Incidence of Hypercortisolism and Dexamethasone Resistance Increases with Age among Wild Baboons

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While many features of the adrenocortical axis are unchanged with age in humans, there is a pattern of senescent hypercortisolism. This occurs basally, following threshold doses of dexamethasone, and in synergy with depression or Alzheimer's disease. An understanding of neuroendocrine aging is important, for both its gerontological implications, and determination of normative values for comparison with neuropsychiatric states. We have investigated whether aging is associated with hypercortisolism in a population of wild primates. The subjects were 108 yellow baboons (Papio cynocephalus) *that have been under long-term study of Amboseli National Park in Kenya. Animals were anesthetized by blowgun under similar circumstances that allow for determination of basal cortisol concentrations. Sixty minutes later, 5.0 mg dexamethasone was administered to each animal, and cortisol determinations were made on serum collected immediately before administration and 6 hr later. Basal cortisol concentrations rose with age ($p < 0.028$; $r = 0.23$). This occurred in a nonprogressive manner, in that there were no differences in concentrations among the youngest three quartiles of animals, whereas animals in the oldest quartile (older than approximately 16 years) had significantly higher values. In addition, there was a significant increase in postdexamethasone cortisol concentrations with age ($p < 0.01$; $r = 0.31$). This feature emerged progressively with age in both sexes. A number of possible artifactual causes of this senescent pattern could be eliminated, including medication confound, coincident disease, and body weight. These findings suggest that hypercortisolism and glucocorticoid feedback resistance might be general features of primate aging.*

Introduction

Neuropsychiatric disorders in which there is hypersecretion of glucocorticoids have long been of interest to biological psychiatrists. This has particularly been the case for depression and Alzheimer's disease, in which approximately 50% of sufferers are basally hypercortisolemic and/or dexamethasone resistant. Progress has been made in uncovering the neuroendocrine bases of such hypersecretion, as well as in explaining why it occurs in only a subset of individuals. One important variable appears to be age of subjects. For

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both major depression and Alzheimer's disease, hypercortisolism (either basal or during the DST) becomes more common with age (reviewed in Sapolsky 1990; Sapolsky and Plotsky 1990).

Naturally, the emergence of senescence as a predisposing factor towards hypercortisolism in these disorders raises the issue of whether hypercortisolism is also a feature of aging itself. This needs to be known both for issues of the biology of aging, and to better determine what constitutes normal function for comparison with disease states. There has been some confusion as to whether aged humans are hypercortisolemic. As is detailed (see Discussion), an older literature suggests that the aged adrenocortical axis is essentially intact in its function. In contrast, more recent work suggests that hypercortisolism and dexamethasone resistance are subtle but consistent features of human aging.

The present report examines the effects of aging upon the adrenocortical axis in a novel primate population. Rather than study captive animals, in which adrenocortical function can be distorted by experimental history and by the stress of captivity, we have studied a population of wild baboons living in a national park in East Africa. We observe that basal hypercortisolism and relative dexamethasone resistance emerge with age.

Methods

Background on Baboon Life Histories and Social Organization

The present research was conducted on yellow baboons, *Papio cynocephalus*, in Amboseli National Park of southern Kenya during 1989 and 1990. Baboons are among the largest, most sexually dimorphic, and most terrestrial of the monkeys. They live in semiclosed matrilocal social groups consisting of males and females of all ages. Baboons are omnivores that, in their savannah habitats, forage long distances daily. Detection of and protection from predators is such an important benefit of group-living for these animals that individuals make extreme efforts to keep up with their group, even a few minutes or hours after parturition or when incapacitated or slowed down by illness or aging.

Like most anthropoid primates, baboon females stay in their group of birth throughout their lives and from about 6 years of age until death they produce a single infant per gestation at 1–2 year intervals. After a subadult period from 6–8 years of age, most males leave their natal group and, if successful, reproduce in one or a succession of other groups; immigration is most commonly into nearby groups (Samuels and Altmann 1991). Under stable demographic conditions, animals over 6 years old usually constitute half of the 60 or so animals in a group, and a few of each sex were usually over 16 years old (Altmann and Altmann 1979; Strum and Western 1982; Altmann et al 1985).

