Does Histamine Play a Role in the Secretogogic Effect of Avian Pancreatic "Gastrin"?[1] (37696)

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In the process of isolating and purifying insulin from the chicken pancreas, Kimmel *et al.* (1) described the presence of a consistent polypeptide contaminant. This pancreatic polypeptide (APP) was subsequently isolated, purified, and shown biochemically to be distinct from chicken glucagon and insulin, and/or fractions thereof (2). The pancreatic polypeptide can not be extracted from tissues other than the pancreas, has been found in at least 8 avian species, is present at levels greater than insulin in the same pancreas, and is a normal circulating plasma polypeptide in chickens (3). Studies of the biological activity of APP indicated it also to be distinct physiologically from insulin and glucagon. The pancreatic polypeptide was shown also so have a potent proventricular (gastric) stimulatory effect, one which was mediated neither by the vagus nerve nor by alterations in systemic cardiovascular parameters (4).

The fact that histamine is a potent gastric secretogogue in mammals is well documented (5, 6). Also, it has been reported that histamine increases the volume and content of proventricular secretion in chickens (7–10). It was important, therefore, to consider the possibility that the effect of APP on proventricular secretion in birds was mediated by an histamine action, especially if histamine is the final common mediator of all gastric secretogogues as has been postulated by Code (11).

This report presents results of a study comparing the effects of APP and histamine in chickens previously injected with an anticholinergic agent known to block the gastric stimulatory effect of histamine.

Methods. Fed, adult, female Single-Comb White Leghorn chickens were anesthetized with sodium pentobarbital (Nembutal). An incision was made on the left side of the bird just posterior to the rib cage. The proventriculus (secretory stomach) was isolated and cannulated, and a ligature was placed around the gut between the proventriculus and the gizzard. A ligature was also placed inferior to the corp, allowing the esophageal-proventricular segment thus produced to be flushed with warm saline to remove food particles. Proventricular secretions were then allowed to flow into collection tubes for three 10-min collection periods to establish basal secretory levels. The first of these samples was discarded (thereby avoiding possible dilution by residual flush volume). At this time the birds receiving the blocking agent were given (iv) glycopyrrolate bromide (A. H. Robins, *Robinul,* 100 μg/kg), and those not receiving the blocking agent were given (iv) APP (25 μg/kg) or histamine (sc, 100 μg Eli Lilly Co. histamine base/kg). Ten minutes later the birds preinjected with the blocking agent were given the same doses of APP or histamine as indicated above. In all studies each APP- or histamine-injected bird was grouped simultaneously with a corresponding glycopyrrolate-injected bird. After the injection of APP (or histamine), six 10-min proventricular collections were made, the volume and pH (by glass electrode) were determined immediately, and the sample remainder frozen for subsequent pepsin and protein determinations by Bucher's modification (12) of the method of Anson and the Lowry method (13), respectively.

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Appropriate statistics were applied to mean group differences by Student's t test.

Results. Data from pilot studies (not presented here) indicated what dose level of histamine base, when injected subcutaneously provoked proventricular secretion in birds equal in magnitude to that previously found with 25 μg APP injected/kg body wt. Also, varying amounts of glycopyrrolate were evaluated as to the effectiveness of each in blocking histamine-induced proventricular secretion in chickens. A dose was selected which would reduce markedly the gastric effect of the histamine dose selected, yet not abolish it totally. It is evident from Fig. 1 that injection of chickens with either histamine (100 μg/base/kg) or APP (25 μg/kg) alone produced responses which were equivalent as far as the four proventricular parameters measured; these responses were evident for at least 60 min. It is also evident that injection of glycopyrrolate (10 min prior to histamine injection) effectively blocked the histamine effect on the proventriculus ($p < 0.001$, each point), but was without effect in those birds which were injected subsequently with APP. Use of different doses of the anticholinergic agent, histamine, or APP did not qualitatively alter this pattern.

Comparison of the preinjection control values with the respective accumulative values for the two histamine groups (Table I) indicated that glycopyrrolate reduced the total histamine effect over 60 min from an increase of 700 to 55%, from 500 to 26%, from 1600 to 75%, and from 900 to 43% for secretory volume, acid, pepsin, and total protein levels, respectively ($p < 0.001$ for each comparison). Similar statistical evaluation of the 60-min accumulative data for the two APP groups indicated no significant differences existed between groups for any parameter comparison (700 *vs* 670%, 677 *vs* 890%, 1370 *vs* 1550%, and 675 *vs* 616% for secretory volume, acid, pepsin, and total protein, respectively).

