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Regulation of the hypothalamic-pituitary-adrenal axis in free-living pigeons

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Abstract We studied feral free-living pigeons (*Columba livia*) to determine whether either unstressed or stress-induced corticosterone release was altered during a prebasic molt. The pigeons were at various stages of molt throughout the study, but corticosterone responses in molting and nonmolting birds did not differ. This was further reflected in equivalent adrenal responses to exogenous adrenocorticotropic hormone (ACTH), suggesting equivalent steroidogenic capacity of adrenal tissues during both physiological states. There was a slight change, however, in pituitary regulation during molt. Whereas exogenous arginine vasotocin (AVT) elevated corticosterone levels in nonmolting birds, during molt an equivalent dose of AVT was ineffective, suggesting that the pituitary is less sensitive to an AVT signal during molt. AVT also appears to be more effective than corticotropin-releasing factor at eliciting ACTH release in pigeons. Overall, these data indicate that pigeons regulate their corticosterone release differently during molt than other avian species studied to date.

Key words Stress · Corticosterone · Adrenocorticotropin · Molt · Birds

Abbreviations *ACTH* adrenocorticotropic hormone · *AVT* arginine vasotocin · *CRF* corticotropin-releasing factor · *HPA* hypothalamic-pituitary-adrenal · *MT* mesotocin

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Introduction

The adrenocortical response to stress has been used extensively as a way to evaluate the ability of wild animals to successfully cope with adverse environmental conditions. The focus has been primarily on an individual's ability to secrete glucocorticoids (e.g., Wingfield et al. 1997), one of the hallmarks of the vertebrate stress response. An individual's ability to mount a stress response has been correlated with a variety of life-threatening events. Deforestation (e.g., Wasser et al. 1997), pollution (e.g., Hopkins et al. 1997; Norris et al. 1997), and adverse weather conditions (e.g., Wingfield et al. 1983; Smith et al. 1994; Romero et al. 2000) have all been associated with increased circulating glucocorticoid levels.

It is becoming increasingly clear, however, that plasma glucocorticoid levels vary according to an annual cycle (e.g., Dawson and Howe 1983; Wingfield 1994; Wada et al. 1999). The time when plasma samples are taken during the annual cycle can make an enormous difference in both the unstressed and stress-induced glucocorticoid levels that are measured. A recent set of experiments on several avian species (Romero et al. 1998a, 1998b, 1998c; Romero and Wingfield 1998) has begun to explore the physiological mechanisms regulating these seasonal changes by focusing on potential regulatory mechanisms at different levels of the hypothalamic-pituitary-adrenal (HPA) axis that could lead to changes in corticosterone release (the primary avian glucocorticoid, Holmes and Phillips 1976). In each species, corticosterone release showed the most dramatic change during the prebasic molt. Interestingly, this change was regulated at different levels of the HPA axis in each species.

All of the previous mechanistic studies, however, have been done on arctic-breeding passerines: Lapland longspurs (*Calcarius lapponicus*, Romero et al. 1998b), redpolls (*Carduelis flammea*, Romero et al. 1998c), snow buntings (*Plectrophenax nivalis*, Romero et al. 1998a), and white-crowned sparrows (*Zonotrichia leucophrys gambelii*, Romero and Wingfield 1998). These species undergo a rapid prebasic molt at the end of breeding in order to allow migration before the onset of the Arctic winter. In the present study we expand the work to pigeons (*Columba livia*). Pigeons differ from the species in the earlier studies in several respects that makes them attractive for this study: they are columbids instead of passerines, thereby providing taxonomic diversity; they are a temperate latitude rather than arctic breeder; and, most importantly, they maintain a lengthy molt throughout part of the year rather than the more-rapid molt of the earlier-studied species (Johnston 1992; Johnston and Janiga 1995). In this study we compare both the baseline corticosterone levels and the corticosterone levels in response to the stress of capture and handling in feral free-living pigeons that were or were not undergoing a prebasic molt. We also explore whether regulation of the HPA axis differs in these two physiological states.

Materials and methods

Subjects

Feral pigeons (*C. livia*) were captured on the University of Washington campus in May 1994, January through March 1995, May 1996, and July 1996. Birds were accustomed to feeding in the seed refuse surrounding the aviaries of the University of Washington Department of Zoology. Fresh seed was used to bait walk-in traps. All birds were captured in the late morning (between 0900 hours and 1200 hours) when routine aviary cleaning was normally finished and refuse seed was usually available. This controlled for potential diurnal changes in corticosterone concentrations. Approximately 50 pigeons routinely fed at this site.

