Naloxone Attenuates Serum Corticosterone and Augments Serum Glucose Concentrations in Broilers Stimulated with Adrenocorticotropin

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ABSTRACT

The effects of exogenous naloxone and adrenocorticotropin (ACTH) on circulating concentrations of corticosterone and glucose in broilers were determined. Birds were injected i.m. at 0 and 2 h with either saline or naloxone, then i.v. at 2.5 h with either saline or ACTH. Control birds received saline at each injection. Blood samples were taken before the experiment started (0 min) and 30, 60, and 90 min after the last injection. Intramuscular injections of naloxone significantly reduced subsequent ACTH-stimulated increases in serum corticosterone; however, when followed by saline, naloxone elevated corticosterone by 90 min after the final injection of saline. Glucose levels were significantly elevated at 60 min in birds receiving ACTH i.v., but remained elevated through 90 min in birds pretreated with naloxone. Naloxone pretreatment attenuated serum corticosterone but augmented serum glucose concentrations in ACTH-stimulated broilers.

(Key words: adrenocorticotropin, broiler, corticosterone, glucose, naloxone)

INTRODUCTION

Adrenocorticotropin (ACTH) and β-lipotropins (β-LPH) are two of several peptides produced from a single precursor molecule called proopiomelanocortin (POMC; Nakanishi et al., 1979). Upon entering the blood stream, ACTH can stimulate the production of glucocorticoids and β-LPH can be broken down into a number of smaller peptides, one of which is β-endorphin, an endogenous opioid. Boscaro et al. (1990) reported that under most physiological and pathological conditions, stimuli that alter ACTH secretion also alter β-LPH in a parallel manner. Morley (1981) has reported that endogenous opioids appear to inhibit the secretion of ACTH in humans. Furthermore, exogenous α-endorphins have been found to depress cortisol secretion in sheep under stress conditions (Przekop et al., 1990) and in frogs (Zerani and Gobbetti, 1992). Therefore, if β-endorphin receptors are blocked, then further production of β-endorphin might occur with possible concomitant increases in related molecules such as ACTH, a peptide associated with POMC. Administration of naloxone, a general opiate antagonist, which can likewise block the binding of β-endorphins, has been shown to significantly increase circulating β-endorphin concentrations in humans (Plewe et al., 1987). Researchers have also previously reported that naloxone affects the hypothalamic-pituitary-adrenocortical axis by increasing ACTH secretion (Meites et al., 1979; Pleffer and Herz, 1984), augmenting hypothalamic corticotropin-releasing hormone secretion (Eisenberg, 1984), or possibly by increasing the adrenal’s responsiveness to ACTH (Lymangrover et al., 1981). However, the precise mechanisms of action of opioids and their antagonists on the hypothalamic-pituitary-adrenal axis are still unknown (Zerani and Gobbetti, 1992). It is suggested that increased ACTH may be a result of an increase in POMC synthesis in response to lower perceived concentrations of β-endorphins.

Blood glucose levels would be expected to increase in response to ACTH-stimulated elevations in glucocorticoids (Compton et al., 1991). Increases in blood concentrations of glucocorticoids in response to naloxone have been demonstrated in a number of species, including humans (Guo et al., 1991), cynomolgus monkeys (McCubbin et al., 1993), pigs (Rushen and Ladewig, 1991), the newt, Triturus carnifex (Zerani and Gobbetti, 1992), the frog, Rana esculenta (Zerani and Gobbetti, 1991), the rat (Tanaka et al., 1983), dehydrated sheep (Thornton and Parrott, 1989), and normal sheep (Przekop et al., 1990). Studies of the effects of naloxone in birds have been limited to its control of ingestive
behavior. The attenuation of drinking and feeding behavior in response to naloxone has been noted in chickens (McCormack and Denbow, 1987; Firman and Volmert, 1991). However, the effects of naloxone on circulating corticosterone and glucose concentrations in the chicken are unknown; therefore, the present study was conducted in order to investigate the relationship between exogenous ACTH and naloxone and subsequent circulating concentrations of corticosterone and glucose in chickens.

