Cardiovascular Response of Chickens to Administration of Nonavian Insulin[1] (35727)

ROBERT P. PITTMAN AND ROBERT L. HAZELWOOD

Department of Biology, University of Houston, Houston, Texas 77004

Avian resistance to exogenous insulin has been reported with the median lethal (convulsive) dose being at least 20 times greater for the chicken than for the rat (1). The criteria for "resistance" has been the absence of the typical mammalian response to pharmacological doses of insulin namely, nervous system dysfunction, a comatose state and/or subsequent death. Insulin resistance in the domestic fowl has been found to be mediated partially through avian plasma factor(s) which prevent full biological expression of injected nonavian insulin (2). It appeared of interest to quantitate further this insulin "resistance" in birds by measuring certain cardiovascular parameters known to be adversely affected in mammals which are injected with insulin.

Materials and Methods. Female single-comb white leghorn chickens (weighing 1.2–1.8 kg each) used in these experiments were maintained at 24 ± 1° and were kept under a periodic light schedule of 12 hr of light/day; feed (growing mash) and water were given *ad libitum*.

Blood glucose (3), plasma volume [T-1824 method (4)], blood volume (calculated using plasma volume and microhematocrit), and extracellular water [SCN^- method (5)] were determined. Both plasma volume and SCN^- space were obtained from a single 0.5-ml blood sample.

Cardiovascular measurements were recorded on an E & M Physiograph. Heart rates and mean electrical axis deviation values were taken from electrocardiograph (EKG) records of standard EKG leads II and III (6). Respiratory rates were determined from impedance pneumograph recordings with needle electrodes inserted at the level of the end of the keel on either side of the chest. End-on blood pressure from the femoral artery was measured by a Statham strain gauge.

Commercial insulin (Iletin, Regular U-40, Eli Lilly) was injected into the alar vein of anesthetized (sodium pentobarbital) birds at dose levels of 1, 10, or 50 units of insulin/kg of body weight. Blood glucose levels, hematocrit (HCT) values, and a 3-min physiograph output (blood pressure, respiratory rate, and EKG) were obtained every 15 min for 105 min. Body fluid volumes were determined at 60 min postinjection on saline-injected animals and on birds injected with 1 and 10 units of insulin/kg of body weight.

Statistical analysis of significance was calculated using both the single classification analysis of variance (ANOVA) and multigroup discriminant analysis, the latter test acting both to reduce the data complexity and to characterize better the cardiovascular observations. Discriminant analysis was performed for seven variables on four experimental groups. The computer output for discriminant analysis includes a two-dimensional plot on which is located the position of the grand mean of all groups, four adjusted means for each of the groups, and seven vectors (extending out from the grand mean) representing each of the seven variables. It should be noted that the two-dimensional plot in this case is actually a representation of a seven-dimensional hyperspace which is mathematically rotated in such a way as to maximize the distance between the groups and at the same time minimize the size of swarm of points of each group. The length and direction of each of the character vectors is determined by the

[1] This work was supported by NSF: GB-6012 and NASA award NsG(T)-52, Sup. 2.

TABLE I. Effect of Insulin on Cardiovascular Observations in Adult Chickens.[a]

