Basal and stress-induced corticosterone levels of garden warblers, *Sylvia borin*, during migration

Hubert Schwab1*, Franz Bairlein2, and Eberhard Gwinner1

1 Max-Planck-Institut für Verhaltensphysiologie, Vogelwarte, W-8138 Andechs, and
2 Zoologisches Institut, Lehrstuhl Physiologische Ökologie, Universität Köln, W-5000 Köln 41, FRG

Accepted May 3, 1991

Summary. Plasma levels of the metabolically and behaviorally active corticosteroid hormone, corticosterone, were studied in garden warblers in the laboratory and in the field during the autumnal migratory phase. Garden warblers showing nocturnal migratory activity in the laboratory had elevated levels of corticosterone at the end of the dark phase and low levels during daytime. When nocturnal migratory activity was experimentally disrupted by food deprivation and subsequent refeeding or after spontaneous termination of migratory activity this rhythm was absent. Garden warblers stopping over in the Sahara desert during autumnal migration had low levels of corticosterone. Levels were negatively correlated with fat stores and body mass in birds sampled throughout the day. These levels were generally lower than those associated with stress in response to repeated handling and blood sampling. The results suggest (1) the existence of diel changes in adrenocortical hormonal activity that could be involved in regulation of migration, and (2) that garden warblers carrying large fat depots are not stressed by prolonged flight or lack of appropriate feeding areas during migration over the desert.

Key words: Adrenal – Fat – Flight – Nocturnal – Starvation

Introduction

Long-distance migratory birds have evolved behavioral and physiological adaptations to prepare for, and regulate physiology and behavior during, migration. During the preparatory phase increased appetite and food consumption [termed hyperphagia (King 1961; Moore and Simm 1985)], and altered diet selection and increased utilization of nutrients (Bairlein 1990), result in lipogenesis and deposition of large fat reserves as primary fuel for flight over inhospitable environments. Many birds, including garden warblers, migrate at night. With the onset of the migratory phase nocturnal migrants develop nocturnal restlessness in captivity (Gwinner 1986). During migration fat is used as fuel for flight and metabolism changes from lipogenesis to lipolysis (Blem 1976; Ramenofsky 1990). Exhausted fat depots may be replenished during stop-overs causing delays of several days dependent on body condition and food availability (Bairlein 1985; Biebach et al. 1986; Cherry 1982; Moore and Kerlinger 1987; Laveé and Safriel 1974; Safriel and Laveé 1988). Supplementary regulation of migratory behavior (Wingfield et al. 1990) by body condition and food availability can be demonstrated in the laboratory. Food restriction of migratory active birds results in depletion of fat depots and enhanced migratory restlessness. Subsequent refeeding results in fat deposition and suspended migratory restlessness for several days (Biebach 1985; Gwinner et al. 1985; Gwinner et al. 1988; Ramenofsky et al., in prep.).

The hypothalamus-pituitary-adrenal axis is intimately involved in animal homeostasis and responses to environmental challenge. Corticosteroid hormone levels change in response to environmental conditions and participate in regulatory mechanisms in a variety of ways, ranging from metabolic to behavioral effects (Axelrod and Reisine 1982). Corticosterone, the major avian corticosteroid, has been implicated in regulation of bird migration at various levels of control. Its diel pattern of secretion in relation to the secretion of other hormones, particularly prolactin, may induce seasonal migratory states (Meier and Farner 1965). Histochemical studies suggest that synthesis of corticosterone by the adrenal gland is high prior to migration (John 1965; Naik and George 1963). In vitro secretion by the adrenals is higher during migratory phases (Péczely 1976). Exogenous corticosterone induces foraging (Wingfield et al. 1990) and fattening in various species (Wingfield and Silverin 1986; Gray et al. 1990). During stressful episodes such as starvation, dehydration, and exercise corticosterone plasma levels increase (Harvey et al. 1984).
In a previous study on the regulation of lipogenesis and lipolysis of garden warblers during autumnal migration, corticosterone levels did not increase in response to several days of food withdrawal (M. Ramenofsky, H. Schwabl and E. Gwinner, unpublished results). This suggests that fasting does not represent a stressor in migratory birds carrying large fat stores, or that the acute adrenal response of corticosterone secretion to stressors is reduced during the migratory phase. Seasonal and inter-individual modulations of corticosterone secretion in response to stressors have been previously reported, although their functional significance is unclear (Wingfield et al. 1982; Schwabl et al. 1985; Schwabl et al. 1988).

This paper investigates 1) the diel pattern of corticosterone levels during the autumnal migratory phase in garden warblers with spontaneous, experimentally interrupted, and spontaneously terminated migratory restlessness; 2) levels of corticosterone of garden warblers trapped during autumn migration at stopping-over sites in the Sahara desert; and 3) corticosterone secretion in response to capture and handling.

