Gluconeogenesis in the Eel—IV

Gluconeogenesis in the Hydrocortisone-Administered Eel*¹

Yasuo INUI*² and Motoyoshi YOKOTE*²

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Gluconeogenesis of the hydrocortisone-administered eel was studied. Hydrocortisone administration produced a marked hyperglycemia. The liver of the treated eel showed an increase in weight with a concomitant increase in the amount of liver glycogen and in the activities of liver GOT, liver FDP, and liver PFK. Muscle glycogen also increased slightly. However, hydrocortisone produced no appreciable change in plasma amino nitrogen level or in liver GPT activity. Histologically, islet B cells of the hydrocortisone-treated eel exhibited a slight degranulation and some accumulation of glycogen.

These results clearly show that hydrocortisone activates gluconeogenesis in the eel. However, the effect of the hormone on amino acid mobilization seems to be small.

In earlier papers¹⁻³⁾ of this series, the authors have discussed the role of insulin in gluconeogenesis of the eel. For better understanding the hormonal control of gluconeogenesis, the present paper deals with the effects of glucocorticoid on gluconeogenesis of the eel.

Glucocorticoids are known to produce a negative nitrogen balance^{4,5)} and an activation of gluconeogenesis^{6,7)} in mammals. In fishes, also, a similar action of this hormone has been brought to light. CHESTER JONES *et al.*⁸⁾ briefly reviewed on this subject. However, the precise role of glucocorticoids on the nitrogen metabolism in relation to gluconeogenesis is much obscure.

The present paper describes changes of blood glucose level, plasma amino nitrogen content, amounts of liver and muscle glycogen, and activities of liver glutamic oxaloacetic transaminase (GOT), liver glutamic pyruvic transaminase (GPT), liver fructose-1,6-diphosphatase (FDP), and liver phosphofructokinase (PFK) of hydrocortisone-administered eels. Histology of the islet tissue, adrenal cortex, liver, and skin was also studied.

Materials and Methods

Animals A total of 40 Japanese eels (*Anguilla japonica*) weighing 95 g on the average were used in the study. They were obtained from those cultured in a pond of Freshwater Fisheries Research Laboratory and fasted for one day prior to the experiment.

Experiment Experimental procedures are summarized in Table 1. For each lot, 10 animals were kept without feeding in two aquaria, each 30 × 30 × 60 cm, supplied with

*¹ Contribution from Freshwater Fisheries Research Laboratory, No. 350

*² Freshwater Fisheries Research Laboratory, Hino-shi, Tokyo. (乾 靖夫・横手元義: 淡水区水産研究所)

Table 1. Experimental procedure

Lot	Injection*	Dose
Intact control	No	
Saline control	Every day	0.75 % NaCl
Hydrocortisone 1	Every day	Hydrocortisone 2 mg/kg
Hydrocortisone 2	1st and 5th days	Hydrocortisone 10 mg/kg

* Injection was made with 2 ml of solution per kg.

running well water. The water temperature was maintained at $25 \pm 0.5^\circ\text{C}$ during the experiment. Five and ten days after the start of the experiment, 4 or 5 fish were taken out from each lot and served for the study.

Hydrocortisone A hydrocortisone acetate suspension, prepared by Nippon Merck Banyu Co., was diluted to 1/10 and 1/25 in volume by 0.75 % NaCl solution. The preparation was injected intramuscularly to the animals.

Informations on the biochemical and histological procedures are referred to the previous papers.^{1,2)}

Results

Blood glucose As is shown in Fig. 1, hydrocortisone administration produced a marked hyperglycemia in the eel. The blood glucose level of the saline control was a little higher than that of the intact one.

Fig. 1. Glycemic levels of hydrocortisone-treated and control eels. Each histogram (□ the intact controls; ▤ saline controls; ■ H.C. 2 mg/kg/day; ▨ H.C. 10 mg/kg/5 days) with a vertical line represents mean $\pm$ S.E.M. T test was applied between the hormone-treated groups and controls.

Plasma amino nitrogen Hydrocortisone produced no significant change in plasma amino nitrogen content of the eel (Fig. 2).

Hepa-somato index As is seen in Fig. 3, liver weight of the hydrocortisone-treated eel showed a marked increase.

Liver glycogen Change in amount of liver glycogen is presented in Fig. 4. Hydrocortisone produced a rapid and marked increase of liver glycogen content of the eel.

Fig. 2. Plasma amino nitrogen content of hydrocortisone-treated and control eels. Each histogram (□ the intact controls; ▤ saline controls; ■ H.C. 2 mg/kg/day; ▨ H.C. 10 mg/kg/5 days) with a vertical line represents mean $\pm$ S.E.M.

