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# Alpha- and Beta-Adrenergic Control of Pancreatic Polypeptide and Insulin Secretion in Adult Chickens[1] (41279)

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*Abstract.* Adult, unanesthetized but restrained chickens were cannulated via appropriate femoral arteries and veins to assess alpha, beta-adrenergic regulation of pancreatic secretion of insulin (IRI) and pancreatic polypeptide (APP) during glucose infusions alone or with simultaneous infusion of either phentolamine (alpha blocker) or propranolol (beta blocker). Plasma samples were obtained over 30 or 60 min and assayed for glucose, insulin, and pancreatic polypeptide. Glucose (0.88 g/kg, bolus or infusion—30 min) provoked an immediate rise in IRI even when plasma glucose levels peaked at 350 mg/dl in contrast with results of previous *in vitro* studies. Sustained glucose infusion was necessary to decrease APP levels from normal fed levels of 8-10 to 4-5 ng/ml within 3–5 min; bolus injection was without effect on APP levels even though plasma glucose levels reached 730 mg/dl. Neither phentolamine nor propranolol altered basal IRI, APP, or glucose levels. Phentolamine also was without effect on the glucose-induced rise in IRI or decrease in APP levels. However, propranolol pretreatment obtunded the usual glucose-induced IRI release concomitant with total blockage of the glucose-induced suppression of APP. It is concluded that chickens may be much more sensitive to glucose-induced insulin release *in vivo* than reported earlier and that adrenergic receptor regulation of IRI and APP release in the basal state is relatively unimportant. The beta receptor site appears stimulatory to glucose-induced insulin release, while being inhibitory to the glucose-induced depression normally observed in APP levels.

Alpha, beta-adrenergic modulation of insulin secretion in mammals has been studied extensively by many laboratories over the last 10 years in an effort to delineate what role these pancreatic receptors play in both the basal and stimulated state. Thus, it is generally accepted that in mammals alpha receptor activation suppresses glucose-induced insulin release, while beta receptor stimulation increases insulin secretion (1–4). Additionally, alpha-adrenergic blockade increases basal (resting) insulin release in man, while beta-adrenergic blockade decreases it regardless of normoglycemia (2).

The influence of adrenergic mechanisms in regulating avian pancreatic hormone secretion has not received the attention which mammalian systems have, even though greater circulating levels of glucose (2- to 3-fold), ketones (2-fold), insulin (2- to 3-fold), glucagon (3- to 5-fold), and pancreatic polypeptide (40- to 50-fold) obtain in Aves in the basal state (5, 6). From what little work which has been done, it appears that alpha adrenergic stimulation (via the catecholamines) inhibits insulin release but stimulates glucagon secretion in ducks (7). Isoproterenol has been demonstrated to produce an hyperinsulinemic state in birds, while suppressing plasma glucagon levels (8). To our knowledge, no literature exists relative to adrenergic modulation of avian pancreatic polypeptide (APP) either in the basal state or in the glucose-stimulated state.

The general goals of the present study were to investigate *in vivo* the role, if any, of adrenergic mechanisms in regulation of pancreatic insulin and/or pancreatic polypeptide secretion from the adult chicken pancreas.

**Methods.** *Animals.* All birds were De Kalb white leghorn chickens, weighing 1.63 ± 0.21 kg each, obtained from Rich-Glo Lab Animal Farm, El Campo, Texas. The birds were housed with water and feed (Purina, Laying mash) *ad libitum* on a 12D:12L light regimen at 21 ± 1°.

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*Surgery.* Chickens were restrained gently on surgical boards with Ace elastic bandage strips; paper towels were placed over the head. Surgery was performed under local anesthesia (Lidocaine). A femoral artery was cannulated for removing blood samples, and the femoral vein cannulated for infusion of saline, glucose, or the adrenergic blockers. Use was made of Sage infusion pumps for the latter purpose (Model 341 or 351).

