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Effects of Adrenaline Dose on Plasma Insulin and Glucagon Concentrations in the Hen

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Summary

Effects of two doses of intravenously injected adrenaline (0.10 and 0.25 mg/kg body weight) on plasma insulin, glucagon, glucose and free fatty acid concentrations were investigated in paused White Leghorn hens.

Plasma insulin concentrations decreased corresponding to the dose of adrenaline injected but no significant difference was observed between the two doses. On the contrary, plasma glucagon increased dose-dependently. Plasma glucose and free fatty acid concentrations were not obviously affected by the two doses of adrenaline.

The action of adrenaline to the fowl is thought to be the same as to the mammal. In our previous experiment[1], however, the intravenous injection of adrenaline (0.05 mg/kg of body weight) did not have a clear influence on the insulin and glucagon concentrations in the plasma.

The amount of 0.05 mg/kg of adrenaline has been proven to be enough to affect the blood insulin and glucagon concentrations in sheep[2].

The purpose of the present investigation was to know the effects of different doses of intravenously injected adrenaline on the concentration of plasma insulin or glucagon from the view-point of dose-response relationship.

The influences of adrenaline dose on the plasma glucose and free fatty acid were also measured at the same time.

Materials and Methods

Fowls: Fourteen paused White Leghorn hens, aged 215 days, were used. They were separately housed in cages under a controlled air temperature of 18-22°C with a condition of 12 h light (06 : 00-18 : 00) and 12 h dark (18 : 00-06 : 00).

They were fed with 120 g of commercial formula feed once daily at 09 : 00. Water was available continuously.

A multi-purpose catheter (Atom multi-purpose 4F, 1.35 mm, o.d.) for adrenaline injection and for drawing blood samples was inserted into the left jugular vein under local anesthesia with procain hydrochloride a day before the experiment. The surgical method for fixing the catheter to the vein was the formerly reported procedure[1].

The catheter was flushed several times in a day until the start of the experiment and was filled with 100 U/ml of heparin dissolved in sterile saline solution.

Experimental procedure : Just before the start of the experiment the hen was moved to a wooden holding stand to keep her in a standing posture.

Each 3 ml of blood was sampled through the catheter at −5, 5, 10, 20 and 40 min after the injection of adrenaline.

The experiment was started at 13 : 30 and lasted for 45 min.

Each group, consisting of five hens, was allocated to (1) adrenaline (0.10 mg/kg) and (11) adrenaline (0.25 mg/kg) injection test.

The remaining four hens were given saline injections (0.5 ml/kg) as a control group. Adrenaline (Sigma Co) was dissolved in saline solution (0.2 and 0.5 mg/ml).

Analyses were made on plasma insulin, glucagon, glucose, free fatty acid (FFA) and protein concentrations. The hematocrit, respiration rate and rectal temperature were measured simultaneously.

Total amount of the increase (glucagon, glucose and FFA) and the decrease (insulin) above or below the base line value due to adrenaline injection are expressed as the area covered with the concentration curve of each of the blood constituents for forty minutes. The concentration obtained at the time of −5 min was used as the base line value of the above calculation.

Analysis : Two ml of blood taken out from 3 ml of blood sample was transferred into a heparinized syringe and was placed on ice. The analytical method used for the estimation of hematocrit was a microhematocrit tube, for plasma protein was a refractometer, for plasma glucose was colorimetry (Wako glucose test kit) and for plasma FFA was ITAYA and UI[3] method. The plasma for glucagon assay which was added to aprotinine (500 kallikrein inhibitor units/ml blood) and for insulin assay were stored −20°C until analysis.

Plasma glucagon was determined by a radioimmunological method[1].

Plasma insulin was determined by the method of HERBERT et al[4].

Statistics : Mean values and SE were given in both tables and figures. The significant difference of data between baseline and subsequent time within each injection and between injections at each sampling time were determined using paired *t*-test.