State of animal	After injection (min)	Blood glucose (mg/100 ml)	Heart rate (beats/min)	Mean blood pressure (mm Hg)	Pulse pressure (mm Hg)	Mean axis deviation (°)	Hematocrit (%)	Respiratory rate (breaths/min)
Saline injected (13)[b]	15	—4.2 ± 2.9	—9.5 ± 9.4	8.0 ± 2.3	2.2 ± 1.8	0.1 ± 1.9	—0.3 ± 0.4	3.1 ± 0.9
	30	—4.0 ± 2.9	—4.8 ± 7.4	2.0 ± 2.5	—3.4 ± 1.7	2.8 ± 1.8	1.1 ± 0.3	—0.2 ± 1.0
	45	—4.2 ± 3.7	—8.5 ± 6.2	5.2 ± 3.1	—0.8 ± 2.1	2.0 ± 2.1	0.1 ± 0.4	—0.2 ± 1.0
	60	—5.9 ± 4.2	—6.9 ± 8.5	—5.5 ± 5.2	—0.7 ± 2.1	1.9 ± 1.3	0.4 ± 0.5	1.1 ± 1.1
	75	—4.5 ± 4.5	—3.9 ± 8.2	—6.2 ± 3.6	0.2 ± 3.0	1.7 ± 1.3	0.3 ± 0.4	1.5 ± 1.2
	90	—5.7 ± 4.8	1.1 ± 8.1	—7.6 ± 3.3	0.3 ± 3.1	3.0 ± 1.5	—0.1 ± 0.6	1.6 ± 1.2
	105	—5.3 ± 4.5	17.5 ± 9.7	—11.1 ± 2.6	3.7 ± 2.9	3.7 ± 1.8	—0.3 ± 0.6	3.8 ± 1.5
Insulin, 1 unit/kg of body wt (13)	15	—38.8 ± 3.7	17.8 ± 5.0	—10.5 ± 2.2	5.3 ± 3.5	2.5 ± 1.4	0.6 ± 0.2	2.5 ± 0.9
	30	—59.4 ± 5.7	17.8 ± 4.7	—13.7 ± 3.3	13.2 ± 2.9	3.3 ± 1.4	0.9 ± 0.3	4.8 ± 1.1
	45	—70.5 ± 7.1	—5.5 ± 12.0	—1.6 ± 3.8	5.8 ± 2.2	2.2 ± 0.9	0.4 ± 0.4	7.2 ± 1.8
	60	—73.7 ± 8.4	0.3 ± 12.4	—3.2 ± 4.8	3.0 ± 3.3	2.5 ± 1.6	2.2 ± 0.4	6.7 ± 1.4
	75	—80.0 ±10.4	—0.2 ± 13.0	—1.2 ± 4.4	1.5 ± 3.3	3.2 ± 1.6	1.4 ± 0.3	6.0 ± 1.5
	90	—81.1 ± 8.6	1.7 ± 13.6	—2.0 ± 4.8	5.6 ± 2.4	1.5 ± 1.1	1.0 ± 0.2	6.2 ± 1.8
	105	—79.5 ±10.2	20.2 ± 13.5	—5.8 ± 3.8	7.9 ± 2.9	3.7 ± 1.6	0.7 ± 0.3	5.3 ± 1.8
10 units/kg of body wt (9)	15	—32.6 ± 4.9	11.6 ± 7.4	—18.8 ± 8.7	12.7 ± 8.3	2.6 ± 1.7	1.0 ± 0.3	2.0 ± 0.8
	30	—51.2 ± 5.4	13.9 ± 13.8	—23.6 ± 9.6	10.5 ± 7.3	4.6 ± 1.7	1.4 ± 0.4	3.0 ± 1.7
	45	—68.2 ± 6.8	10.9 ± 16.6	—19.8 ±10.3	3.3 ± 6.9	3.4 ± 2.0	1.0 ± 0.4	4.0 ± 2.2
	60	—71.4 ± 6.8	15.9 ± 16.4	—24.8 ± 9.7	—5.0 ± 4.1	2.6 ± 1.7	3.8 ± 0.6	3.9 ± 1.9
	75	—76.4 ± 7.6	21.6 ± 18.2	—16.7 ±11.9	—0.7 ± 5.6	2.2 ± 1.2	2.4 ± 0.4	5.3 ± 1.8
	90	—82.2 ± 8.5	31.6 ± 16.1	—12.2 ±10.7	—4.5 ± 5.0	3.1 ± 1.6	2.9 ± 0.4	8.0 ± 2.0
	105	—81.1 ± 9.0	45.6 ± 12.0	—16.6 ±11.0	—0.5 ± 6.7	3.9 ± 1.4	1.9 ± 0.5	10.0 ± 3.6
50 units/kg of body wt (5)	15	—38.4 ± 1.6	25.0 ± 10.3	—7.8 ± 6.0	12.8 ± 5.0	2.0 ± 2.6	1.3 ± 0.4	6.4 ± 3.0
	30	—54.4 ± 2.2	32.8 ± 14.2	—6.4 ± 7.1	10.8 ± 7.0	3.0 ± 2.1	1.9 ± 0.4	4.6 ± 3.6
	45	—67.2 ± 2.2	30.4 ± 17.0	—9.9 ± 8.4	11.0 ± 7.5	3.0 ± 3.0	1.6 ± 0.3	7.0 ± 4.1
	60	—79.6 ± 4.1	40.0 ± 13.9	—13.1 ± 7.0	8.8 ± 7.0	3.8 ± 2.4	2.0 ± 0.4	9.4 ± 4.5
	75	—80.8 ± 5.0	44.8 ± 16.0	—14.8 ± 6.2	8.8 ± 8.4	4.4 ± 2.9	2.1 ± 0.2	9.4 ± 4.8
	90	—88.2 ± 7.8	49.0 ± 14.6	—12.5 ± 7.2	5.4 ± 8.7	2.6 ± 2.4	1.7 ± 0.5	9.3 ± 4.8
	105	—86.5 ± 7.2	63.2 ± 19.6	—11.0 ± 4.4	7.2 ± 5.6	4.0 ± 3.5	1.4 ± 0.4	10.2 ± 4.8

[a] Data expressed as change from initial and recorded as mean ± SEM. Note that statistical treatment for these data is in Table II.
[b] Sample size given in parentheses.

degree of correlation among the characters and by the relative difference between the group means. The computer program also includes computations for the degree of overlap between groups which in turn indicate the significance of the discrimination between group means and the degree of overlap present (7).

