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Hyperinsulinemia and Its Role in Maintaining the Hypothalamic Hyperphagia in Chickens

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SONODA, T. *Hyperinsulinemia and its role in maintaining the hypothalamic hyperphagia in chickens.* PHYSIOL BEHAV 30(3) 325–329, 1983.—Electrolytic lesions of the ventromedial hypothalamus including nucl. hypothalamicus inferior caused the cockerels to become hyperphagic. The plasma insulin level of the control birds was low before feeding and it increased after feeding, while that of the hyperphagic birds was higher before feeding and increased even more after feeding. Under the fasting condition on the 19th day, the insulin secretion of the hyperphagic birds remained at a high level, and there was a high correlationship between the insulin value and the food consumption. Hyperactivity of insulin secretion in the hypothalamic hyperphagic birds was inhibited by administration of diazoxide and their insulin levels declined to a level similar to that of the controls. As a result the hyperphagia was interrupted and the daily food consumption declined to the same level as the controls. Therefore, the hyperinsulinemia seemed to be necessary for maintaining the hypothalamic hyperphagia in chickens. The role of glucagon in this phenomenon is also discussed.

Ventromedial hypothalamus Hyperphagia Insulin Chickens

MANY authors have reported that circulating insulin levels were increased in hypothalamic hyperphagic rats [4,6], and that the inhibition of hyperinsulinemia by diabetogenic agents was accompanied by depression of food intake [1, 3, 25]. These findings suggest the importance of hyperinsulinemia in maintaining the hypothalamic hyperphagia in rats. However, no evidence has been reported so far about the change in insulin levels in hypothalamic hyperphagic chickens. The present experiments were performed to confirm the hyperactivity of insulin secretion in hypothalamic hyperphagic chickens by observing the changes in insulin levels after feeding. To examine the relationship between hyperinsulinemia and hyperphagia, the changes in insulin levels under fasted conditions and after the administration of a diabetogenic agent were studied.

METHOD

Animals

Thirty White Leghorn cockerels of six months in age, whose average body weight was 1.9 kg, were raised separately in a temperature-conditioned room (22°C), under 12 hr lighting schedule, and were given commercial mash feed (New Layer, Chubu Shiryo Ltd., M.E. 2.7 kcal/g) at 0900 every morning and fed ad lib. Food consumption was recorded daily and body weight was measured on the third day after surgery and thereafter weekly. The birds were handled daily so as to allow the birds to adapt to handling, and thereby to reduce the stress to the birds during blood withdrawal.

Hypothalamic Lesion

The operation was performed under nembutal anethesia (25 mg/kg, intravenously). A stainless-steel electrode of 0.6 mm o.d., insulated with Casu except about 0.5 mm of the tip, was inserted into the ventromedial part of the hypothalamus, i.e., nucl. hypothalamicus inferior (IH), probably the satiety center in chickens [14, 19, 22], using a stereotaxic instrument, following the brain atlas of van Tienhoven and Juhasz [24], while the indifferent electrode was attached to the tip of the comb. Electrolytic lesion was made by an anodal current of 2 mA for 20 sec. Six birds were sham-operated for control experiments.

The cockerels were autopsied 30 days after surgery. The serial frozen sections of the brain were stained with cresyl-violet and the sites of the lesion were ascertained three-dimensionally using a small projector.

The hypothalamic lesion sites in eight operated birds, as shown in Fig. 1, were distributed in the ventromedial part of the hypothalamus and their common area was in IH. These birds showed the typical signs of hypothalamic hyperphagia with 50% increase in food intake after the hypothalamic destruction. On the other hand, the other 16 operated birds, whose lesion sites included little or no IH, did not show any increment of food intake.

Variations of Food Intake and Body Weight of the Hyperphagics

A two-fold increase in the daily food consumption was observed in the hyperphagic birds from the 9th day following the hypothalamic lesion, though from the 25th day a reduc-

tion in food intake. Estimation of body weight indicated a 300 g increase from the 3rd day to the 30th day following the lesion.

Treatment

Variation of insulin level after feeding. Blood samples were drawn via the wing vein of the concious birds, just before feeding (0900) and 2, 3 and 5 hours after feeding, on the 14th to 18th day after operation. Blood was collected every other day, as not to irritate the birds.

Changes in insulin level after fasting. Some experimental birds were fasted on the second and 19th day after operation, for 15 hr from 1800 in the evening to 0900 in the next morning.