Material and methods

Laboratory studies. First-year garden warblers were captured with mist-nets in Baden-Württemberg, southern Germany, in July/August and transported to Andechs. They were held in individual activity cages on a constant 12L:12D photoperiod and fed a diet described previously (Gwinner et al. 1988).

During the autumnal migratory phase (characterized by increased body mass, fat deposits, and nocturnal migratory restlessness), birds were food-deprived until body mass was 18 g and then refed ad libitum. This protocol interrupted nocturnal migratory activity for several days. After birds had regained body mass migratory activity was resumed as described earlier (Gwinner et al. 1985, 1988).

During spontaneous and experimentally interrupted migratory activity blood samples were taken every 4 h for 24 h from different birds. Samples were obtained within 1–3 min after entering the housing box. In April, after birds had terminated migratory restlessness and were in prenuptial molt, additional samples were taken at key time points prior to and 4 h after lights on (09:00 hours) and prior to lights off (21:00 hours).

The response to handling stress was investigated in late August (premigratory phase), late September (migratory phase), and May (postmigratory phase) by sampling individual birds at intervals of 10 min up to 1 h. Between sampling birds were placed in a cloth bag. These samples were taken during mid-day.

Field studies. Blood samples prior to migration were taken in August in Baden-Württemberg, southwest Germany. Birds were captured with mist-nets and bled immediately after capture up to 1 h at 10-min intervals. Garden warblers crossing the Sahara desert during migration were collected in September and October at two sites in the Algerian desert: Ain Hassi Hadjar, located 100 km southwest of Quarqla in the northern Algerian Sahara, about 600 km south of the Mediterranean coast. This place contains small areas of vegetation surrounded by stony and sandy desert. Two birds were sampled at Gueltul Aflalé in the central Ahaggar mountains, 1000 km south of Ain Hassi Hadjar. Garden warblers land in these areas during late night/early morning and rest for the day. Most of them stay only 1 day. Due to the specific placement of mist-nets and trapping activity it is assumed that the birds sampled had arrived that same morning after a night flight (Bairlein, in prep.). Most samples were collected in the morning around sunrise, although few samples were obtained later during the day and before sunset (Fig. 2).

Hormone assays. Blood samples taken in the field were centrifuged and the plasma obtained (10–40 µl) was measured and stored in a freezer after addition of 100 µl EtOH. Plasma from captive birds was frozen without EtOH. Corticosterone was extracted with 6 ml petroleum benz:diethylether (1:1) on extrelut columns. This method removes most of the plasma lipids that might interfere with the assay (Hall et al. 1988). The dried extract was resuspended in 500 µl phosphate-buffered saline and assayed in duplicates of 200 µl by RIA. Tritiated corticosterone (2000 cpm) was added to each sample as internal standard prior to extraction for estimation of extraction efficiency and recovery. Recoveries were ≥80%. The applied antiserum (DDV Diagnostika) crossreacts with deoxycorticosterone to 6.1% but below 1% with other steroids. Intra- and interassay variations were within a range of 5–14%, and the least detectable amount was 8 pg per tube.

Data were analyzed by one-way ANOVA and ANOVA after appropriate transformation to obtain equal variances and normal distribution.

Results

Basal levels of corticosterone

During spontaneous autumnal migratory restlessness corticosterone levels varied significantly with time of day (ANOVA, $F = 8.75$, $P < 0.001$) in birds sampled within 1–2 min. Levels were highest at the end of the night just prior to lights on (09:00 hours), and lowest at 13:00 hours, 4 h after lights on (Fig. 1). When migratory restlessness was interrupted during refeeding a diel pattern of corticosterone was absent ($F = 0.55$, $P = 0.74$), and levels were not elevated at night (Fig. 1). In April, after birds had spontaneously terminated migratory restlessness, corticosterone levels were also low at the end of the night (Fig. 1). Free-living warblers resting in the desert during fall migration had rather low levels of corticosterone comparable to those measured in the laboratory (Fig. 2).

Response to handling stress

Corticosterone levels of captive birds increased about 2 min after capture (Fig. 3). There was no statistically significant difference in this initial increase between the three phases (pre-migratory, migratory, post-migratory). During sampling up to 60 min corticosterone levels varied significantly with time (all phases combined, $F = 8.93$, $P < 0.01$). Initial levels were significantly lower than all subsequent values. From 30 to 60 min levels increased again (Fig. 4). During the migratory phase levels increased more rapidly within the first 10 min than during the pre-migratory or post-migratory phases ($F = 6.43$, $P = 0.007$; Student-Newman-Keuls test, $P < 0.05$; Fig. 4).

Free-living birds captured during August in southwest Germany and during September/October at stopping-over sites in the Sahara desert reacted rapidly to handling, although the responses were variable (Fig. 5).

Body mass and fat

Body mass of captive birds was significantly higher during the migratory phase than during the pre-migrato-