An Alternative Method for Delivering Adrenocorticotropin Hormone in Birds

C. Morgan Wilson¹ and Rebecca L. Holberton²

Department of Biology, University of Mississippi, University, Mississippi 38677

Accepted March 5, 2001

In birds, intrajugular injection (ij) of exogenous adrenocorticotropic hormone (ACTH) has been used to challenge adrenocortical tissue when investigating various dynamics of the hypothalamic-pituitary-adrenal (HPA) axis. Although this method has been shown to deliver ACTH efficiently, ij injection can be difficult and potentially damaging to the bird, especially to young birds and small species. Intramuscular (im) injection has been shown to be an alternative method for delivering ACTH to large birds; however, small songbirds have relatively less muscle mass in which to absorb im injections and, in very small birds, even small needles may injure the delicate muscle tissue. Therefore, intraperitoneal (ip) administration of ACTH was investigated as a means of avoiding the potential problems associated with ij and im injections when conducting adrenocortical pathway studies on small birds. Dark-eyed Juncos, Junco hyemalis, were first treated with dexamethasone to suppress the endogenous signal cascade for corticosterone release. Twelve hours later, birds were sampled for baseline corticosterone concentration and then immediately given an ip injection of either ACTH or physiological saline. A second blood sample was taken from all birds 30 min later to assess the effectiveness of ip ACTH administration on adrenocortical tissue. While saline-treated birds showed no significant increase in endogenous plasma corticosterone in response to the capture and handling protocol, ACTH-treated birds showed a significant increase in plasma corticosterone concentration, illustrating the effectiveness of the ip administration of exogenous ACTH. The adrenocortical response of ACTH-treated birds was similar in magnitude and rate of increase to that found in free-living, noninjected Juncos. Intraperitoneal injection of ACTH is thus a valid alternative to ij and im injections when challenging adrenocortical tissue in small birds.

Key Words: intraperitoneal injection; adrenocorticotropic hormone; adrenocortical response; stress; Junco hyemalis.

A rapidly growing area of research is the study of behavioral and endocrine responses to environmental stressors in vertebrates, particularly birds (cf., Cherel et al., 1988; Wingfield, 1994; Wingfield et al., 1992, 1995; Holberton et al., 1996; Silverin, 1998; Fowler, 1999). An increasing number of studies have focussed on how the avian hypothalamic-pituitary-adrenal (HPA) axis participates in the processes of energy regulation and the maintenance of homeostasis, particularly through its influence on glucocorticoid secretion (Astheimer et al., 1994; Romero et al., 1998a, b; Sims and Holberton, 2000).

The production and release of corticosterone, the primary avian adrenocorticosteroid, is under the control of the HPA axis (Holmes and Phillips, 1976; Harvey et al., 1984). In birds, in response to the secretion of corticotropin-releasing factor and/or other secretagogues from the hypothalamus (such as arginine va-

1 To whom correspondence should be addressed. E-mail: cmwilson@olemiss.edu.

2 Current address: Department of Biological Sciences, 5751 Murray Hall, University of Maine, Orono, ME 04469-5751. E-mail: rebecca.holberton@maine.edu.
sotocin and mesotocin; Castro et al., 1986; Romero et al., 1998b), adrenocorticotropin hormone (ACTH) is released from the anterior pituitary, stimulating the secretion of corticosterone from adrenocortical tissue. The level of control within the HPA axis at which variation in the strength of the corticosterone response is regulated has been studied in several bird species (Astheimer et al., 1994; Romero et al., 1998a, b; Sims and Holberton, 2000). These studies have found that while some individuals often show a reduced adrenocortical response, they retain the ability to respond robustly to ACTH.