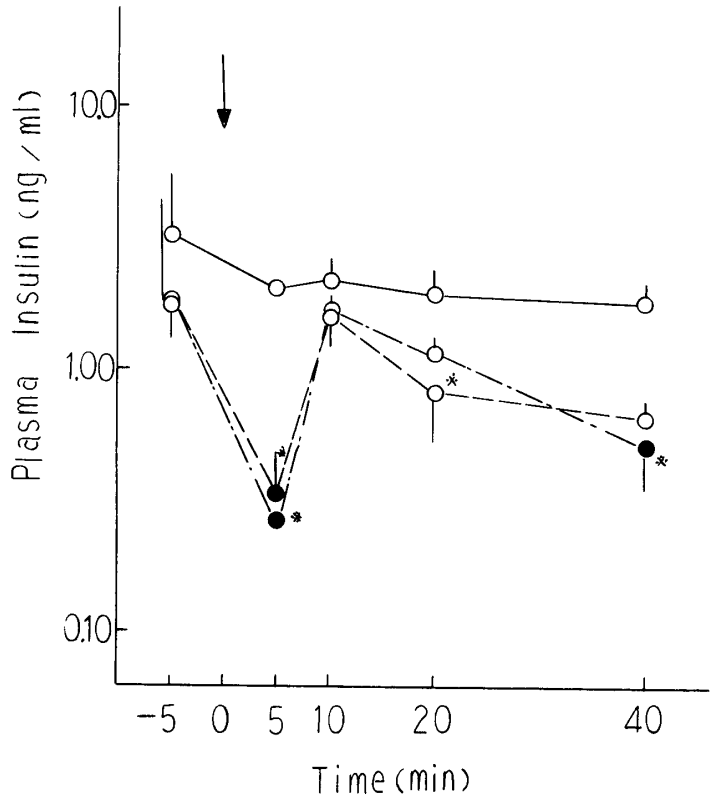

FIG. 1. Effects of intravenous injection (arrow) of saline (——), 0.10 mg/kg adrenaline (— —) and 0.25 mg/kg adrenaline (— · —) on plasma insulin concentration in hens. The mean values for five are shown, The vertical line represents the SE. Closed circle indicates the significant differences ($P<0.05$) from the pre-injection values.
*Asterisk indicates the significant differences of the same time values befween saline and each injection at the level of $P<0.05$.

Results

The pattern of decrease of plasma insulin concentrations with a sharp drop at 5 min after injection was the same with the two doses of adrenaline (FIG. 1).

Plasma glucagon increased in response to the dose of adrenaline.

At 40 min after the injection, the higher level of concentration than the base line level of glucagon was still maintained with the 0.25 mg/kg of adrenaline injection but the same level as control was observed with the 0.10 mg/kg injection (FIG. 2).

Significant increase of plasma glucose above the initial level was only demonstrated with the 0.25 mg/kg of adrenaline injection.

The increased concentration of glucose was continuously observed throughout the experimental period. In case of the 0.10 mg/kg of adrenaline injection, however, significant increase compared to the initial level was only observed at 5 min after injection (FIG. 3).

The plasma FFA increased with the two doses of adrenaline and with the saline injection. No significant difference was observed between the three injections (FIG. 4).

The incremental (glucagon, glucose and FFA) and decremental (insulin) areas are shown in Table 1. Though the incremental area of glucagon, glucose and FFA depended on the dose of injection of adrenaline, the significant difference was only found in that of glucagon.

As shown in Table 2, the rectal temperature decreased significantly at 40 min after 0.25 mg/kg of adrenaline injection but did not change after the 0.10 mg/kg of adrenaline or saline injection.

