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RAPID COMMUNICATION

EFFECTS OF HYPOPHYSECTOMY ON PLASMA LEVELS OF PANCREATIC POLYPEPTIDE (APP) AND INSULIN IN ADULT CHICKENS*

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Abstract. Adenohypophysectomy (hypox) was carried out in adult chickens in an attempt to assess what role, if any, the anterior pituitary gland plays in maintaining basal levels of plasma insulin (IRI) and avian pancreatic polypeptide (APP) both before and immediately after a fast-refeed regimen. Each bird was tube-fed 61 gms twice daily, body weights were taken daily, and blood samples drawn daily just before the second feeding. All birds were fasted for 24 hr on days 4-5, another blood sample taken, and then refed the usual gruel. Blood samples were taken at 5,15,30,90 and 180 min after refeeding. Hypox caused an immediate and sustained decrease in plasma IRI and a significant but transient increase in plasma APP which lasted 3-4 days before returning to normal; plasma glucose was marginally decreased. Refeeding resulted in a trend of less response (increase) in all three parameters studied in the hypox group. It is suggested that in chickens, hypox may lead to a release phenomenon from a normal inhibitory role which the anterior pituitary gland plays on APP release.

Introduction. The role which the pituitary gland may play in maintaining basal levels as well as in the rapid elevation of plasma pancreatic polypeptide in human and non-human vertebrates after meal eating is not known. As in man (1), plasma avian pancreatic polypeptide (APP) rises within minutes after eating to levels anywhere from 300-500% greater than overnight fasting levels, only to subside somewhat and then remain 200-300% elevated over a period of several additional hours (2). While the immediate increase in PP represents 15-20% of the total integrated response to feeding, and is probably due to cholinergic input (3), the sustained response is probably a result of a mixture of neurogenic (distention), gut secretagogues (peptide hormones), nutrient absorption (mainly amino acids), and metabolic inputs (yet to be elucidated) to the PP cells. Hypophysectomy increases liver lipid, cholesterol, free fatty acids, phospholipids and triglycerides in plasma of adult chickens. The birds become obese even though food intake is decreased (4). Because of the established post-prandial rise in APP (2,5) and the peptide's reported hypoglycerolemic, anti-lipolytic and postulated lipogenic action in chickens (6,7), studies were carried out in hypophysectomized adult chickens to elucidate any possible regulatory role the pituitary gland might play.

Methods. Adult female white leghorn chickens (Rich-Glo Farms, El Campo, Texas) were maintained on a 12:12 hr light:dark cycle at 21-22°C with food and water *ad libitum*. Surgical adenohypophysectomy was performed by an oral approach; sham-operated control birds merely had the site exposed. After surgery, all birds were tube-fed *per os* twice daily with 61 gm of gruel consisting of Purina Chicken layer mash (41% food by weight). Completeness of hypophysectomy was judged by recovery of the intact organ at time of surgery, as well as by headchecks and ovary and oviductal tissue weights at time of sacrifice. Blood samples were taken daily by an alar vein-right

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atrium catheter (or sometimes via cardiac puncture) just before the daily 1430 hr gruel feeding (days 1-4), representing 7-hr postprandial conditions. After a 24 hr fast (day 4-5), all birds had a blood sample drawn at 1430 hr. They were then tube-fed the usual 61 gm of gruel-feed. Blood samples then were taken at exactly 5, 15, 30, 90 and 180 min after refeeding, the plasma separated at 4°C, and stored for analyses at -20°C. Immunoreactive APP (IRAPP) and insulin (IRI) were determined on all plasma samples in triplicate by established methods (8,9). Chicken hormone standards and specific antibodies were gifts of J.R. Kimmel, Univ. of Kansas Medical Center. APP least detectable concentration was 0.176 = 0.05 ng/ml, intra-assay coefficient of variance was 5.17% and the inter-assay 3.21%. Least detectable concentration of IRI was 65.8 = 7.3 pg/ml, intra-assay coefficient of variance was 1.26%, and the inter-assay 5.45%. Glucose was determined by a glucose oxidase method (BMC Reagent Set kit) on 25 ml of plasma. All data were treated by analysis of variance (ANOVA) and p-values less than .05 were considered significant. Data are expressed as mean values = SEM.

<u>Results</u>. Twenty hypophysectomized (hypox), five additional birds which subsequently proved to be partially hypophysectomized, and nine control sham-operated chickens were followed five days prior to a test meal refeeding. (The test meal refeeding had been shown in a pilot study to elevate plasma APP and glucose levels in intact chickens to the same extent as had been reported earlier (2)). During this period, mortality was greatest in the hypox birds, 50% dying by two days post-operative. Such a surgical loss following complete hypox in adult chickens is well established in the literature (and by A.V. Nalbandov, personal communication). No deaths occurred in the other two bird groups although the venous catheters became occluded in some birds. The twice daily gruel feedings maintained the body weights of the hypophysectomized and sham-operated chickens at day '0' fasting levels throughout the experiment. No significant differences between the two groups were detected (p>0.05).