Subjects and Observational Methods

The subjects of this study were the members of three baboon groups whose adjacent home ranges include Amboseli National Park and that are a subset of the larger Amboseli basin baboon population. All members of the three groups are identified by individual physical characteristics and have been part of longitudinal research projects. The history of almost all females, and of those males that were born into one of these study groups are known since birth (see, e.g., Altmann et al 1988; Altmann and Muruthi 1988). For animals born into the study groups, birthdates are known within a few days, and usually to the day, based on our almost-daily census and neonatal assessment records (Altmann 1980). Ages of immigrant males in each group are usually known, at least to within the

Table 1. Cortisol Concentrations in Baboons, Regardless of Age and Sex[a]

Sample	Cortisol (μg/dl)
0 hr ("Basal")	14.8 ± 1.1
1 hr (prior to dexamethasone)	16.9 ± 1.4
4 hr (3 hr postdexamethasone)	13.7 ± 1.2
7 hr (6 hr postdexamethasone)	10.8 ± 1.4

[a]Sample sizes for successive samples were 90, 91, 76, and 72.

year, either because they were born into one of the other study groups or because they had been individually identified in their previous group during periodic censuses of the groups adjacent to the study groups. In some cases, males are first identified when they immigrate into a study group; in those instances, age is estimated upon entry, based on our extensive information on visual morphological assessments during maturation and aging of known-age animals in study groups.

Acquisition of Blood Samples and Dexamethasone Suppression Test

Plasma was obtained by anesthetizing subjects with Telazol (tiletamine hydrochloride and zolazepam) (250 mg for older juvenile and adults [male weight range: 16–38 kg; female weight range: 10–24 kg] and one fourth to one half that for smaller animals) injected from a propelled syringe fired from a blowgun at 10 m. No pregnant females were darted except a few in the first trimester. Animals were darted only when their backs were turned, so as to preclude anticipatory stress. All subjects were darted between 7:30 and 10:30 AM, during the summers of 1989 and 1990, to control for seasonal and circadian hormone fluctuations. A first blood sample was obtained as rapidly as possible; in all cases, this was within 15 min of darting. Darting itself is not sufficiently stressful to increase cortisol secretion in baboons; rather, it is the disorientation just before anesthetization that is stressful (Sapolsky 1982). Thus, the first sample taken probably reflects basal concentrations of cortisol.

A second sample was obtained 1 hr after the initial darting. Immediately following that, animals were administered dexamethasone (Decadron phosphate, 5 mg IM) and subsequent samples were taken 3 and 6 hr later. Thus, this protocol differs from the classic DST (Carroll et al 1981; of neccesity, animals had to be anesthetized at the time of the test: moreover, because animals could not be anesthetized for longer than the greater part of a single day, the lengthy postdexamethasone follow-up done in the typical DST could not be carried out).

Animals were allowed to recover in a cage near their group and were released the following morning, when fully conscious. No loss of habitation to observers or difficulty in rejoining troops has been observed. A total of 62 males and 46 females were anesthetized in this manner. The dexamethasone study was carried out only on individuals during the 1989 season; thus, only values for basal and stress cortisol concentrations were available from animals darted in 1990 (n = 22).

Determination of Cortisol Values

Samples were centrifuged on site and plasma frozen in dry ice until return to the United States. Cortisol concentrations were determined by radioimmunoassay as described previously (Krey et al 1975) with an antibody with <0.1% cross-reactivity with dexameth-

Table 2. Effects of Age on Basal Cortisol Concentrations in Male and Female Baboons[a]

Age	Male	Female
0–2000 days	10.5 ± 2.0	15.0 ± 2.0
2000–4000 days	17.0 ± 2.8	13.5 ± 3.1
4000–6000 days	12.4 ± 4.3	15.7 ± 5.3
6000–days	28.1 ± 5.3	25.0 ± 7.0

[a]Cortisol concentrations are expressed as μg/dl. Samples sizes, from youngest to oldest: male, 17, 20, 10, 3; females, 21, 12, 5, 2. In both this and the following Table, ages correspond to immature, young adult, middle adult and older adult categories from Figures 1 and 2.

asone (Antibody F21-53, Endocrine Sciences, Tarzana, CA). Intraassay and interassay coefficients of variation were 0.07 and 0.11, respectively.