*Infusion solution.* Saline was infused (heparin added) for 10–15 min following the end of surgery. Subsequently, according to the experimental design, a bolus of glucose (0.88 g/kg body wt), an infusion of glucose (0.88 g/kg, 0.0293 g/kg/min for 30 min), or both glucose bolus and infusion, phentolamine–HCl (alpha blocker, CIBA Co., bolus of 0.07 mg/kg followed by 0.007 mg/kg/min for 60 min, or 0.28 mg. kg followed by 0.028 mg/kg/min for 60 min), or L-propranolol (beta blocker, Ayerst Co., bolus of 0.07 or 0.14 mg/kg followed by 0.0011 or 0.0022 mg/kg/min, respectively) was infused over the 60-min experimental period. In several studies the 30-min glucose challenge was superimposed on the last 30 min of the 60-min adrenergic blockade period.

*Analyses.* Glucose was measured by the glucose oxidase enzymatic method on plasma diluted 1:4. Homologous immunoassays for plasma insulin (IRI) (diluted 1:2) and pancreatic polypeptide (APP) (diluted 1:6) were carried out by the double-antibody method of Hales and Randle (9) on plasma. Dr. J. R. Kimmel (University Kansas Medical Center) provided the chicken hormone standards. Heart rate, blood pressure, and ECG were monitored during all experiments involving propranolol and phentolamine.

Blood samples (two) were obtained during the control saline infusion, and again after the test substance infusion and/or bolus at 5, 10, 20, and 30 min (propranolol or phentolamine), 1, 3, 5, 10, 15, and 30 min (glucose only). Thus, when glucose was infused in addition to an on-going blocker infusion, samples were taken as well at the 31-, 33-, 35-, 40-, 45-, and 60-min times.

*Statistics.* Comparisons for significance between groups were made by Student's $t$-test. A level of $P < 0.05$ was considered significant.

**Results.** *Glucose studies.* Injection of a bolus (0.88 g/kg) of glucose into adult chickens caused an immediate release of IRI within 30 sec, which remained at significantly higher levels than control values for at least 30 min (data not shown). Peak blood glucose levels were nonphysiological at 725, 605, and 524 mg/dl at 1, 3, and 5 min, respectively, postinjection. Despite this pharmacological hyperglycemia, plasma APP levels were unperturbed.

Infusion of the same quantity of glucose over a 30-min period increased plasma glucose levels to physiological levels of 307, 342, and 374 mg/dl at 5, 10, and 15 min, respectively, after infusion commenced (data not shown). However, the immediate rise in IRI levels concomitant with significant and immediate depression of APP levels (as early as 1 min) were events observed in both infusion and bolus plus infusion studies. Figure 1 shows the impact of a glucose bolus followed by a glucose infusion on plasma IRI and APP levels. Very significant increases in IRI concomitant with marked depression of APP occurred as early as 1 min following the initial glucose load. At this time the plasma glucose level was 684 mg/dl and remained thereabouts for an additional 15 min (Fig. 1).

*Adrenergic studies.* Administration (bolus plus infusion) of phentolamine at the low dose level (0.007 mg/kg/min) was without effect on basal IRI and APP levels and did not alter the rapid increase in IRI observed as soon as glucose (bolus + infusion) was introduced (data not shown). Glucose levels peaked at 637, 604, and 590 mg/dl at 1, 3, and 5 min, respectively, after the glucose challenge. APP levels were suppressed by the addition of glucose to the system.