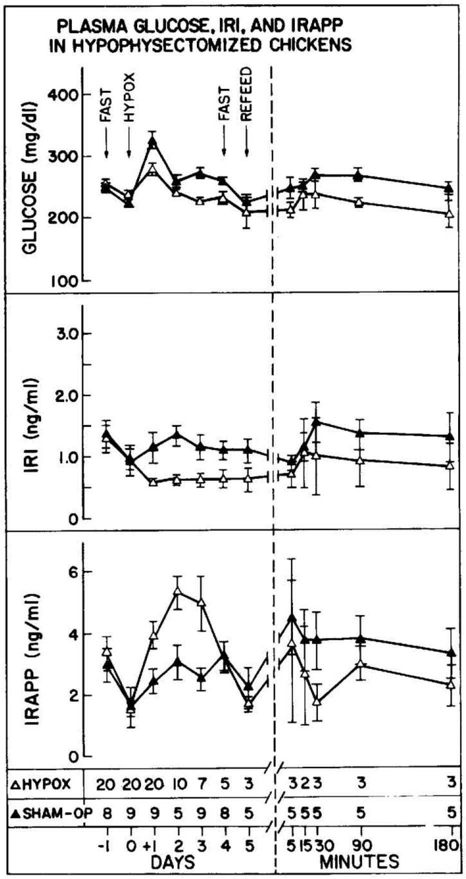

Figure 1. Effects of hypophysectomy on basal plasma IRI, APP and glucose levels and on the fast-refeeding response in adult chickens. All birds were fasted day 4 to day 5 and then fed a gruel <u>per os</u> as detailed in Methods. Blood samples taken daily pre-test feeding, them at five different time intervals following feeding. Number of observations indicated at bottom, opposite data symbols and vertical bars are SEM.

The effects of hypox on basal plasma glucose, IRI and IRAPP are shown in Figure 1 where it can be seen that glucose levels were significantly depressed (ANOVA, $p < .01$) during the five days following surgery, regardless of whether or not the birds were fed (days 1-4 <u>vs</u> 5). Plasma IRI was depressed in the hypox birds after surgery and remained significantly depressed ($p<.01$) every day throughout the five-day observation period. In contrast, IRAPP levels were elevated significantly ($p<.01$) as early as day 1, and remained elevated for at least two additional days. By the start of the pre-test meal fast (days 4-5), IRAPP levels had returned to normal (Fig. 1). Partially hypox birds had glucose and IRAPP values virtually identical to those of the sham-op control group, while the IRI data were close to, or slightly above, control values during the five days preceding the test meal.

Refeeding all three groups after a 24 hr fast resulted in hypox plasma levels of all three parameters being relatively undisturbed (glucose and APP) or elevated less (insulin) than that of the sham-op control and partially ablated birds over the 180 min observation period. The small number in each group (N=3,4,5) make it impossible to establish statistical significance at many points but the lower trend of the hypox chickens is clearly evident in all parameters analyzed (Fig. 1).

Discussion. The role of the pituitary gland in regulating (either directly or by synergistic action) metabolic patterns in mammals is well accepted. Lipid metabolism is particularly affected by no less than five of the gland's hormones. Hypophysectomy in birds, an extremely difficult task in adult forms, leads to increased fat deposition, obesity, hepatic lipid accumulation and reduced appetite (4). APP is postulated to stimulate hepatic lipogenic pathways (6,7,10), thus a reasonable expectation of interplay between hypophyseal secretions and APP exists. Such an interaction in fast-refed vertebrates is yet to be studied. Also, no data are available on the effects of hypox on basal levels of pancreatic hormones in adult chickens.

In the present study, all birds were force-fed the same amount of food daily to negate this parameter as a contributing/ interfering factor. The amount fed had proved previously to be an effective releaser of APP in intact adult chickens. Contrary to the observed decrease in basal plasma glucose and IRI both in the fed and the fasted state, hypophysectomy caused a significant elevation in IRAPP for at least 3 to 4 days. Which of the absent hypophyseal secretions were responsible for the rise in APP levels is not evident though the prompt rise in the plasma peptide would suggest a "release-type" mechanism. Stress of many forms is known to increase PP levels in mammals (1) but were that the case in this study, the two control groups would have been expected to demonstrate a similar, transient APP rise. Reattainment of normal plasma IRAPP levels on day 4 and 5 suggests either a compensatory decrease in modulation by target gland secretions or a metabolic readjustment obviating a "need" for elevated levels of APP. Pancreatic somatostatin may well be involved in modifying plasma APP levels immediately after hypox (11), the D-cell regaining its full secretory-paracrine capacity by the fourth post-operative day. Estrogen secretion, prior to deterioration of the ovary following surgical ablation of the adenohypophysis, may be responsible for the early rise in IRAPP, only to subside as ovarian tissue atrophies concomitant with that of oviductal tissue. Estrogen, like hypox, in chickens is known to have a pronounced lipogenic effect, particularly at the liver.

Hypophysectomy in ducks has been shown to decrease plasma glucose levels. In the absence of growth hormone and corticosterone, glucagon has reduced hyperglycemia potential in pituoprivic ducks. Further, hypophysectomy markedly lowers plasma free fatty acids and insulin levels in ducks (12). Thus, the IRI and glucose data presented herein for hypox chickens are in accord with observations made in ducks.

Refeeding hypox and sham-op control birds resulted in little perturbation of plasma glucose, IRI or APP in the former except at the first (5 min) sample in the case of APP. Such results could reflect the reduced efficiency of alimentation known to follow hypox; alternatively, it could represent an absence of a secretion required for elevation of the anabolic peptide.

Conclusion. The results presented herein indicate a transitory increase in plasma APP due to hypophysectomy in adult chickens, an increase which is largely obliterated after a fast-refeed of a test meal. Whether this increase is due to a release phenomenon and the failure to increase after a test meal a result of the absence of a necessary secretogogue, is yet to be investigated.

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