Data Analysis

General linear models analyses and paired *t*-tests were performed using SAS 6.04 (SAS Institute 1988). The paired *t*-tests were used to examine stress response and dexamethasone sensitivity. General linear models (GLM) procedures were used to evaluate sex, body weight, and age as factors predicting cortisol levels, stress responses, and dexamethasone sensitivity.

For purposes of visual presentation, we then grouped data by sex (Tables 2 and 3) and by age classes of 2000 days (Figures 1 and 2, Tables 2 and 3). These classes correspond in a general way to immature, young adult, mid-adult, and older adult life stages, and are referred to as such. Data are presented as mean ± SEM. Cortisol "stress responsiveness" is defined as the increase in cortisol concentrations from the initial sample to the one taken at the 1-hr mark. "Dexamethasone responsiveness" is defined as the circulating cortisol concentration 6 hr following dexamethasone administration.

Results

Table 1 presents the mean cortisol concentrations for all subjects. There was only a nonsignificant trend ($p < 0.10$; paired *t*-test) towards an increase over the first hour in response to the stressor of anesthetization. This contrasts with our previous report of a significant cortisol stress-response in wild baboons subjected to this anesthetization protocol (e.g., Sapolsky 1982). This probably reflects the switch from the earlier use of the anesthetic Sernylan (phencyclidine hydrochloride) to the current use of Telazol, as the latter contains a benzodiazepine (in addition to tiletamine hydrochloride), which is known

Table 3. Effects of Age and Sex on Cortisol Concentrations in 6 hr after Dexamethasone[a]

Age	Male	Female
0–2000 days	8.5 ± 1.8	7.9 ± 2.3
2000–4000 days	9.1 ± 2.9	16.7 ± 7.5
4000–6000 days	15.5 ± 6.3	8.3 ± 4.2
6000–days	17.5 ± 4.2	33.0

[a]Values represent cortisol concentrations (in μg/dl) 6 hr after administration of 5.0 dexamethasone. Sample sizes, from youngest to oldest: males, 15, 20, 9, 4; females; 14, 5, 4, 1.

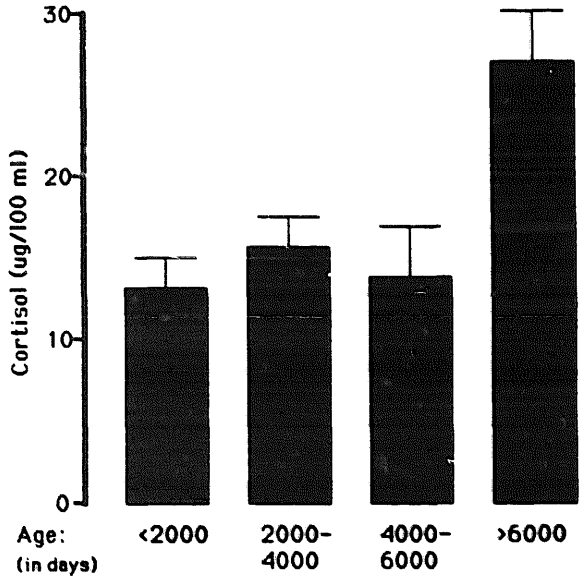

Figure 1. Basal cortisol concentrations in animals up to 2000 days of age (n = 38), those >2000–4000 days (n = 32), those >4000–6000 (n = 15) and those older than 6000 days (n = 5).

to inhibit the adrenocortical axis (Antoni 1986). Subjects were responsive to dexamethasone, in that cortisol concentrations declined significantly following its administration ($p < 0.0001$; paired t-test).

We then examined these parameters with respect to age, body weight, and sex. Age was the only of these factors that contributed significantly to variability in any of the parameters ($p > 0.10$ for the other cases); the relationship to age is reported as r values and p values, below.