MATERIALS AND METHODS

Forty broilers (Arbor Acres × Arbor Acres), 2 wk of age, were randomly assigned to one of four treatment groups with five replicates per group and two birds per replicate. Birds were housed in Petersime brooder batteries,3 exposed to continuous lighting, and offered a standard diet that met or exceeded NRC (1995) recommendations. Feed and water were available for ad libitum consumption. Birds were allowed to adapt to cages and to handling for 1 wk prior to testing. Blood samples were obtained from the brachial vein for baseline data (0 min) and at 30, 60, and 90 min after the last treatment injection. Blood (500 µL) was collected into nonheparinized tubes at each designated time and then immediately centrifuged at 1,000 × g for 10 min to express serum. Individual serum samples were pooled within each replicate prior to storage. All pooled serum samples remained fluid prior to freezing, and all five samples per treatment and sampling time were assayed. Serum samples for all treatments were stored at -20 C after centrifugation.

Treatments

Beginning at 1300 h on 1 d, birds were given intramuscular (i.m.) injections at 0 and 2 h and i.v. injections at 2.5 h. At 0, 2, and 2.5 h, one replicate pen from each treatment was selected at random for injection. This procedure was continued five times until birds in all treatment replicates were injected. Control birds received saline at each injection (SAL-SAL). Other treatments consisted of: 1) naloxone i.m. at 0 and 2 h then saline i.v. at 2.5 h (NAL-SAL); 2) saline i.m. at 0 and 2 h then ACTH i.v. at 2.5 h (SAL-ACTH); and 3) naloxone i.m. at 0 and 2 h then ACTH i.v. at 2.5 h (NAL-ACTH). Naloxone and ACTH were administered at 5 mg/kg (Firman and Volmert, 1991; McCormack and Denbow, 1987) and 10 IU/kg of BW, respectively. The dosage of naloxone was chosen based on dosages known to affect drinking and feeding behavior in chickens.

RESULTS AND DISCUSSION

The birds treated with saline at each injection had baseline corticosterone concentrations that were considered to be in the normal physiological range at all time periods (Satterlee et al., 1980) and were not different from the concentrations in all birds prior to treatment at 0 min (Figure 1). Mean corticosterone concentration in all birds at 0 min was 3.41 ng/mL. There were significant (P ≤ 0.0001) main effects due to treatment for periods (Satterlee et al., 1980) and were not different from the concentrations in all birds prior to treatment at 0 min (Figure 1). Mean corticosterone concentration in all birds at 0 min was 3.41 ng/mL. There were significant (P ≤ 0.0001) main effects due to treatment for

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Serum corticosterone was determined by radioimmunoassay according to the procedure of Satterlee et al. (1980). The corticosterone antibody was supplied by Endocrine Sciences.5 Five samples within each treatment and sampling time were assayed in duplicate. All samples were run within one assay to avoid interassay variation. The intra-assay coefficient of variation was 9.2% with the 80 samples assayed in duplicate. The r² value of the standard curve was 0.9863.

Glucose Analysis

The glucose analysis was based on the application of 10 µL of serum on a Kodak Ektachem slide6 specific for glucose. This procedure was performed according to the method of Elliot (1984), as described by Latour et al. (1996).

Statistical Analysis

The experimental units were arranged in a completely randomized design. The data were subjected to a 2 (i.m. injection of either saline or naloxone) × 2 (i.v. injection of either saline or ACTH) factorial arrangement of treatments split in time (0, 30, 60, and 90 min). Tests for differences between the main effects of i.m. injection with either saline or naloxone at 0 and 2 h and i.v. injection with either saline or ACTH at 2.5 h, and between the means from interactions of the main effects and sampling time were performed. Interaction means were partitioned by Student-Newman-Keuls test (Steel and Torrie, 1980). The data were analyzed using the General Linear Models procedure of SAS® (1995). Statements of significance were based on P ≤ 0.05 unless otherwise noted.