Diazoxide administration. In the present experiment, diazoxide (American Scherring) at a dose of 20 mg/kg was added to the diet and was given for four days from the 22nd to 25th day after operation to five hyperphagic and five control birds. Blood samples were taken at 1400 every other day.

Insulin Determination

Plasma insulin values were determined by a double-antibody radioimmunoassay [7], using a commercial insulin assay system (anti-guinea pig labelled porcaine, Dainabot/Tokyo). Chicken insulin was used as the standard (gift from Prof. Hazelwood, CH-Panc-I-172).

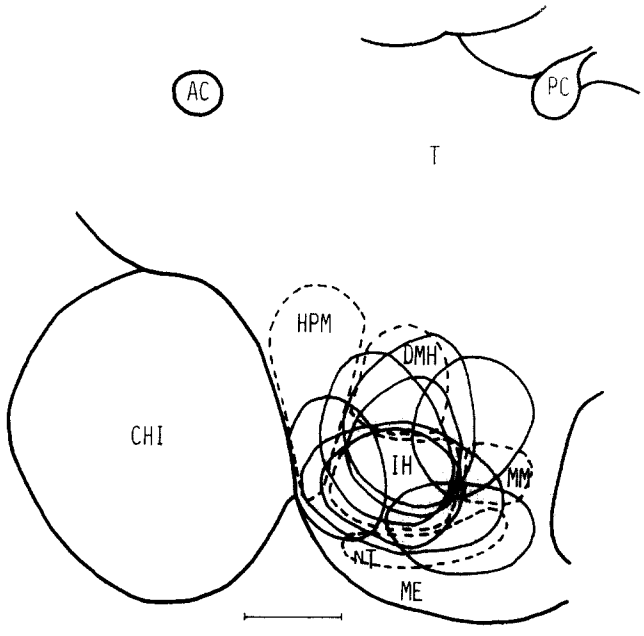

FIG. 1. The sites of lesion in hypothalamic hyperphagic cockerels (saggital section). Dotted line: nucleus, solid line: boundary of the lesion in each bird, AC: anterior commisure, CHI: optic chiasma, DMH: nucl. hypothalamicus dorsomedialis, HPM: nucl. hypothalamicus postrior medialis, IH: nucl. hypothalamicus inferior, ME: median eminence, MM: nucl. mammilaris medialis, nT: nucl. tuberis, PC: posterior commisure, T: thalamus. Calibration bar = 1 mm.

RESULTS

Variation of Insulin Levels after Feeding

The plasma insulin concentration of the controls was 17.1 μU/ml before feeding. The insulin level increased significantly after feeding ($p<0.05$), the values at the 2nd, 3rd and 5th hour after feeding being 30.1, 46.9 and 31.5 μU/ml, respectively. On the other hand, the insulin concentration in the hyperphagics was 38.2 μU/ml before feeding and increased significantly ($p<0.05$) to 58.1, 56.7 and 56.1 μU/ml at the 2nd, 3rd and 5th hour after feeding, and each value was significantly higher ($p<0.05$) than that of the controls except for the 3rd hour ($p<0.10$). Each insulin value of the non-hyperphagic birds did not differ from that of the controls (Table 1).

Changes in Insulin Level after Fasting

Under fasting condition on the second day after operation the insulin value of the fasted hyperphagic birds was 30.9 μU/ml and did not differ from that of the controls. There was a slight correlationship ($r=0.39$, $n=26$, $p<0.05$) between the insulin value and the body weight increase of the whole experimental birds for one week from the 4th to 10th day after operation (Table 2).

On the 19th day the insulin value of the fasted hyperphagic birds was 36.3 μU/ml, and significantly higher ($p<0.05$) than that of the fasted control birds. There was a close correlationship ($r=0.67$, $n=21$, $p<0.01$) between the insulin value and the food consumption of the whole experimental birds for 30 days after operation (Table 3).

Changes in Insulin Level and Food Intake Following Administration of Diazoxide

After administration of diazoxide, the average insulin level of the controls showed a slight decrease in the 4th day. The insulin level (53.8 μU/ml) of the hyperphagics was significantly higher ($p<0.05$) than that (31.3 μU/ml) of the controls before diazoxide administration. However it decreased after administration and the values on the 2nd and 4th day were 35.6 and 37.8 μU/ml, respectively (Fig. 2).