Basal cortisol concentrations rose with age ($r = 0.23$; $p < 0.028$). Displaying the data as a histogram of quartiles showed clearly that the relationship was nonprogressive (Figure 1), in that there was an abrupt increase in cortisol concentrations in old age,

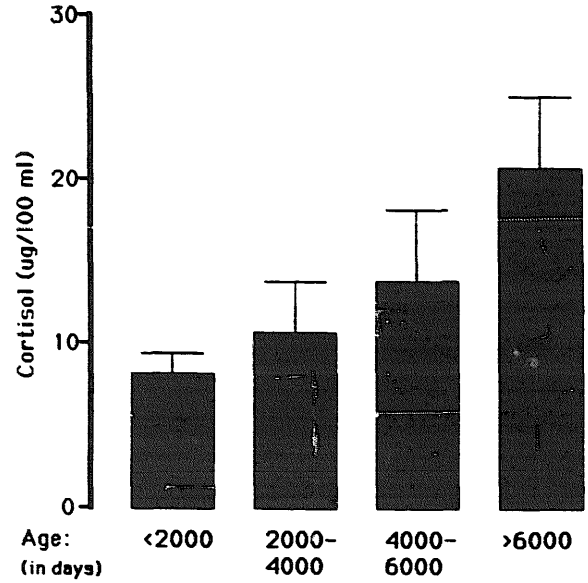

Figure 2. Cortisol concentrations 6 hr after administration of 5.0 mg dexamethasone. Quartiles of age groups were as described for Figure 1, with sample sizes of 29, 25, 13, and 5, respectively (sample sizes were smaller than in Figure 1 because dexamethasone suppression tests could not be carried out on all individuals).

rather than a gradual rise over the lifetime; however, sample sizes were not adequate to distinguish among various linear and nonlinear models.

Dexamethasone responsiveness also decreased with age, in that circulating cortisol concentrations 6 hr after dexamethasone administration were higher in more aged animals ($r = 0.31$; $p < 0.01$). A quartile analysis showed this to be a trait that emerged progressively with age (Figure 2).

Despite both basal hypercortisolism and dexamethasone unresponsiveness in aged animals, there was only a trend towards a correlation between elevated basal cortisol concentrations and elevated cortisol values 6 hr after dexamethasone administration ($p < 0.078$).

Discussion

Aged rats tend to hypersecrete glucocorticoids basally, and during the poststress recovery period, and are resistant to the inhibitory feedback effects of both dexamethasone and corticosterone itself (reviewed in Sapolsky 1990). In contrast, it is less clear whether aged primates or humans are hypercortisolemic. Although there is an age-related decrease in glucocorticoid production, there is also a decrease in the glucocorticoid clearance rate; the net result is unchanged basal concentrations of cortisol with age (as well as of 17-hydroxycorticoids and CBG). Adrenocortical responsiveness to various stressors and to dexamethasone remains intact, as is adrenal sensitivity to ACTH and pituitary sensitivity to CRF (reviewed in Zimmerman and Coryell 1987; Sapolsky 1990).

Despite these findings, there is still a subtle pattern of hypercortisolism in aged humans. (1) *Extremely* aged humans tend to be hypercortisolemic, and the earlier literature that concluded that human aging is not associated with dexamethasone resistance relied upon now-dated notions of what constitutes an "aged" human (for example, in the review on the subject by Zimmerman and Coryell 1987, a mean age of 68 years was the oldest of any study considered). (2) The threshold for feedback resistance may be lower in aged humans. When 0.5 mg dexamethasone is used in the DST in order to unmask "borderline" cases of resistance, rather than the standard 1.0 mg, senescence is associated with dexamethasone resistance. (3) Aged humans may have normal feedback sensitivity, and yet be near the threshold of resistance, such that if aging coincides with a disorder of borderline resistance, the two should combine to increase the incidence of resistance. This occurs in depression and Alzheimer's disease (all of these issues are reviewed in Sapolsky 1990).

Thus, there appears to be some degree of overt or borderline hypercortisolism in aging primates. Our data support this view. Among these wild primates, basal cortisol concentrations rose with age. Significantly, the increase was not progressive over the lifetime, but emerged abruptly only in the most aged quartile; this agrees with the human literature just cited in which basal hypercortisolism emerges nonlinearly with only extreme senescence. In human syndromes of hypercortisolism, particularly in depression, the basal hypersecretion is most pronounced during the circadian trough (i.e., the evening) (Sacher et al 1980). Because of the experimental constraints of the present study, evening basal cortisol concentrations could not be obtained.