1Petersime Incubator Co., Gettysburg, OH 45328.
2Endocrine Sciences, Calabasas, CA 91301.
3Eastman Kodak Co., Rochester, NY 14650.
Naloxone at 0 and 2 h (i.m.) and ACTH at 2.5 h (i.v.). \(n = 5\) samples

estimated variance.


delayed response until 90 min in the NAL-SAL birds might have

been necessary to allow enough time for significant

concentrations by 90 min after a final i.v. injection of

saline. The delay in corticosterone increase may be partly

attributed to a slower absorption of naloxone due to i.m. injection. The effects of naloxone on corticoster-
one in the NAL-ACTH birds were evident by 30 min

after the last injection of ACTH; however, the delayed

response until 90 min in the NAL-SAL birds might have been necessary to allow enough time for significant concentrations of ACTH to be reached after stimulation of its synthesis and secretion. This response would also not have manifested itself until sufficient numbers of \(\beta\)-endorphin receptors were blocked by naloxone in NAL-SAL birds. That is, the sporadic increase in corticosterone at this later time may be viewed as a response to a compensatory elevation in ACTH elicited by the perception of a lowered \(\beta\)-endorphin concentration in association with the blocking of specific \(\beta\)-endorphin receptors by naloxone. An increase in corticosterone or cortisol in response to naloxone has also been observed in studies with other species (Morley, 1981; Thornton and Parrott, 1989; Guo et al., 1991; Rushen and Ladewig, 1991; Zerani and Gobbetti, 1991, 1992; McCubbin et al., 1993). Naloxone injected subcutaneously at 1.5 \(\mu g/g\) BW to adult frogs led to a 1.5-fold increase in corticosterone at 60 min after injection (Zerani and Gobbetti, 1992), whereas a 5.5-fold increase was noted at 2 h after an i.m. injection of naloxone in the present study. Sheep that have not experienced dehydration are an exception to this, in that they do not exhibit an increase in cortisol after naloxone treatment (Przekop et al., 1990).

Birds treated with saline followed by ACTH exhibited elevated corticosterone concentrations compared to control animals at all sampling periods after the last i.v. injection. These results are similar to those of Beuving and Vonder (1978), who observed an increase in plasma corticosterone in young and old laying hens and roosters within several minutes after the i.v. injection of ACTH. It is interesting that the increase was more rapid in younger than older birds, but that the increase lasted longer in the older birds. The response to ACTH in the young broilers in this study would, likewise, be expected to be rapid. An approximate 17-fold increase occurred at 40 min after injection in layers of both sexes in the previous study compared to a 35-fold increase at 60 min in the birds of the present study. The duration of increase, which lasted through 90 min in the broilers, was also longer than that observed in the young laying hens. Corticosterone concentrations between 30 and 90 min in birds treated with naloxone prior to ACTH changed similarly to those treated with saline followed by ACTH. Nevertheless, NAL-ACTH birds exhibited significantly lower corticosterone concentrations than SAL-ACTH birds at 30, 60, and 90 min. Naloxone decreased ACTH-stimulated corticosterone secretion within each of the sampling times. Comparison of these latter two treatments also suggests that naloxone pretreatment dampens the corticosterone response to ACTH as early as 30 min after ACTH injection and that this effect persists for at least another 60 min. Because naloxone’s effects were limited to a depression in corticosterone after exogenous ACTH-stimulated secretion, it is suggested that naloxone may only modify the responsiveness of the adrenal gland to ACTH. Opioids can affect in vitro adrenal steroidogenesis (Guaza et al., 1986), and specific naloxone-sensitive opioid receptors have been found to mediate the effects of opioids in the hypothalamus and adrenal gland (Lotti et al., 1969; Guaza and Borrell, 1984; Al-Damluji et al., 1990).