The daily food consumption of the controls was 80 to 100 g before administration and did not change clearly after administration of diazoxide. On the other hand, the daily food consumption of the hyperphagics was about 150 g before diazoxide administration and was much higher than that of the controls ($p<0.01$). It decreased greatly following the start of administration, with recovery after the end of diazoxide administration.

DISCUSSION

Many authors have reported increased secretion of insulin from the pancreatic islets in hypothalamic hyperphagic rats [4,6] and the interruption of hyperphagia by the inhibition of excessive insulin secretion [1, 3, 25]. Since administration of insulin to rats causes hyperphagia and obesity [16,18], the above-mentioned findings lead us to suspect that the hyperinsulinemia should play an essential role in the occurrence or maintenance of the hypothalamic hyperphagia.

In the present experiment, the insulin level of the fed hypothalamic hyperphagic chickens was about two times higher than that of the controls, indicating facilitation of insulin secretion. To examine whether this facilitation of insulin secretion is a primary effect of hypothalamic destruction or a secondary result of increased food intake, the hyper-

TABLE 1

VARIATION OF PLASMA INSULIN LEVEL IN CONTROL AND HYPOTHALAMIC HYPERPHAGIC AND NON-HYPERPHAGIC COCKERELS BEFORE AND AFTER FEEDING (μU/ml)

Group	Hours After Feeding 0	2	3	5
Control	17.1 ± 8.2(4)	30.1 ± 8.8(4)†	46.9 ± 26.2(4)†	31.5 ± 14.7(4)
Hyperphagic	38.2 ± 21.7(4)*	58.1 ± 8.2(4)*†	56.7 ± 28.3(4)	56.1 ± 17.3(4)*†
Non-Hyperphagic	18.1 ± 0.9(2)	37.2 ± 14.7(6)†	50.8 ± 22.2(2)	37.8 ± 13.7(6)

Mean ± S.D. (NO.), *$p<0.05$ (vs control), †$p<0.05$ (vs 0 time).

TABLE 2

PLASMA INSULIN LEVEL IN CONTROL AND HYPOTHALAMIC HYPERPHAGIC AND NON-HYPERPHAGIC COCKERELS UNDER THE FASTED CONDITION (THE 2ND DAY AFTER OPERATION), AND ITS RELATIONSHIP TO THE DAILY FEED CONSUMPTION AND BODY WEIGHT INCREASE FROM THE POSTOPERATIVE 4TH TO 10TH DAY

Group	No.	Insulin μU/ml	Daily Feed Consumption g/day	Body Weight Increase g
Control	5	26.0 ± 4.3	62.1 ± 19.3	72 ± 75
Hyperphagic	8	30.9 ± 10.6	117.4 ± 13.4*	119 ± 101
Non-Hyperphagic	13	34.7 ± 9.8	82.6 ± 27.6	95 ± 99
Relative Coefficient			r=0.33	r=0.39

Mean ± S.D., *$p<0.01$ (vs control).

TABLE 3

PLASMA INSULIN LEVEL IN CONTROL AND HYPOTHALAMIC HYPERPHAGIC AND NON-HYPERPHAGIC COCKERELS UNDER THE FASTED CONDITION (THE 19TH DAY AFTER OPERATION), AND ITS RELATIONSHIP TO THE DAILY FEED CONSUMPTION AND BODY WEIGHT INCREASE FROM THE POSTOPERATIVE 30 DAYS

Group	No.	Insulin μU/ml	Daily Feed Consumption g/day	Body Weight Increase g
Control	5	18.6 ± 14.0	70.0 ± 2.3	−42 ± 107
Hyperphagic	7	36.3 ± 8.8†	122.3 ± 6.0*	341 ± 114*
Non-Hyperphagic	9	16.0 ± 7.7	90.2 ± 15.2†	142 ± 212
Relative Coefficient			r*0.35	r*0.35

Mean ± S.D., *$p<0.01$, †$p<0.05$ (vs control).