In addition, aged baboons of both sexes were relatively dexamethasone resistant. An absolute cutoff value of 5 μg cortisol per 100 ml (Carroll et al 1981) was not appropriate to use as the criterion for dexamethasone responsiveness because of the brief follow time post-dexamethasone. Nevertheless, cortisol concentration 6 hr after 5.0 mg dexamethasone rose progressively with age. As discussed above, the dexamethasone resistance of

aged humans is revealed far more readily with a threshold dexamethasone dose (i.e., 0.5 mg instead of 1.0 mg). Given that baboons weigh far less than do humans, the 5.0 mg used in this study represents considerably more than a threshold dose, and an even more dramatic dexamethasone resistance might be demonstrable in aged baboons with a lower dexamethasone dose. (Because of the methodological constraints of the present study, it was not possible to explore some of the subtleties of the feedback resistance in aged animals—e.g., do they also show "early escape" from the suppression? Finally, as another departure from the more traditional use of the DST in clinical settings in humans, in the present case, dexamethasone was administered 1 hr following the darting stressor, and thus represents feedback inhibition of secretion during a rather poorly defined stressor.)

A number of possible artifactual causes of this DST result can be eliminated, while a few potential ones must be noted. Obviously, this aged population did not have the confound of medication common to many aged humans. Moreover, the aged baboons were probably in relatively better health than aged humans in Western societies, making it less likely that the DST results were an artifact of some coincident disease. This is because aged wild baboons in less than robust health will not survive the exigencies of predators and the physical demands of foraging (i.e., the survivorship curve among wild baboons is less rectangular than in Western societies). Among animals 2000 days of age or older, greater age was not associated with greater body weight. Therefore, it could not be the case that the oldest animals simply weighed the most and thus had the dexamethasone most diluted by increased blood volume. Differences in the pharmacokinetics of dexamethasone explain some variability in DST results in humans (Lowry and Meltzer 1987). Since dexamethasone concentrations were not measured in these animals, the importance of that variable in the present case is not known. The early pregnancy status of a few of the females might have added to the variability in cortisol determinations, given the effects of pregnancy upon CBG (Freinkel 1985); however, this was only a handful of animals. Finally, our neccesary anesthetization of these subjects introduces a number of possible confounds limiting comparison to the traditional DST in humans. Telazol is a relatively new drug whose endocrine effects are not yet characterized. Furthermore, the amount of drug administered was likely to differ by animal (reflecting accuracy of body weight estimates and varying efficacy of delivery of the drug with the blowgun system); in addition, the effects of age upon anesthetic pharmacokinetics is not known. Because of the rapidity with which the initial, basal sample was obtained, interpretation of those data are likely to be less confounded by the anesthetic issues than are the DST data.

While both basal hypercortisolism and relative dexamethasone resistance emerged with age, the two could dissociate, in that there was only a near-significant trend towards the two traits correlating. This dissociation occurred among the middle-aged baboons, given the nonlinearity of the basal cortisol data (Figure 1) and the linearity of the dexamethasone data (Figure 2). In the extremely aged quartile, however, individuals were typically hypersecretory by both criteria (because of the small sample size [$n = 4$], this correlation did not reach significance [$p < 0.13$], but had r value of 0.86). In human instances of hypercortisolism (particularly in depression), there can also be dissociations among various manifestations of hypersecretion (APA Taskforce 1987), and any models explaining the generic trait of hypercortisolism must account for this heterogeneity of dysfunction (Sapolsky and Plotsky 1990).

In conclusion, adrenocortical hyperactivity appears to occur in this (admittedly small) population of senescent wild primates. The hippocampus is among the sites in the brain

that inhibit glucocorticoid secretion and mediate glucocorticoid feedback regulation (Jacobson and Sapolsky 1991); we have shown that the adrenocortical hypersecretion of aged rats is substantially due to senescent hippocampal degeneration (Sapolsky et al 1986). The aged primate hippocampus undergoes neuronal degeneration to the same magnitude as in the rodent (Coleman and Flood 1987), and the primate hippocampus, much like the rodent hippocampus, can inhibit glucocorticoid secretion (Sapolsky et al 1991). Whether such hippocampal degeneration contributes to the hypercortisolism of aged primates, human or otherwise, remains to be tested.

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