Stress protocol

Upon capture, birds were subjected to the stressors of capture and restraint. The stress protocol was a modified version of a protocol by Wingfield et al. (1992) and described in detail previously (Romero et al. 1998b). Briefly, within 3 min of capture a blood sample was taken by puncturing the alar vein and collecting approximately 360 μl blood in heparinized hematocrit tubes. These samples were considered to reflect unstressed baseline corticosterone levels since corticosterone levels generally do not start to increase until 3 min after initiation of a stressful stimulus (Wingfield et al. 1982). Cotton stanched blood flow. Birds were then placed in opaque cloth bags for a 30 min restraint period, after which a second blood sample was collected.

Immediately after collection of the first blood sample, birds were injected with various releasing hormones dissolved in lactated Ringer's solution (Baxter, Deerfield, Ill.). Injections were given into the alar vein on the contralateral wing to the one used for taking the blood samples. Animals received either 100 IU/kg body weight porcine adrenocorticotropic hormone (ACTH; Sigma, St. Louis, Mo.), 3 μg/kg ovine corticotropin-releasing factor (CRF; Sigma), 3 μg/kg arginine vasotocin (AVT; Bachem, Torrance, Calif.), 3 μg/kg mesotocin (MT; Bachem), or a combination of 3 μg/kg each of CRF and AVT. All injections were in 10 μl lactated Ringer's solution, which also served as the vehicle control. Sample sizes for each group are included in the figures.

The CRF, AVT, and MT doses were chosen to mimic the effective in vitro doses from isolated duck (Castro et al. 1986) and chicken (Carsia et al. 1986) pituitaries. These doses have been effective at elevating corticosterone (presumably via ACTH release) in four passerine species (Romero et al. 1998a, 1998b, 1998c; Romero and Wingfield 1998) as well as in pigeons (Westerhof et al. 1992). The ACTH dose was the lowest maximally effective dose in white-crowned sparrows (Romero and Wingfield 1999), and was found to effectively stimulate corticosterone release in avian taxa as diverse as passerines (e.g., Romero et al. 1998b) and strigiformes (owls, e.g., Wasser et al. 1997).

After collection of the second blood sample, birds were scored for molt. Feral pigeons are known to initiate molt throughout the year (Johnston 1992), although it is often slowed or suspended during the breeding season (Johnston and Janiga 1995). Furthermore, completion of the molt can take as long as 8 months (summarized by Johnston and Janiga 1995). Birds in this study were examined for possible molting of both primaries and secondaries, as well as body feathers. Based upon this examination, birds were assigned to either a molting or nonmolting group and were therefore caught at equivalent times of year. Pigeons cannot be sexed by plumage (Johnson 1992), so birds represent a mixture of males and females.

Sample processing and assays

Hematocrit tubes were stored on ice for up to 12 h, sealed on one end with clay, and centrifuged at approximately 400 *g* for 5 min. Plasma was removed, stored at −20 °C, and transported to Tufts University for measurement of corticosterone levels. Corticosterone was extracted from plasma with dichloromethane and assayed by radioimmunoassay as described by Wingfield et al. (1992). Inter- and intra-assay variations were 15% and 8%, respectively, as determined by running a pooled standard in duplicate in each assay.

Statistical analyses

Differences between corticosterone levels after different hormone injections were compared by ANOVA followed by Fisher's protected least-significant difference (PLSD) post hoc tests. Differences between molting and nonmolting baseline and stress-induced corticosterone levels were compared with unpaired *t*-tests, and increases in corticosterone levels in response to stress during molt and nonmolt were compared using Mann-Whitney *U* nonparametric tests. It was not possible to collect baseline samples from every bird, so we could not perform a repeated-measures ANOVA to compare molting and nonmolting stress responses. Although birds were captured during several sampling periods over 3 years, there was insufficient power to test for year or time-of-year effects (we caught too few animals during some sampling periods).

Results

Pigeons do not appear to alter their stress response depending upon whether they are or are not molting (Fig. 1). The baseline corticosterone levels are equivalent in both physiological states ($t=0.11$, $P=0.91$) and the increased levels after 30 min of stress are not significantly different ($t=0.968$, $P=0.34$). There is, however, a significant increase in response to stress during both nonmolt ($Z=-2.991$, $P<0.003$) and molt ($Z=-2.274$, $P<0.025$).

