a series of non-invasive procedures designed to mimic daily monitoring procedures. These included weighing the animal, and taking standard morphological measurements. The procedures typically required 10–15 min to complete. Immediately following these procedures, a second blood sample was taken (“After”). The blood was centrifuged and the plasma frozen for future cortisol (A) and corticosterone (B) radioimmunoassays. (A): *, $t = 3.09$, $P < 0.03$ (AM), and $t = 4.97$, $P < 0.001$ (PM) for baseline (“Before”) vs. stress (“After”). (B): Statistical analysis was performed only on a subset of 5 animals (all in PM) from which sufficient plasma was available for both “Before” and “After” measurements. For those animals, *, $t = 3.89$, $P < 0.02$.

Kunz, '93). It is likely that stress activates the sympathetic branch of the autonomic nervous system in bats as it does in other mammals (Havel and Taborsky, '89), and this is a significant contributing factor in the development of hyperglycemia. The types of stress that bats were subjected to in our study are similar to those that would be encountered in the routine capture, handling,

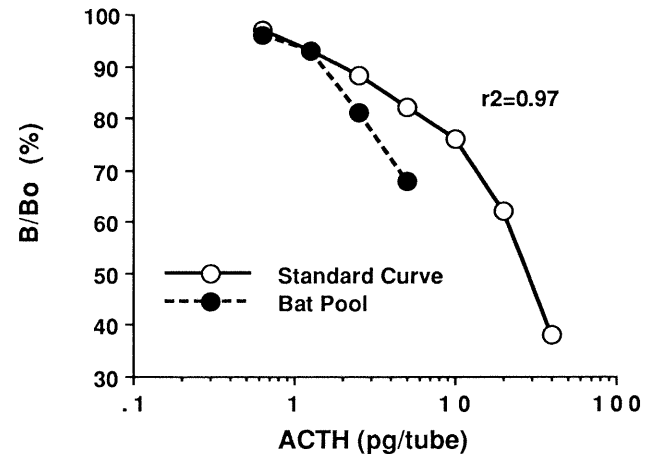


Fig. 2. Serial dilutions of bat plasma. A pool of blood from several *P. hypomelanus* was serially diluted with assay buffer to test for parallelism with a standard curve. The initial volume of plasma was 50 μ l. Volumes were normalized with assay buffer. The coefficient of determination (r^2) for B/Bo (bat pool vs. standard curve over four dilutions) was 0.97.

transport, and monitoring of captive bats. Both handling and restraint caused pronounced and significant increases in circulating glucocorticoid levels in *P. hypomelanus* and *P. pumilus*, indicating that these activities constitute an acute stress to the animal. Although the timing of the restraint-stress samples was based on our earlier report (Widmaier and Kunz, '93), it is possible that we have underestimated the magnitude of the response to handling, since no timecourse on the

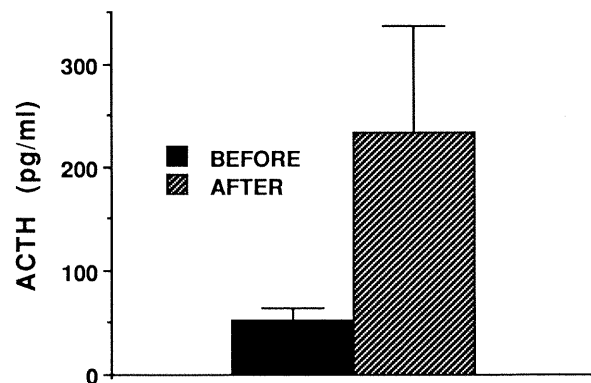


Fig. 3. Effects of restraint stress on plasma levels of ACTH. Individual *P. hypomelanus* were removed from their roosts, bled within 3 min (“Before”) and then placed into restraint cages for 1 h. At the end of this period bats were removed from the holding cages and quickly bled a second time (“After”). Both samples were obtained using 1 ml plastic syringes and plastic collection tubes in the presence of EDTA. $n = 4$ (“Before”) and 6 (“After”).

effects of this stress and recovery from it have ever been performed.

Plasma levels of both cortisol and corticosterone were extremely high in *P. hypomelanus*, confirming our previous report (Widmaier and Kunz, '93). The ratio of circulating cortisol/corticosterone was ~4:1, typical of other mammalian species in which both steroids are normally produced (e.g., Albers et al., '85; Keller-Wood et al., '83). Slightly lower, but still exceptionally high steroid levels were observed in the other megachiropteran included in this report, and also in the megachiropteran *P. vampyrus* reported previously (Widmaier and Kunz, '93). These levels are approximately 5 to 10 times greater than those observed in the microchiropteran tropical fruit-eating species, and 3 to 50 times greater than those of the temperate insectivorous bat *Myotis lucifugus* (depending on the season of sampling). The explanation for these differences within and between sub-orders is presently unclear, but it is apparent that even when every measure is taken to minimize non-specific stress, basal levels of glucocorticoids are consistently high in the Megachiroptera and within the typical mammalian range in the Microchiroptera. However, in the absence of field measurements from wild, free-ranging animals, we cannot conclude that the basal levels measured in this and our earlier study (Widmaier and Kunz, '93) are truly representative of basal circulating steroid levels in the wild.