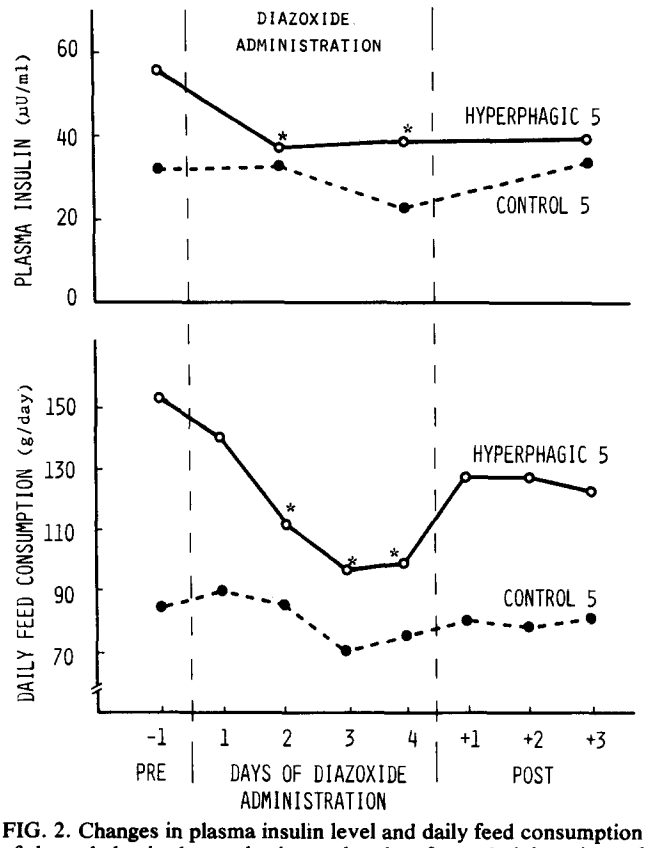

FIG. 2. Changes in plasma insulin level and daily feed consumption of hypothalamic hyperphagic cockerels after administration of diazoxide. Solid line: hyperphagic birds (5 birds), dotted line: control (5 birds), *$p<0.05$.

phagic birds were fasted. High insulin levels were observed after fasting on the 19th day after operation, suggesting hypothalamic destruction as the primary cause of increased insulin secretion. The results corresponded to those reported in rats [4,23]. In addition, high correlation between the insulin value and the food consumption would show the possibility of the facilitated level of insulin to cause the hypothalamic hyperphagia in chickens.

Hustvedt and Løvø [9] reported that the insulin secretion in the ventromedial hypothalamic lesioned rats increased on the 2nd day after operation and this resulted in body weight gain during the following three days. In the present experiment, the insulin level of the chickens, whose satiety center was destroyed, was not higher than that of the sham-operated controls on the 2nd day after operation. However, the insulin levels of the control and non-hyperphagic birds were higher on the 2nd day after operation, when compared to that on the 19th day. The high level of insulin, even slightly correlated with body weight increase during the following seven days, was probably the result of operative stress-induced transient variations in some hormonal trends and metabolism.

Insulin secretion from the rat pancreas can be inhibited by administration of diabetogenic agents: alloxan, diazoxide, streptozotocin, etc. [5,13]. However these substances have little effect on chicken pancreas, and only diazoxide has a modest diabetogenic effect (Langslow *et al.* [12]). In the present experiment, diazoxide reduced the insulin secretion and the increased food intake of hypothalamic hyperphagic birds to a level similar to that of the controls. The present results indicate a close relationship between insulin secretion and food intake and the necessity of hyper-insulinemia in maintaining the hypothalamic hyperphagia in chickens as well as in rats.

Exogenous insulin administration is known to be incapable of inducing hyperphagia in chickens in spite of inducing severe hypoglycemia in the same manner as in mammals [8,15]. The following features concerning pancreatic hormones and metabolism in chickens are different when compared to mammals. The plasma glucose level in chickens is about 250 mg/dl, a value much higher than that of mammals, pancreatic A cells are more numerous than B cells [17,20], and the principal hormone for lipolysis is not catecholamine but glucagon [10]. These features may suggest the possibility that glucagon would play a key role in glucose and lipid homeostasis [11], and may possibly explain the failure to induce hyperphagia by exogenous insulin in chickens.

Intravenous glucose load induces a transient depletion of plasma free fatty acids [12]. On the other hand, stress induces an elevation of free fatty acids, attributable to glucagon secretion [2]. The present author observed that plasma free fatty acids in the hypothalamic hyperphagic chickens did not become depleted after glucose loads and did not increase due to stress as a result of repeated blood sampling [21]. These findings show the possibility of disturbance of normal lipolysis by glucagon in hypothalamic hyperphagic birds.

In conclusion, the hyperactivity of insulin secretion is necessary for maintaining the hypothalamic hyperphagia in chickens, but further research is required to investigate the significance of glucagon as another factor.

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