Quadrupling the phentolamine concentration (bolus and infusion) to 0.028 mg/kg did not influence basal glucose, IRI, or APP levels. (It should be noted that the higher of the two phentolamine doses blocked the increase in heart rate induced by the alpha agonist, phenylephrine. Mean blood pressure decreased 10% below basal control

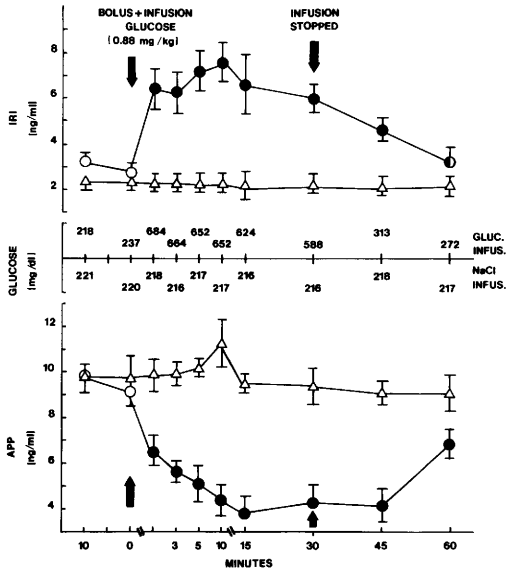


FIG. 1. Effect of glucose (bolus plus infusion) on plasma hormones of adult chickens. Plasma IRI, glucose, and APP response to a bolus followed immediately by an infusion of NaCl (△) and to glucose (○). Five birds were tested in the glucose group and four birds were tested in the NaCl group. Vertical bars represent SEMs. Comparisons were made between the glucose experimental group and the NaCl control group using Student's $t$ test: ◑, $P < 0.05$; ●, $P < 0.01$; ○, $P > 0.05$.

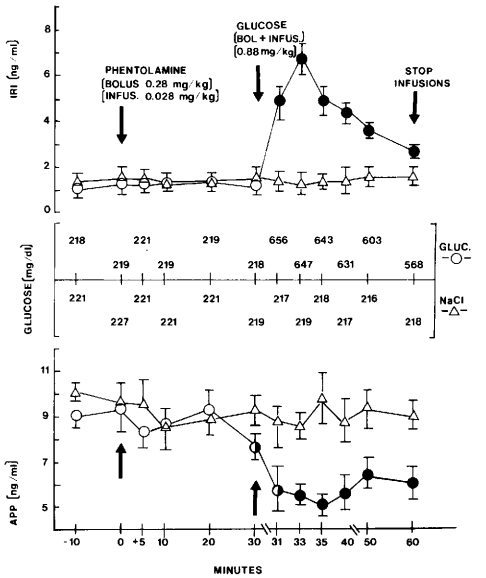


FIG. 2. Effect of infusion of phentolamine 0.028 mg/kg/min followed by glucose (bolus plus infusion) on hormones of adult chickens. Plasma IRI, glucose, and APP response to a phentolamine (bolus plus infusion) followed by a NaCl (bolus plus infusion) (△) and to a phentolamine (bolus plus infusion) followed by a glucose (bolus plus infusion) (○). Five birds were tested in both the experimental and the control groups. Vertical bars represent SEMs. Comparisons were made between the glucose experimental group and the NaCl control group using Student's $t$ test: ◑, $P < 0.05$; ●, $P < 0.01$; ○, $P > 0.05$.

levels when monitored at the 35 min sampling). Neither did such a concentration modulate the IRI response or APP suppression induced by glucose infusion (Fig. 2). The effects observed in presence of the alpha blocker were virtually identical to those observed in its absence over the first 30 min (Fig. 1 vs Fig. 2); however, IRI levels returned to control levels more quickly between the 30- to 60-min period.

Propranolol was employed at two infusion levels to examine the effects of this beta-adrenergic blocker on glucose-induced changes in plasma IRI and APP (Fig. 3). The higher dose (0.0022 mg/kg/min) limited increases in mean blood pressure to 6% or less when the beta agonist isoproterenol was infused. Neither propranolol concentration affected basal IRI or APP levels in the chickens; additionally, the lower dose reduced by approximately 50% the glucose-induced increase in plasma IRI (data not shown). Contrarily, both propranolol doses obliterated the usual glucose-induced depression APP levels and the higher concentration reduced the usual increase in IRI even further as can be seen by comparing Figs. 1 and 3. This latter decrease in IRI amounted to an approximate 75% reduction of the normal response to glucose (Fig. 1).