Discussion. The anticholinergic agent, glycopyrrolate bromide, has been demonstrated to block effectively histamine-stimulated gastric secretion in mammals without exerting any cardiovascular influence. Current evidence indicates that this gastric effect is accomplished by selective blockade of histamine–gastric receptor sites (14–16). Data presented here demonstrated that glycopyrrolate also provides an effective blockade against histamine-stimulated proventricular secretion in chickens. Contrarily, it was observed that the magnitude of the APP-stimulated proventricular secretion was not affected by glycopyrrolate injections (Fig. 1, Table I). It can be concluded, therefore, that the effect of APP on proventricular secretion is not mediated by the release of histamine. Also, as a result, the hypothesis that histamine is the final common mediator of gastric stimulation does not obtain in chickens.

Ruoff and Sewing (17) concluded that "gastric" acid secretion in chickens was under the control of a mechanism other than gastrin. These conclusions were based upon the demonstration that extracts of glandular, stomach, gizzard, and duodenum were free of acid stimulatory potential; however, these workers did not examine the pancreas for such a component. The pancreatic polypeptide is found only in the avian pancreas (3) and has been shown to exert a proventricular effect without vagal or cardiovascular involvement (4). These observations are similar to what has been observed for porcine gastrin II in mammals (6). The present study adds further evidence for the direct effect of APP on the proventriculus and, in doing so, possibly documents its role as avian "gastrin." Other evidence reported by Grieder and McGuigan (18) indicates that gastrin has been detected in both the normal and pathological human pancreas. Such data add strength to the hypothesis of the existence of the avian pancreatic gastrin-like substance described herein.

Summary. Investigation was made of what role histamine plays in the gastric secretion induced by a new avian pancreatic polypeptide (APP). Use of an anticholinergic agent (glycopyrrolate bromide) was made to block the gastric effects of histamine in adult chickens. However, this agent was without effect on the stimulatory action of APP as measured by secretory volume, H^+, pepsin, and protein levels. The pancreatic polypep-

TABLE I. Effect of Glycopyrrolate Bromide[a] on APP- and Histamine-Stimulated Proventricular Secretion.