Results. The results presented in Table I are expressed as changes from initial levels (before saline or insulin injection). The following are initial values with ranges in parentheses for the seven variables: blood glucose, 195.6 mg/100 ml (173–231); heart rate, 300.0 beats/min (222–372); mean blood pressure, 113.2 mm Hg (82–150); pulse pressure, 43.1 mm Hg (30–51); mean electrical axis deviation, —98.7° (95–105); hematocrit, 25.9% (19–35); and respiratory rate, 25.1 breaths/min (12–33). It is apparent from Table I that the decrement in blood glucose did not differ significantly among the three insulin-injected groups (over the 105-min observation period). At each time period, ANOVA was used to determine significant differences (where present) among the saline-injected birds and the three insulin-injected groups of birds (Table II). ANOVA was computed also for the three insulin groups omitting the saline-injected group, the result being that none of the insulin-injected groups were significantly different from each other except for hematocrit values 60 min after insulin injection (significant at $p<0.05$). Multiple-group discriminant plots for 15 and 75 min after insulin injection are shown in Figs. 1 and 2 with blood glucose acting as an extremely good discriminator. As the degree of discrimination among groups is followed through time, the 50-unit insulin group separates out from the other groups 60 min after insulin with heart rate serving as an important discriminator. There was some distinction among groups during the early stages of the experiment, but the degree of continuing overlap through time does not allow any significant separation of the group clusters. Plasma volume, blood volume, and SCN^- space expressed as percentage of body weight

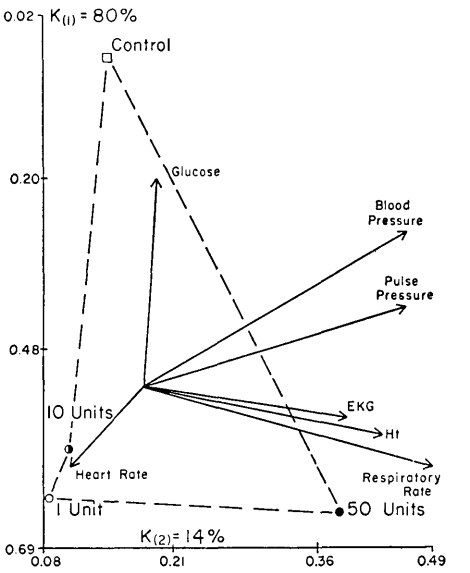

FIG. 1. Discriminant analysis plot (15 min after insulin injection).

TABLE II. ANOVA Summary for Cardiovascular Data.
Three insulin groups plus one control; F values $df = 3.36$.

After injection (min)	Blood glucose	Heart rate	Mean blood pressure	Pulse pressure	Mean axis deviation	Hematocrit	Respiratory rate
15	21.73[c]	3.41[a]	6.74[b]	1.42	0.50	3.87[a]	1.76
30	33.69[c]	2.54	4.43[b]	3.87[a]	0.18	1.15	2.74
45	32.62[c]	1.32	3.36[a]	1.56	0.13	2.03	3.43[a]
60	28.13[c]	1.90	2.21	0.38	0.16	8.16[b]	3.27[a]
75	15.74[c]	1.99	1.14	0.73	0.40	5.45[b]	2.64
90	23.68[c]	3.08[a]	0.68	1.22	0.46	4.12[a]	2.86[a]
105	19.94[c]	1.82	1.32	0.21	0.01	1.65	1.04

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.