**Discussion.** The very significant increase in IRI in response to a glucose load *in vivo* (infusion-only data not shown; bolus plus infusion data of Fig. 1) suggests that the normal chicken pancreas may be far more sensitive to this glycemic stimulus than previously reported. King and Hazelwood (10) found that the isolated perfused pancreas required levels of perfusate glucose in excess of 500 mg/dl to provoke significant insulin release and not until 700 mg/dl levels were reached did the characteristic two-phase release of IRI occur. Similar resistance to glucose induction of insulin release

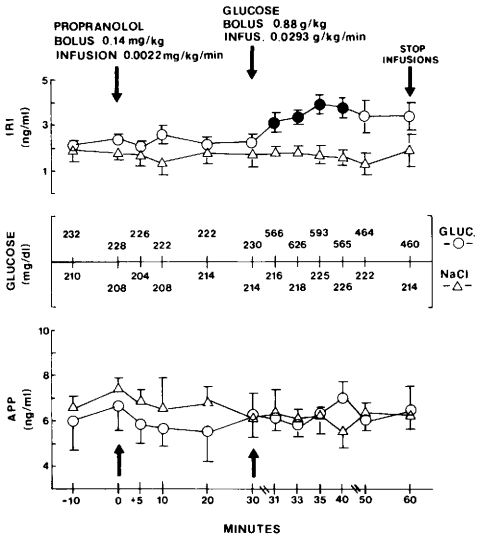


FIG. 3. Effect of infusion of propranolol 0.0022 mg/kg/min followed by glucose (bolus plus infusion) on plasma hormones in adult chickens. Plasma IRI, glucose, and APP response to a propranolol (bolus plus infusion) followed by a NaCl bolus and infusion (△) and to a propranolol (bolus plus infusion) followed by a glucose bolus and infusion (○). Five birds were tested in both the experimental and control groups. Vertical bars represent SEMs. Comparisons were made between the glucose experimental and the NaCl control groups using Student's $t$ test: ●, $P < 0.05$; ○, $P > 0.05$.

has been reported by Weir and associates (e.g., (11)) employing perfused chicken duodenopancreas preparations and Naber and Hazelwood employing small pieces (18–24 mg) of pancreatic tissue *in vitro* (12). Thus, while avian glucose levels *in vivo* rarely exceed 350 mg/dl after alimentation, it would appear that reasonable amounts of insulin are released *in vivo* which then may facilitate the ultimate distribution of this nutrient. The present studies do not address the question whether or not absorption per se across the gut is important for this glucose-induced IRI response; thus, one cannot rule out the possibility of glucose (or a metabolite thereof) acting as a secretogogue at the gut level.

The rapid depression in APP levels observed herein in response to a glucose load is similar to that observed in mammals (13). This depression does not appear to be related to peak glucose levels but rather to duration of hyperglycemia (bolus glucose data vs infusion data). Also, the depression in plasma APP appears to be unrelated to IRI release because the glucose bolus failed to alter APP levels, yet it increased rapidly the existent plasma IRI levels. Thus, glucose may exert a direct inhibitory action on the PP cell of the chicken pancreas, or alternatively, sustained plasma glucose elevation may be detected by an extrapancreatic glucosensor forming an afferent neural, possibly vagal, limb. Our experiments with perifusion of isolated microfragments of chicken pancreas with varying levels of glucose would indicate that a direct effect on the PP cell is unlikely (14).