The respiration rate was unchanged following the adrenaline and saline injections. The hematocrit value decreased significantly at 40 min after saline

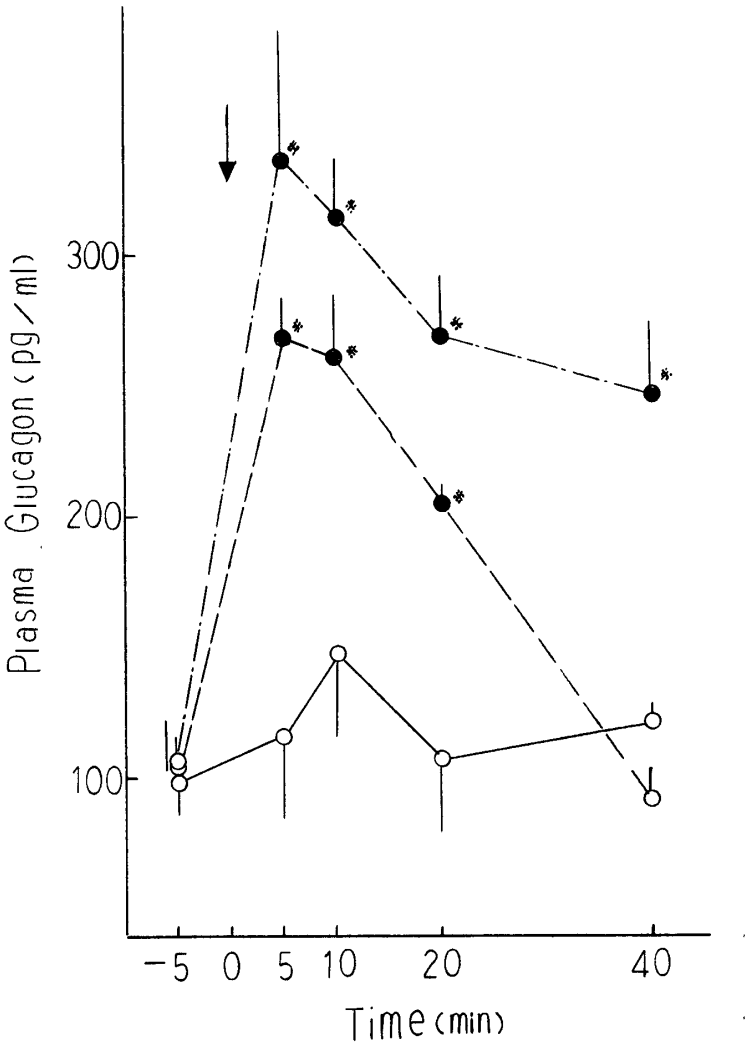

FIG. 2. Effects of intravenous injection (arrow) of saline (——), 0.10 mg/kg adrenaline (— —), 0.25 mg/kg adrenaline (— - —) on plasma glucagon concentration in hens. Symbols are the same as explained in Fig. 1.