In previous studies of challenging adrenocortical tissue in birds, exogenous ACTH was delivered by intrajugular injection (ij). Although this method has been shown to deliver ACTH efficiently in a variety of birds (Astheimer et al., 1994; Romero et al., 1998a, b; Sims and Holberton, 2000; and see Spelman et al., 1995), this procedure can be difficult and potentially damaging, especially to small species or young birds of larger species. Spelman et al. (1995) addressed adrenocortical responsiveness in the American Black Duck (Anas rubripes) and opted for intramuscular (im) injections of either porcine ACTH or cosyntropin (a synthetic form of the hormone), rather than ij injections. While the im injection of cosyntropin resulted in a significant increase in plasma corticosterone concentration compared with that in control birds injected with physiological saline, the im injection of porcine ACTH produced variable results. There was no effect with low and medium doses of ACTH (2 and 5 IU/kg, respectively), while some of the ducks (but not all) receiving the high dose of ACTH (10 IU/kg) showed elevated plasma concentrations of corticosterone. Therefore, while the rate and delivery efficiency of the exogenous hormone to its target tissue as a result of the im injection method used by Spelman et al. (1995) is unknown, the results of their study indicate that ACTH (or a synthetic ACTH substance) does not have to be injected intravascularly to have an effect on corticosterone release from adrenocortical tissue.

The im injection method may not be the most appropriate method for delivering ACTH to small birds, however, because they have relatively less muscle mass in which to absorb im injections. Further, in the case of very small birds, even small needles may injure the delicate muscle tissue. Because many avian studies use young birds or small species of songbirds, a way in which ACTH delivery might be accomplished without direct injection into the jugular or muscle was investigated. In this study, the potential for intraperitoneal (ip) administration of ACTH was investigated in Dark-eyed Juncos, Junco hyemalis, as a means of avoiding the potential drawbacks associated with ij and im protocols.

METHODS

Dark-eyed Juncos overwintering in northern Mississippi in February and early March, 2000, were captured using mist nets and potter traps. The birds were brought into the laboratory and housed in individual cages (40 cm × 36 cm × 44 cm). All birds were given water and food (a 50:50 mixture of millet and commercial chick starter) ad libitum and held under a natural photoperiod. The period of injections took place while birds were exposed to a 13.5 light:10.5 dark photoperiod.

Following a 3- to 4-week acclimation period, ten Juncos were first treated with dexamethasone (DXM; No. D-1756, Sigma Chemical Co., St. Louis, MO) to suppress the endogenous activity of the HPA axis. The DXM solution (0.18 μg DXM/μl vehicle) was prepared by dissolving 0.018 g DXM in 5 ml of 95% EtOH, followed by the addition of 95 ml of physiological saline (0.9% NaCl). This dose of DXM (based on Westerhof et al., 1994, and Joseph and Ramachandran, 1992) effectively suppresses the release of endogenous ACTH, and thus endogenous corticosterone, in Dark-eyed Juncos for up to 48 h following injection (Holberton et al., manuscript in preparation). One hour prior to lights being turned off [18:00 Central Standard Time (CST)], each bird was given a single ip injection of 500 μl of DXM solution using a 1-cc tuberculin syringe and a 26-gauge needle held at a 45° angle from the abdomen, 3–4 mm inferior of the sternal keel. For all injections, care was taken to insure that the solution was delivered into the body cavity (just under the peritoneal wall) without puncturing abdominal organs or air sacs.

Fourteen hours following the DXM injection (08:00 CST on the day after injections), each bird was captured and, within 2–3 min of entering the bird room, a
small blood sample (approximately 80 μl) was collected from the brachial vein into a heparinized capillary tube following venipuncture with a 26-gauge needle. Within 1–2 min of sampling for baseline corticosterone, five birds were chosen at random and given an ip injection of 0.72 μg of porcine ACTH/100 μl physiological saline. This dose of ACTH (No. A-6303, Sigma) is 10 times greater, per gram body mass, than that used in experiments in which ij injections were used (Romero et al., 1998a, b; Sims and Holberton, 2000). The five remaining birds were given a 100-μl ip injection of physiological saline as a control treatment. All birds were then placed in individual cloth bags and held for 30 min (from the time of initial disturbance as a result of entering the room). A second cloth bags and held for 30 min (from the time of initial treatment. All birds were then placed in individual

To detect differences between the two groups at each sampling time (0 and 30 min), a single-factor ANOVA was used with Fisher’s protected least-squares difference used for post hoc analysis. To detect a change in plasma corticosterone concentration over time within each treatment group, a single-factor repeated measures ANOVA was used. Statistical analyses were performed using StatView version 4.51 (Abacus Concepts, Inc., Berkley, CA).