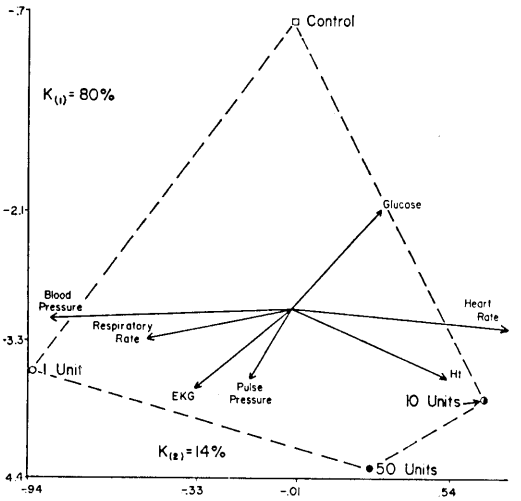

FIG. 2. Discriminant analysis plot (75 min after insulin injection).

60 min after injection are tabulated in Table III for saline injected and for 1 and 10 units of insulin injected/kg of body weight. There were no significant shifts in fluid volumes for the two doses of insulin used.

Discussion. The avian reaction to exogenous insulin differs from that observed in other animals (1). The "resistance" of Aves to insulin could be a reflection of utilization by the central nervous system (CNS) of glucose other than that provided by plasma or dependence of the CNS upon substrates other than carbohydrate (fatty acids, ketone bodies, etc.). Table I indicates that "effective" plasma insulin concentrations were established in all three groups, even though quantitative differences in the degree and duration of hypoglycemia were not significant when comparing any two of the experimental groups. It is known that the chicken pancreas is well endowed with α-cells (8), that at least six times as much glucagon can be extracted per gram of fresh tissue weight as that from mammalian pancreas (9), and that glucagon (but not catecholamine) is a powerful lipolytic agent in birds, readily releasing free fatty acids from depots (10–12). If glucagon were released as in response to hypoglycemia the results would be hepatic glycogenolysis and an increased release of fatty acids and ketone bodies for possible CNS metabolism. That this may not obtain is indicated by the report that the hyperglycemic rebound (and presumably fatty acid release, too) following repeated insulin injections is not prevented by previous pancreatectony in birds (13). However, insulin increases plasma free fatty acids in chickens (14) and our laboratory has observed a concomitant depression in cerebrospinal fluid (CSF) levels of stearic, oleic, and linolenic acids during a 47% reduction in plasma glucose levels 60 min after insulin injection (Hunzicker and Hazelwood, unpublished data). There are no reports, however, relative to the acute effects of glucagon on fatty acid utilization by the avian CNS, an area which merits further investigation. Finally, the observation that insulin lowers both avian plasma and CSF glucose renders unlikely the possibility that constant CSF glucose levels in face of an hypoglycemic crises provides metabolic stability to the avian CNS, thereby protecting the bird from neural dysfunction (15).

Evidence has been presented by Hazel-

TABLE III. Effect of Beef Insulin on Body Fluid Volumes in Adult Chickens. Data as percentage body wt 60 min postinsulin.[a]

Group	Plasma vol	Blood vol	SCN^- space
Saline control (16)[b]	4.7 ± 0.2[c]	6.3 ± 0.2	24.6 ± 1.0
1 Unit/kg of body wt (17)	5.1 ± 0.1	6.9 ± 0.1	26.6 ± 0.9
10 Units/kg of body wt (13)	4.1 ± 0.2	5.9 ± 0.3	24.8 ± 1.2

[a] Note that none of the values are significantly different from each other.
[b] Number of observations in parentheses.
[c] Mean ± SEM.

wood *et al.* (2) that avian plasma factors reduce (but do not abolish) the effective plasma insulin concentration. Such plasma factors could bind irreversibly to insulin or to peripheral receptor sites for insulin. Low molecular weight, heat labile avian plasma factors have been shown to depress significantly the ability of beef insulin to encourage glucose uptake; however, these factors alone cannot account entirely for the avian resistance to insulin (2).

Cardiovascular dependent shifts in body fluid volumes (as a compensation to insulin injection) conceivably could alter plasma insulin levels by plasma volume expansion and thereby diluting circulating hormone levels. Plasma volume determinations obtained in the present investigation were supplemented with measurements of blood volume and SCN^- space which act as a check on plasma volume data. Also, cardiovascular measurements were monitored because such changes result from predictable inputs from the endocrine and nervous systems, thus making cardiovascular observations a useful device to locate additional *in vivo* mechanisms employed by the bird during insulin hypoglycemia.