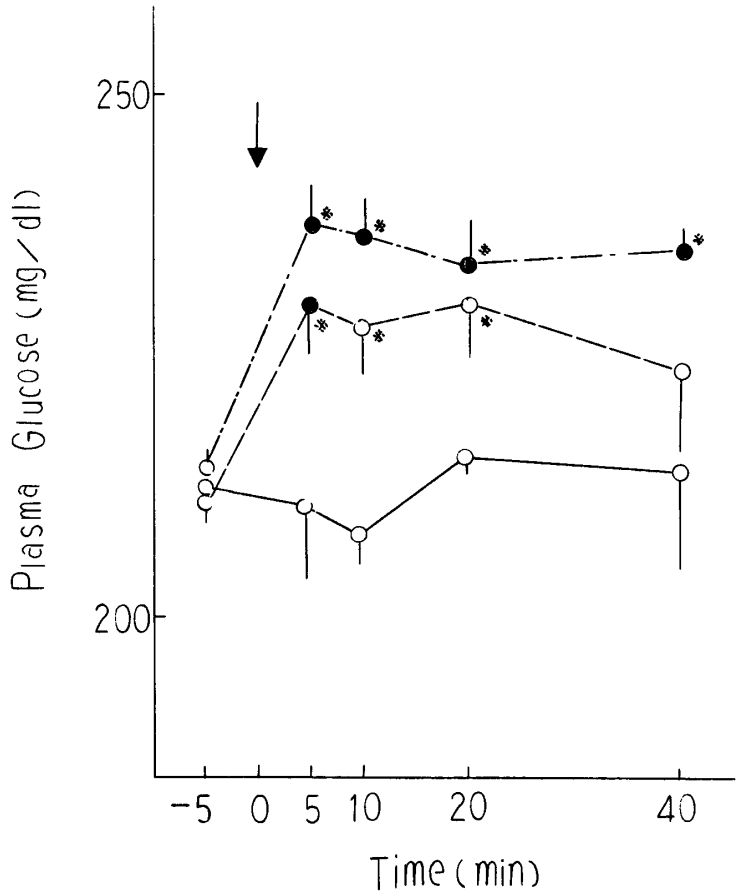

FIG. 3. Effects of intravenous injection (arrow) of saline (——), 0.10 mg/kg adrenaline (— —), 0.25 mg/kg adrenaline (— - —) on plasma glucose concentration in hens. Symbols are the same as explained in Fig. 1.

injection, and the plasma protein concentration was maintained at a constant level throughout the experimental period with all injections

Discussion

As shown in Table 1, the plasma concentration of insulin decreased with the injection of adrenaline. The observation was similar to the results obtained in sheep as weel as a number of other animal species[2]. We have no data to speculate on the mechanism of the above results but they could be explained in that insulin secretion was inhibited through the action of α-adrenergic receptor in B cells in pancreas[5] which was stimulated by the injected adrenaline. In addition, the elevated blood glucose concentration induced by adrenaline could not reach a level high enough to stimulate insulin secretion.

The plasma glucagon increased significantly and dose-dependently with the injection of adrenaline. It is known that glucagon secretion is increased by stimulation of α-adrenergic receptor in A cell in ducks[6]. It may be reasonable