Both the adrenal gland and the pituitary maintain sensitivity to their respective releasing signals when pigeons are not molting (Fig. 2). Exogenous ACTH-stimulated corticosterone release above concentrations resulting from capture and handling ($F=6.217$, $df=5$, $P<0.0001$). AVT was the only pituitary stimulant that resulted in increased corticosterone concentrations ($P<0.004$); injecting CRF ($P=0.76$), MT ($P=0.68$), and a combination of CRF and AVT ($P=0.34$) was ineffective. The patterns of adrenal and pituitary sensitivities were similar when pigeons were molting (Fig. 3).

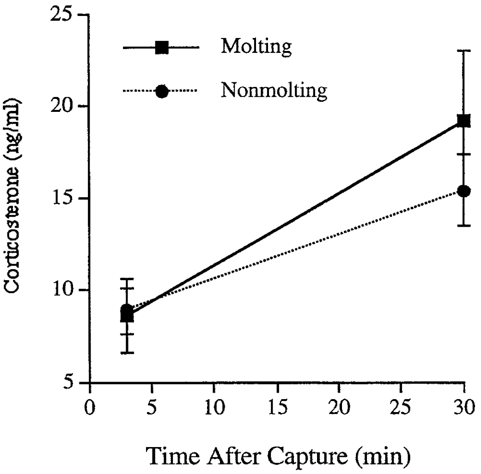

Fig. 1 Increase in corticosterone in response to capture and restraint in molting and nonmolting feral pigeons. Samples taken before 3 min are grouped together for statistical purposes. Each *point* represents the mean ± SEM of $n = 12$ and $n = 31$ for molting and nonmolting baseline samples, respectively, and $n = 9$ and $n = 23$ for molting and nonmolting samples, taken at 30 min, respectively

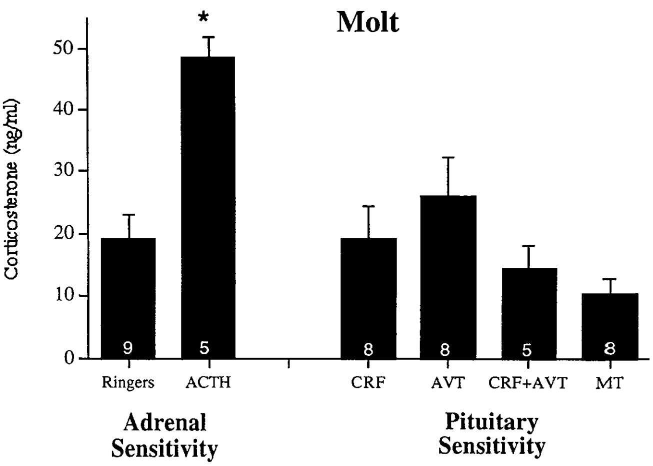

Fig. 3 Adrenal and pituitary sensitivities to their respective releasing factors in molting feral pigeons. Each *bar* represents the mean ± SEM for the sample sizes indicated in each *bar*. The *asterisk* indicates a significant difference ($P < 0.0002$) compared to the Ringer's injected controls. Note that all samples were taken 30 min after capture and restraint

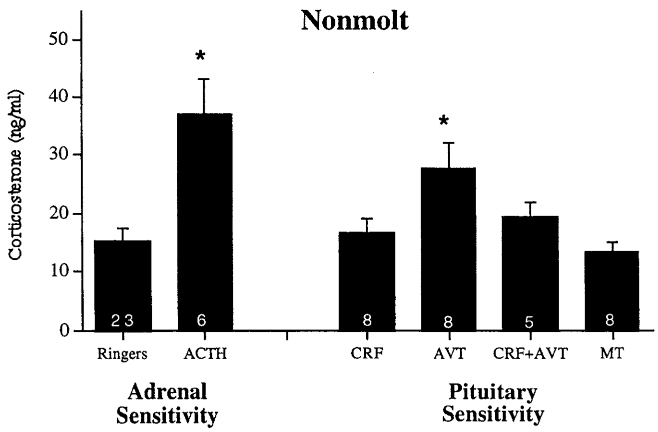

Fig. 2 Adrenal and pituitary sensitivities to their respective releasing factors in nonmolting feral pigeons. Each *bar* represents the mean ± SEM for the sample sizes indicated in each *bar*. (*ACTH* adrenocorticotropic hormone, *AVT* arginine vasotocin, *CRF* corticotropin-releasing factor, *MT* mesotocin). The *asterisk* indicates significant differences compared to the Ringer's injected controls ($P < 0.0001$ for ACTH and $P < 0.004$ for AVT). Note that all samples were taken 30 min after capture and restraint

Exogenous ACTH continued to stimulate corticosterone release ($F = 6.213$, $df = 5$, $P < 0.0002$), but none of the ACTH-releasing factors was effective ($P = 0.99$ for CRF, $P = 0.28$ for AVT, $P = 0.45$ for CRF and AVT combined, and $P = 0.22$ for MT).