Volume:		Deviation from control levels (ml/10 min)								Total per
Group	Dose (μg/kg)	Control	Gp[a]	10	20	30	40	50	60	60 min
Hist	100	0.62[b] ±0.25	—	0.79 ±0.15	0.85 ±0.19	0.90 ±0.19	0.81 ±0.14	0.75 ±0.17	0.25 ±0.18	4.35 ±0.21
Hist + Gp	100 + 100	0.65 ±0.29	−0.05 ±0.10	0.06 ±0.10	0.08 ±0.09	0.10 ±0.09	0.05 ±0.05	0.02 ±0.05	−0.05 ±0.01	0.36 ±0.09
APP	25	0.58 ±0.25	—	0.75 ±0.16	0.74 ±0.14	0.68 ±0.17	0.62 ±0.10	0.55 ±0.10	0.42 ±0.13	4.06 ±0.21
APP + Gp	25 + 100	0.55 ±0.21	−0.09 ±0.10	0.70 ±0.17	0.76 ±0.22	0.70 ±0.19	0.61 ±0.17	0.50 ±0.21	0.41 ±0.32	3.68 ±0.43
Acid:		**Deviation from control levels (mEq H^+/10 min)**								**Total per**
Group	Dose (μg/kg)	Control	Gp[a]	10	20	30	40	50	60	60 min
Hist	100	138 ±30	—	90 ±11	115 ±11	140 ±16	138 ±17	119 ±19	92 ±12	694 ±25
Hist + Gp	100 + 100	125 ±21	−15 ±9	5 ±3	15 ±8	25 ±8	2 ±3	1 ±6	−15 ±5	33 ±11
APP	25	140 ±25	—	143 ±11	195 ±16	219 ±21	185 ±12	125 ±10	126 ±12	948 ±32
APP + Gp	25 + 100	120 ±21	−21 ±11	195 ±23	227 ±18	225 ±19	179 ±15	130 ±21	119 ±22	1075 ±48
Pepsin:		**Above control levels (P.U.Hb × 10^4/10 min)**								**Total per**
Group	Dose (μg/kg)	Control	Gp[a]	10	20	30	40	50	60	60 min
Hist	100	55[b] ±12	—	120 ±16	121 ±19	220 ±19	200 ±21	142 ±21	120 ±18	923 ±41
Hist + Gp	100 + 100	69 ±16	−3 ±9	11 ±10	9 ±11	12 ±10	8 ±11	5 ±10	6 ±12	52 ±22
APP	25	70 ±16	—	115 ±21	250 ±18	175 ±17	175 ±20	150 ±20	143 ±21	961 ±35
APP + Gp	25 + 100	66 ±13	−1 ±8	112 ±16	256 ±13	200 ±20	196 ±20	138 ±18	126 ±16	1028 ±65
Total protein:		**Above control levels (mg/10 min)**								**Total per**
Group	Dose (μg/kg)	Control	Gp[a]	10	20	30	40	50	60	60 min
Hist	100	1.85[b] ±0.28	—	2.28 ±0.38	2.50 ±0.30	3.90 ±0.48	3.75 ±0.29	2.10 ±0.35	2.00 ±0.35	16.53 ±0.95
Hist + Gp	100 + 100	1.99 ±0.25	−0.10 ±0.30	0.10 ±0.39	0.11 ±0.25	0.25 ±0.24	0.15 ±0.19	0.15 ±0.28	0.09 ±0.30	0.85 ±0.65
APP	25	2.19 ±0.20	—	2.38 ±0.45	3.10 ±0.45	3.85 ±0.61	3.25 ±0.21	1.21 ±0.20	1.00 ±0.09	14.79 ±0.98
APP + Gp	25 + 100	2.10 ±0.21	−0.06 ±0.25	1.90 ±0.29	2.25 ±0.35	3.50 ±0.29	2.10 ±0.29	2.10 ±0.16	2.09 ±0.31	12.94 ±0.99

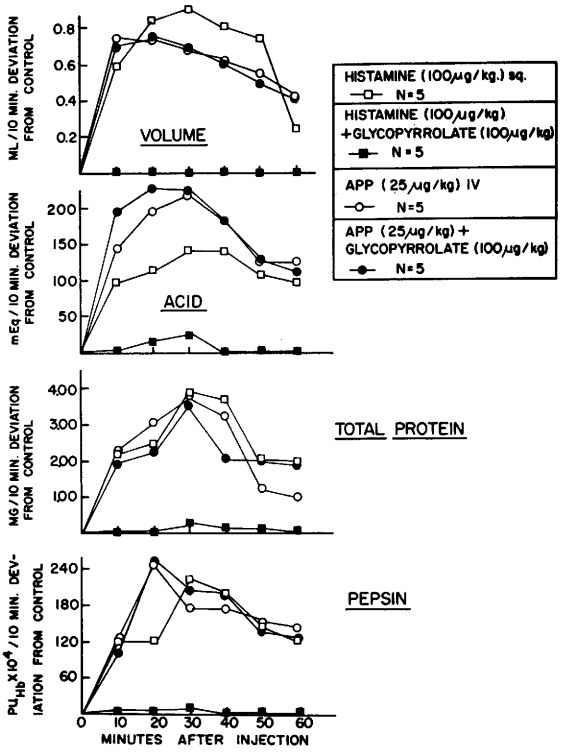

FIG. 1. Response of nonfasted adult female chickens to APP and histamine injection in the presence or absence of the blocking agent, glycopyrrolate bromide (GB). GB injected (iv) 10 min prior to either APP (iv) or histamine base (sc). Three 10-min collections were made prior to '0' time; five birds in each group. Statistically (○—○, ●—●, and □—□) data for all four parameters measured were not significantly different from each other, though all were significantly different ($p < 0.001$) from (■—■) data at all six time periods.

tide action, therefore, is not mediated by histamine release in chickens, and, in fact, APP may be avian gastrin.

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[a] Glycopyrrolate bromide (*Robinul*, A. H. Robins, Inc.) injected iv at time −10 min.

[b] Mean ± SEM for five (5) observations each group, each time.

Note: Histamine (sc) and APP (iv) were injected at time '0', i.e., 10 min *after* glycopyrrolate injection when combination studies were performed.

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