There was a significant \((P \leq 0.0007)\) main effect on serum glucose due to i.v. injection treatment (Table 1). Serum glucose was elevated across time of sampling in

![FIGURE 1. Serum corticosterone concentrations for different injection treatments at 0, 30, 60, and 90 min after the last injection in 2-wk-old broilers. Values ± SEM with different letters among the 0, 30, 60, and 90 min sampling periods differ significantly \((P \leq 0.05)\) by Student-Newman-Keuls test. Individual SEM were based on a pooled estimate of variance. * = Saline at 0 and 2 h (i.m.) and at 2.5 h (i.v.). ▲ = Naloxone at 0 and 2 h (i.m.) and saline at 2.5 h (i.v.). ● = Saline at 0 and 2 h (i.m.) and adrenocorticotropin (ACTH) at 2.5 h (i.v.). ● = Naloxone at 0 and 2 h (i.m.) and ACTH at 2.5 h (i.v.). \(n = 5\) samples within each treatment and sampling time.](https://academic.oup.com/ps/article-abstract/76/3/511/1513031)

<table>
<thead>
<tr>
<th>Injection Treatment</th>
<th>Corticosterone ((\text{ng/mL}))</th>
<th>Glucose ((\text{mg/dL}))</th>
</tr>
</thead>
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<tr>
<td>i.m.</td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>45.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>231.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Naloxone</td>
<td>26.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>236.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>i.v.</td>
<td></td>
<td></td>
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<td>Saline</td>
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<td>225.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>ACTH</td>
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<td>242.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> For each parameter, means within each type of injection with no common superscript differ significantly \((P < 0.05)\) by Student-Newman-Keuls test.

<sup>b</sup> \(n = 40\) samples within each treatment group.

<sup>2</sup> SEM based on pooled estimate of variance.
birds treated with ACTH when compared to those given saline i.v. The ACTH would be expected to increase glucose as a result of a response to elevated glucocorticoids. However, as was found for corticosterone concentration, there was also a significant ($P \leq 0.01$) i.m. by i.v. injection by sampling time interaction for serum glucose concentration. These data are provided in Figure 2. There was no significant main effect (i.m. by i.v. injection) interaction for serum glucose. Serum glucose was unaffected in all treatments at 30 min after the last injection and values were all within the normal physiological range (Latour et al., 1994). However, both i.v. ACTH-treated groups had significantly higher serum glucose concentrations than controls and NAL-SAL treated birds by 60 min. In addition, the glucose concentrations of the NAL-ACTH treated birds remained higher than the other treatment groups by 90 min.

Similar to its effect on corticosterone, naloxone led to greater increases in glucose when it was injected prior to ACTH rather than saline. The increase in glucose by 60 min in the ACTH-treated birds may be indirectly attributed to an ACTH-induced increase in corticosterone (Latour et al., 1993) by 30 min. Bisbis et al. (1994) have demonstrated that exogenous corticosterone effectively induced insulin resistance in chickens. At 90 min in the NAL-ACTH treatment, glucose was still elevated but corticosterone was lowered, perhaps indicating that the naloxone indirectly affected the production of factors other than corticosterone which promoted an increase in serum glucose. This effect may have occurred through naloxone’s influence on the pituitary. Edens and Parkhurst (1994) have provided data indicating that opioid agonists caused transitory increases in plasma growth hormone and prolactin concentrations 30 min after i.m. injection in broiler cockerels. In addition, Paolisso et al. (1987) have suggested that the effects of $\beta$-endorphins on blood glucose are mediated through the pancreas. Naloxone has been reported to inhibit the release of insulin (el-Tayeb et al., 1986a), and exogenous $\beta$-endorphins have been shown to give rise to significant increases in glucagon (el-Tayeb et al., 1986b). Furthermore, the secretion of $\beta$-endorphins has been shown to be stimulated by insulin (Imura et al., 1982).

In conclusion, naloxone elevated corticosterone in broilers as in other species, and subsequently augmented serum glucose concentrations. This study also provides initial evidence that a known $\beta$-blocker, naloxone, may attenuate the corticosterone response to ACTH. Recent evidence (Latour et al., 1996) has shown that elevated corticosterone levels predispose birds to higher than expected levels of low (LDL) and high (HDL) density lipoprotein-cholesterol. Therefore, it would be interesting to test whether naloxone could indirectly effect the circulating LDL and HDL, a study that has never been performed in avian species.

### REFERENCES


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