Discussion

Although several different avian species have dramatically lower corticosterone levels (both baseline and stress-induced) during their prebasic molt (Astheimer et al. 1994; Romero et al. 1998a, 1998b, 1998c), this does not appear to be the case for pigeons. There is a difference, however, in the progression of the prebasic molt in these different species. In all the species studied so far [Lapland longspurs (Cramp and Perrins 1994), snow buntings (Cramp and Perrins 1994; Lyon and Montgomerie 1995), redpolls (Baldwin 1968; Cramp and Perrins 1994), and white-crowned sparrows (Chilton et al. 1995)], molt progresses quickly and is completed in early fall at the end of the breeding season. Pigeons, in contrast, have been known to molt throughout the year (Johnston 1992) and the molt can typically last 5–6 months and for as long as 8 months (Johnston and Janiga 1995). Although it is currently unknown why corticosterone levels are lower during the prebasic molt in the passerines, these data suggest that either the physiology of molt is different in columbids than in passerines, or that the relationship between corticosterone and molt is different in prolonged molts as compared to rapid molts. Further experiments with other columbids would be required to distinguish between these possibilities.

Perhaps not surprising given the equivalent stress responses when molting and nonmolting, the regulation of the HPA axis does not appear to dramatically change in the two physiological states (Figs. 2 and 3). In both groups exogenous ACTH more than doubles circulating corticosterone levels. It should be remembered, however, that this response is in addition to the natural stress-induced release of corticosterone in response to capture, handling, and restraint. All birds in this study were stressed, but the effectiveness of exogenous ACTH indicates that there is an untapped capacity to release corticosterone that is not normally utilized in response to stress. Presumably, a larger endogenous ACTH signal would stimulate more corticosterone release, so that the relatively low levels in the controls indicate that the birds are regulating their corticosterone output by regulating ACTH release in response to stress.

At least in nonmolting pigeons, it appears that endogenous ACTH release is regulated by AVT. AVT is present in the avian median eminence (Mikami and Yamada 1984; Mikami 1986) and is known to stimulate ACTH release directly from isolated pituitaries (Castro et al. 1986) and appears to stimulate ACTH release in vivo as well (e.g., Westerhof et al. 1992; Romero et al. 1998b). Interestingly, these data fit with a growing number of studies that suggest that AVT is more potent at stimulating avian ACTH release than CRF (Castro et al. 1986; Romero et al. 1998a, 1998b), although CRF is undoubtedly important (Carsia et al. 1986; Carsia 1990; Romero et al. 1998c). Again, it is important to remember that CRF may be driving the natural ACTH response to stress, but that the corticotrophs' response to CRF is saturated by capture and handling. It is thus not clear from these data whether AVT is more potent than CRF, or whether only the response to AVT is not saturated during the normal stress response.

It is unclear, however, why AVT in combination with CRF was not successful in elevating corticosterone. Furthermore, it is unclear why AVT was not successful in elevating corticosterone during molt. The lack of response during molt does suggest that the corticotrophs are less sensitive to AVT during molt, although this level of sensitivity is unlikely to be important in regulating the normal ACTH response to stressors such as capture and handling since the normal responses did not differ with physiological state.

The lack of changes in HPA axis regulation during molt in pigeons stands in stark contrast to earlier studies. In the three species studied to date (Romero et al. 1998a, 1998b, 1998c), the sensitivity of the adrenal to an exogenous ACTH signal was greatly reduced during molt. Furthermore, the responsiveness of the pituitary to CRF, AVT, and MT was also altered during molt. None of these changes are evident in molting pigeons, although this is perhaps not surprising given the identical normal corticosterone responses during both physiological states. Regardless, this is further evidence that pigeons regulate their HPA axes during molt differently than the three previously studied passerine species.

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