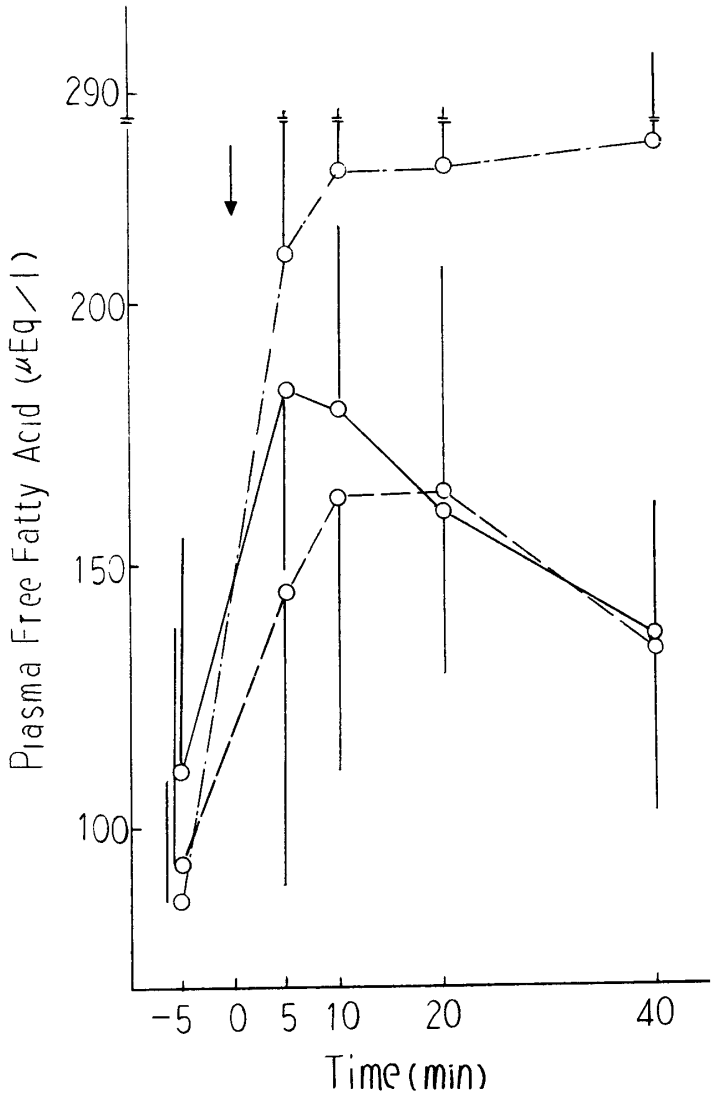

FIG. 4. Effects of intravenous injection (arrow) of saline (——), 0.10 mg/kg adrenaline (— —), 0.25 mg/kg adrenaline (— - —) on plasma FFA concentration in hens. Symbols are the same as explained in Fig. 1.

TABLE 1. *Values of Incremental (Glucagon, Glucose and FFA) and Decremental (Insulin) Areas due to Two Doses of Adrenaline Injections in the Hen*

Adrenaline mg/kg	Insulin ng/min•ml^{-1}	Glucagon pg/min•ml^{-1}	Glucose mg/min•dl^{-1}	FFA Eq/min•l^{-1}
0.10	−2232+329	3864±394	2499±194	3997±2119
0.25	−1908±201	5202±237*	3382±172	14020±2968

The calculating method of area: See text.
Values represent mean with SE of mean
* The significant difference between saline and each injection at the level of $P<0.05$

TABLE 2. *Rectal Temperature, Respiration Rate, Hematocrit Values and Plasma Protein Level before and after Injection of Two Doses of Adrenaline in the Hen*

Hormone	Rectal temperature (°C)		Respiration rate (breaths/min)	
	−5 min	40 min	−5 min	40 min
Saline	41.3±0.22	41.8±0.25	31.3±0.06	30.3±4.51
Adrenaline (0.10 mg/kg)	40.2±0.18	41.7±0.32	33.8±0.97	30.4±2.16
Adrenaline (0.25 mg/kg)	41.9±0.17	41.3±0.10	31.6±2.48	31.6±2.11

Hormone	Hematocrit values (%)		Plasma protein values (%)	
	−5 min	40 min	−5 min	40 min
Saline	27.3±2.38	25.3±2.54*	5.4±0.14	4.9±0.15
Adrenaline (0.10 mg/kg)	28.3±1.90	27.0±1.91	5.5±0.21	5.2±0.25
Adrenaline (0.25 mg/kg)	28.6±1.91	27.7±1.91	5.4±0.24	5.2±0.22

Values respresent Mean with SE of mean

* Significant difference between values 5 min before and 40 min after injection at the level of $P<0.05$

to assume that chickens (*Gallus domestics*) also have the same secretory activities as ducks.

The plasma glucose increased dose-dependently by adrenaline injection though no statistically significant differences were found between the two doses (Table 1), FREEMAN and MANNING[6] reported that the intramuscular injection of 300 μg/kg adrenaline in hens caused an increase of blood glucose as much as 100 mg/kg. In our present study, however, only 30 mg/kg of glucose increase from the control level was observed following the intravenous injection of 250 μg/kg adrenaline. The different responses to adrenaline administration on plasma glucose in the above two experiments might be due to the differences of experimental conditions and of ages of chickens used.

Comparing adrenaline potency on the effects of glucose mobilization between several species, it would be smaller in chickens than rats[7] and sheep[8]. In regards to the mechanism of glucose increase with adrenaline in chickens, the main pathway is assumed to be not the direct glycogenolysis but the indirect action of glucagon which was secreted by the stimulation of adrenaline[6].

No significant difference of FFA was observed between adrenaline and saline injections. The reason of the augumentation of FFA due to saline injection is not known but the shock of injection of cold solution might be one reason for it.

The significant decrease of hematocrit value with the three kinds of solution injections would result from the drawing out of the relatively large amount of blood (about 15 ml in total) within a short period (45 min). In sheep[7], however,

the hematocrit value increased during the injection of adrenaline which caused the mobilization of reserved erythrocytes in the spleen by the stimulation of sympathetic nerve[9,10]

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