Although the presence of a corticosteroid-binding protein has been measured in plasma of several bat species including members of the Pteropodidae, Phyllostomidae, and Vespertilionidae (Kwiecinski et al., '87), it is unknown how its affinity for glucocorticoids compares with other mammalian corticosterone-binding globulins (CBG). High levels of CBG or high affinity CBG could sufficiently reduce unbound (active) levels of glucocorticoids to decrease the negative feedback effect of the steroids on hypothalamic/pituitary function. This, in turn, might be expected to elevate plasma ACTH levels, which would then stimulate the adrenal gland to synthesize increasing amounts of steroid (Keller-Wood and Dallman, 1984). The feedback effects of glucocorticoids have yet to be examined in detail in bats, but histological observations of the pituitary gland of the Indian fruit bat, *Rousettus leschanaulti* (Megachiroptera: Pteropodidae), suggest that after pharmacological adrenalectomy the process of negative feedback may be similar to that observed in other mammals (Bhiwagade et al., '89). Nevertheless, immunoreactive ACTH

levels in *P. hypomelanus* were well within the mammalian range, and thus it is unlikely that CBG levels alone could explain the high circulating concentrations of steroids before and after stress. Future studies will be directed towards determining the free (unbound)/bound ratio of circulating cortisol and corticosterone in chiropteran blood.

The possibility that the high basal and stressed glucocorticoid levels observed in *P. hypomelanus* and the other megachiropteran species might be due to high circulating ACTH levels was tested by measuring plasma ACTH before and after stress. Baseline ACTH levels were at or below the limit of detectability in the radioimmunoassay for each animal tested, and stress-induced levels were moderate and well within the typical mammalian range. These results should be carefully interpreted, however. First, it is possible that actual plasma ACTH levels were underestimated due to poor cross-reactivity in the RIA. This possibility is difficult to determine in the absence of purified chiropteran ACTH. However, the antibodies used were polyclonal and direct against a region of the ACTH molecule (residues 7–18) that is completely conserved in all other mammals in which ACTH has been identified. Moreover, *P. hypomelanus* ACTH diluted roughly in parallel with the standard curve, suggesting that matrix effects due to unidentified plasma factors were not of major consequence. Of great significance is that chiropteran ACTH was detectable using an entirely different method, the immunoradiometric assay for human ACTH. This assay requires an intact molecule that closely resembles human ACTH, since two different antibodies are employed directed against either the N- or C-terminal regions, respectively, of the human ACTH molecule. In order to be detected by this method, ACTH must be able to bind both antibodies. Using this assay, 5 of 6 animals had ACTH levels approximately twice as high as the stressed levels recorded by RIA. One animal had exceptionally high plasma ACTH (2,250 pg/ml). Notwithstanding this one outlier (for which we have no clear explanation at this time), the significance of these findings is that *P. hypomelanus* ACTH appears to very closely resemble human ACTH, and circulates within the normal mammalian range. The relatively high ACTH levels detected by the IRMA may be due in part to the effects of anesthesia and the prolonged duration of blood sampling under those conditions (to obtain sufficient blood for the IRMA and steroid assays, combined).

Our current hypothesis is that the adrenal cortex of *P. hypomelanus* and other Megachiroptera is unusually active, even in the presence of typical mammalian ACTH levels. Whether this is due to high constitutive steroidogenic activity, to the presence of other unidentified circulating adrenocorticotrophic factors, to intracellular differences (e.g., higher rates of cholesterol transport to mitochondria), or to changes in cellular sensitivity to ACTH (e.g., high affinity receptor), awaits detailed in vitro analyses before conclusions can be drawn.

A diurnal fluctuation in the response to stress has been observed in rats which is partly attributable to daily changes in adrenal sensitivity to ACTH (Kant et al., '86; Wilkinson et al., '79). However, there was no apparent AM/PM difference in stress responsiveness in *P. hypomelanus*. Our earlier study (Widmaier and Kunz, '93) suggested that evening glucocorticoid levels tended to be higher in this species, and the present study confirms this observation, although the differences were not significant. However, the magnitude of the response to stress (i.e., difference between stress-induced and basal steroid levels) was similar at the two times of day.

In *M. lucifugus*, on the other hand, there was a statistically significant diurnal rhythm in cortisol, with peak levels just prior to the onset of the active (feeding) phase of the animals' diurnal cycle, a pattern observed in most mammals (e.g., Gibson and Krieger, '81). Thus, in this respect as well as in absolute concentrations of steroid hormones in blood, this and other Microchiroptera more closely resemble other mammals in terms of the regulation of the HPA axis. Of interest is the observation that peak corticosterone levels in *M. lucifugus* occurred synchronously with the nadir in cortisol (i.e., in the morning). In another dual steroid mammal, the hamster, corticosterone is the predominant morning steroid, while the cortisol/corticosterone ratio shifts to 2:1 in the evening (Albers et al., '85). Presumably, the AM/PM shifts in steroid ratios in these species reflect either diurnal control of 17 α -hydroxylase activity or differential clearance rates of each steroid during the day.

Finally, it should be noted that *M. lucifugus* has seasonal cycles of steroid hormone levels that are correlated with hibernation periods (Gustafson and Belt, '81). In the latter study, cortisol levels reportedly were near the nadir of the annual rhythm during July, but still considerably higher than those found in the present report. However,

those samples were obtained in anesthetized animals that had been captured and maintained in the laboratory, whereas our samples were taken in the field without any prior stress, and therefore are probably a more reasonable indicator of true resting glucocorticoid levels in *Myotis lucifugus*.

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