**RESULTS**

There was a significant main effect of TREATMENT on plasma corticosterone concentration ($F_{1,8} = 26.26, P < 0.001$). Specifically, the two groups differed significantly in their adrenocortical response 30 min after the initial disturbance and injection (TREATMENT × TIME, $F_{1,8} = 25.19, P = 0.001$). While there was no significant difference in the initial (time 0) corticosterone sample between the two groups (Table 1; $F_{1,8} = 0.86, P = 0.38$), the DXM + ACTH-injected birds showed a significantly higher 30-min corticosterone concentration when compared to the 30-min corticosterone concentration of DXM + saline-injected birds (Table 1; $F_{1,8} = 25.40, P = 0.001$). Birds treated with DXM + saline showed no significant increase in corticosterone after the initial disturbance and injection (Table 1; a 24% increase above the initial, time 0, sample to the time 30 min sample; $F_{1,4} = 1.46, P = 0.29$). However, DXM + ACTH birds showed a significant increase (2536%) in plasma corticosterone concentration 30 min after the initial disturbance and injection (Table 1; $F_{1,4} = 24.84, P = 0.008$). While

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Time 0 plasma corticosterone concentration (ng/ml)</th>
<th>Time 30 min plasma corticosterone concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXM + ACTH</td>
<td>5</td>
<td>0.49 ± 0.08</td>
<td>12.97 ± 2.48</td>
</tr>
<tr>
<td>DXM + saline</td>
<td>5</td>
<td>0.35 ± 0.09</td>
<td>0.48 ± 0.05</td>
</tr>
</tbody>
</table>

**Note.** Changes in plasma corticosterone concentration (means ± SE) in response to capture and handling in Dark-eyed Juncos treated with dexamethasone (DXM) and followed 14 h later by ip injection of either ACTH or physiological saline.

---

Copyright © 2001 by Academic Press
All rights of reproduction in any form reserved.
ACTH-injected birds appeared to show moderate vasoconstriction within 30 min following the ip injection, there was no difference in these birds’ postexperiment general behavior and well-being when compared to those of the control-injected birds over the period of time before the birds were released.

**DISCUSSION**

These results indicate that, in birds, exogenous ACTH administration via ip injection is a valid alternative to ij and im injection. It is clear that ACTH delivered into the body cavity can stimulate adrenocortical activity, but the exact route of delivery of this source of ACTH to adrenocortical cells is not known.

The ACTH dose used in this experiment was 10 times that used for earlier experiments (cited above) using ij delivery of ACTH to birds. In this experiment, the effect on adrenocortical tissue through ip ACTH administration was rapid and resulted in an increase in plasma corticosterone concentration similar in both magnitude and rate of increase to that observed in free-living, noninjected Dark-eyed Juncos sampled in the same manner in winter, 1998–1999 (initial corticosterone = 3.6 ng/ml ± 1.0 SE, n = 13; time 30-min corticosterone = 14.1 ng/ml ± 1.6 SE, n = 13; Holberton and Able, 2000). This study illustrates that this route of delivery can be used in further studies on birds but, for most studies, preferably after a dose-response curve has been determined. Additional work is necessary to determine the dose–delivery dynamics of ip ACTH treatment that would need to be adjusted for body size, mass, and other parameters. However, investigators should be encouraged to consider this method as a viable, and in some circumstances, a safer alternative to ij and im injections when challenging adrenocortical tissue in small or young birds. Through the continued development of alternative methodologies, more constraints on experimental design can be overcome while continuing to balance the humane and safe treatment of animals with the ongoing need to increase our understanding of endocrine mechanisms.

**ACKNOWLEDGMENTS**

We thank Karen Voltura and Tony Lee for their assistance with the experiment. We also thank Chris Sims, Ben Cash, and anonymous reviewers for their helpful comments on the manuscript. This work was conducted under the guidelines of the University of Mississippi Institutional Animal Care and Use Committee and with state and USFW Permit Nos. MB819656-1 and 22616 for collecting and holding wild birds in captivity issued to R.L.H. This work was funded by the National Science Foundation (R.L.H., Grant No. 9873852).

**REFERENCES**