Competitive blockade of the alpha adrenergic receptor site had no effect on basal IRI or APP secretion even though the doses of phentolamine employed were those known to negate alpha agonist-induced elevations in mean blood pressure and also were similar to those known in man to increase plasma insulin levels (2). However, it should be recalled that Aves have endogenous catecholamine levels several hundred-fold higher than those of mammals and the blockers employed herein may be competing therewith. The lack of an alpha adrenergic blockade on basal IRI levels in chickens may be in discord with those observations of Tyler (15) who concluded from studies on fasted ducks which were infused with catecholamines that reciprocal alpha–beta action of sympathetic system is exerted on the pancreatic insulin- and glucagon-secreting cells. However, considerable differences exist in the experimental design of the two studies making it difficult to reconcile the apparent discordant data (prandial state, species of birds, infusion of catecholamine, plasma levels of catecholamines, etc.). Our results suggest that beta cells of man, but not chickens, may possess significant inhibitory adrenergic tone which is mediated by the pancreatic alpha receptor.

The inability of phentolamine to prevent glucose-induced suppression of APP in chickens suggests that any neural efferent pathway (see above) is not regulated by an alpha adrenergic receptor. The efferent

limb may consist of a reduction of vagal tone to the PP cell, or possibly the paracrine effects of intrapancreatic somatostatin. Such possibilities deserve further investigation.

Propanolol blockade of the pancreatic beta receptor was without effect on basal avian glucose, IRI, or APP levels, observations which agree with those of Cerasi *et al.* on the glucose and IRI parameters (16) but disagree with those of others who employed virtually the same dose of propranolol in man (2). The latter workers observed a 40% decrease in basal insulin levels in man, yet their subjects remained euglycemic (2). Thus, the apparent different results could well reflect differences in degree of adiposity, existing glucose levels, glucagon inhibition, or other stress-related factors.

The reduced glucose-induced insulin release from the chicken pancreas during beta receptor blockade has been observed also in rats and man (17, 18). In studies employing the perfused rat pancreas only the second phase of IRI release was inhibited by pretreatment with propranolol (18). Aryl-substituted secondary aminoethanols, a class of compounds which include the beta receptor blocking agents, as well as D and L isomers of propranolol are equally effective in inhibiting insulin release in response to glucose infusion in mice (18). It appeared that a generalized, overall, reduction in chicken IRI release occurred with the two concentrations of propranolol selected. Biphasic insulin release was not apparent in this study. The higher of these dose levels, still, was but 1/20th that employed in the mammalian studies cited above (18).

Beta-adrenergic receptors are probably closely linked either to the production of, or the stimulatory role of, cyclic 3′, 5′-AMP (19, 20). Whether or not propranolol exerts an inhibitory action over islet function by membrane stabilization is yet to be studied in birds. It should be noted, however, that membrane-stabilizing effects of this blocking agent have been reported in mammals only at levels 20–25 times higher than those used in chickens in the present study.

The beta-adrenergic receptor in man and dogs appears to favor release of pancreatic polypeptide (HPP and CPP). Thus, isoproterenol increases CPP release in dogs (21) and propranolol infusion suppresses the (otherwise) increase in HPP observed in exercising man (22). Involvement of the beta receptor site in mediating glucose-induced suppression of APP in chickens also appears to exist, as presented in Fig. 3, though the effect is opposite to that observed in man. Propranolol infusion totally obliterated the depression expected (Fig. 1) from the bolus plus infusion of glucose in chickens. Since PP is considerably less dependent on the availability of $Ca^{2+}$ as compared to other pancreatic hormones (21), it is unlikely that membrane–$Ca^{2+}$ events are important to the chicken in modification of the glucose-induced suppression of APP. Such a statement does not preclude the possibility that presynaptic, or postsynaptic, beta receptor site activity is an important adjunct in modifying APP release by vagal or peptidergic systems (23).

In conclusion, the avian pancreas *in vivo* is considerably more sensitive to glucose or metabolite-induced insulin release than previously reported. While neither alpha nor beta-adrenergic receptor blockade altered basal IRI or APP levels in adult chickens, the beta blocker profoundly reduced the suppression in APP normally observed with glucose infusion. Finally, blockade of the beta receptor suppresses glucose-stimulated insulin release in chickens.

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