Human and canine cardiovascular systems respond to insulin hypoglycemia with increased systolic pressure, decreased diastolic pressure, slightly decreased mean blood pressure (or no change), increased heart rate, and increased cardiac output (16–20). Cardiovascular responses to decreased blood glucose levels could be the result of the rate of developed hypoglycemia and/or the magnitude of hypoglycemia as has been found in similar studies on ACTH secretion in mammals (21). In the present study observations on heart rate, blood pressure, pulse pressure, and mean electrical axis deviation were taken while the rate of fall of glucose was maximum as well as when the lowest level of blood glucose had been achieved. Data so obtained indicate significant changes in blood pressure and pulse pressure when blood glucose was undergoing the most rapid rate of change with trends toward tachycardia not being observed until 60 min later (Table II). The temporary increase in pulse pressure and the trend toward depressed mean blood pressure (Table I) which are recorded here for birds are in accord with similar studies performed in humans (18). Since the fluid compartments were not affected by insulin administration (Table III) in the present study, such volume shifts or cardiovascular alterations do not appear of sufficient magnitude to explain the so-called "avian resistance" to insulin.

Discriminant analysis (see Methods for rationale) did not reveal any significant information above that given by ANOVA except in the case of the group of birds receiving 50 units of insulin/kg of body weight. This group, receiving the highest dosage of insulin, is separated from other groups most likely because cardiovascular changes incurred lasted a longer period of time after insulin administration. Other than this difference, the reaction of the three groups of chickens to different (pharmacological) doses of insulin was quantitatively similar, indicating attainment of equivalent "effective" plasma insulin concentrations. Conclusions drawn from discriminant analysis are similar to those drawn from ANOVA: relative stability of variables exists indicating that the 50% depression in blood glucose following insulin does not precipitate a physiological crisis in the domestic fowl. However, the insulin-induced tachycardia 60 min after injection indicates possible endocrine homeostatic (catecholamine?) compensation effected by the hypoglycemic crises.

Summary. Alterations in heart rate, blood pressures, pulse pressure, mean electrical axis, hematocrit, plasma volume, interstitial fluid volume and total blood volume were measured and statistically analyzed in adult chickens receiving pharmacological doses of bovine insulin. Multiple-group discriminant analysis techniques proved valuable in a critical analysis of the data obtained. Cardiovascular alterations or shifts in body fluid volumes do not appear of sufficient magnitude to explain the so-called "avian resistance" to exogenous insulin.

The authors express their gratitude for the expert statistical assistance rendered by Roger T. McFadden.

1. Shao, T., and Hill, D. C., Can. J. Physiol. Pharmacol. **45**, 225 (1966).

2. Hazelwood, R. L., Kimmel, J. R., and Pollock, H. G., Comp. Physiol. Biochem. (1971), in press.

3. Somogyi, M., J. Biol. Chem. **195**, 19 (1952).

4. Hunsaker, W. G., Proc. Soc. Exp. Biol. Med. **120**, 747 (1965).

5. Bowler, R. G., Biochem. J. **38**, 385 (1944).

6. Sturkie, P. D., Amer. J. Vet. Res. **10**, 168 (1949).

7. Cooley, W. W., and Lohnes, P. R., "Multivariate Procedures for the Behavioral Sciences." Wiley, New York (1962).

8. Mikami, S. I., and Ono, K., Endocrinology **71**, 464 (1962).

9. Vuylsteke, C. A., and deDuve, C., Arch. Int. Physiol. **61**, 273 (1953).

10. Goodridge, A. G., Amer. J. Physiol. **214**, 902 (1968).

11. Grande, F., Proc. Soc. Exp. Biol. Med. **128**, 532 (1968).

12. Grande, F., and Prigge, W. F., Amer. J. Physiol. **218**, 1406 (1970).

13. Riddle, O., and Opdyke, D. F., Carnegie Inst. Publ. **n569** (1947).

14. Heald, P. J., McLachlan, P. M., and Rookledge, K. A., J. Endocrinol. **33**, 83 (1965).

15. Anderson, D. K., and Hazelwood, R. L., J. Physiol. (London) **202**, 83 (1969).

16. DiSalvo, R. J., Bloom, W. L., Brust, A. A., Ferguson, R. W., and Ferris, E. B., J. Clin. Invest. **35**, 568 (1956).

17. Werk, E. E., Jr., Garber, S., and Sholiton, L. J., Metabolism **10**, 115 (1961).

18. Arner, B., Hedner, P., Karlefors, T., and Westling, H., Acta Endocrinol. **44**, 430 (1963)

19. Goldberg, E., and Rosenblum, I., Amer. Heart J. **72**, 482 (1966).

20. Hiatt, N., Katz, J., and Sheinkopf, J. A., Endocrinology **87**, 186 (1970).

21. Matsui, N., and Plager, J. E., Endocrinology **81**, 737 (1966).

Received Feb. 8, 1971. P.S.E.B.M., 1971, Vol. 137.