Fig. 3. Hepa-somato indices of hydrocortisone-treated and control eels. Each histogram (□ the intact controls; ▤ saline controls; ■ H.C. 2 mg/kg/day; ▨ H.C. 10 mg/kg/5 days) with a vertical line represents mean $\pm$ S.E.M.

Fig. 4. Liver and muscle glycogen content of hydrocortisone-treated and control eels. Each histogram (■ the intact controls; ░ saline controls; □ H.C. 2 mg/kg/day; ▨ H.C. 10 mg/kg/5 days) with a vertical line represents mean $\pm$ S.E.M.

Muscle glycogen As shown in Fig. 4, amount of muscle glycogen of the hydrocortisone-treated eel tended to increase.

Liver GOT Hydrocortisone yielded a marked elevation of liver GOT activity of the eel (Fig. 5).

Liver GPT In contrast to liver GOT activity, liver GPT activity of the hydrocortisone-administered eel showed no significant change (Fig. 5).

Liver FDP It is seen from Fig. 5 that liver FDP activity of the hydrocortisone-treated eel was slightly higher than that of the control.

Liver PFK As is shown in Fig. 5, hydrocortisone produced a marked increase in liver PFK activity of the eel.

Histological observation

Liver Liver preparations of hydrocortisone-treated eels 5 days after the initial injection showed far abundant cytoplasmic glycogen compared to those of controls. However, except this no appreciable change was observed in the livers of both hydrocortisone-administered and control eels.

After 10 days, livers of control eels exhibited symptoms of fasting; liver parenchyma showed a trend of general atrophy, and amount of cytoplasmic glycogen decreased. In contrast to this, the liver of hydrocortisone-treated eels showed no feature of atrophy, and

Fig. 5. Liver GOT, liver GPT, liver FDP, and liver PFK activities of hydrocortisone-treated and control eels. Each histogram ($\square$ the intact controls; $\dots$ saline controls; $\blacksquare$ H.C. 2 mg/kg/day; hatched H.C. 10 mg/kg/5 days) with a vertical line represents mean $\pm$ S.E.M.

its glycogen was as abundant as those at 5 days.

Adrenal cortex Hydrocortisone administration produced no appreciable change in adrenal cortex.

Islet tissue The islet tissue of both hydrocortisone-treated and control eels after 5 days showed a normal histological feature. After 10 days, no appreciable change was

Fig. 6. Number of skin goblet and clavate cells of hydrocortisone-treated and control eels. Each histogram (□ the intact controls; ▨ H.C. 10mg/kg/5 days) with a vertical line represents mean $\pm$ S.E.M.

observed in the islet tissue of the control (Plate I-Fig. 1). However, B cells of the hydrocortisone-treated eel exhibited a slight degranulation and accumulation of a small amount of glycogen (Plate I-Fig. 2).

Skin Changes in number of goblet and clavate cells are given in Fig. 6. Hydrocortisone administration produced no appreciable change in both cells.

Discussion

BUTLER⁹⁾ reported that the administration of glucocorticoids to the North American eel produced hyperglycemia and increase of liver and muscle glycogen. Similar results were obtained in the hydrocortisone-treated Japanese eel in the present study. These data clearly show that glucocorticoids activate gluconeogenesis in the eel.

It is established in mammals that glucocorticoids lead to a net production of amino acids from cellular protein.^{5,10-12)} SMITH and LONG¹²⁾ emphasized that gluconeogenesis in corticoid-treated rat is primarily due to the increased mobilization of amino acids from the extrahepatic tissue. In the present study, however, hydrocortisone-administration produced no change in amount of plasma amino nitrogen. In view of these facts, the action of hydrocortisone to activate amino acid mobilization from body protein is considered to be small in the eel. This problem will be further discussed in one of this series.

In mammals, administration of glucocorticoids is known to produce an enlargement of liver size, accompanied with increase of liver glycogen and protein nitrogen. As for fishes, however, data on corticoid-liver size interaction are very limited. The study by

SWALLOW and FLEMING¹⁶⁾ on the hydrocortisone-treated tilapia is the only report which confirmed an increase of liver size in glucocorticoid-treated fish. In the present experiment, hydrocortisone administration resulted in a marked increase of liver size of the eel. A concomitant increase of liver glycogen content shows that the increase of liver size is partly due to the accumulated glycogen. Concurrent activation of some liver enzymes such as GOT, FDP, and PFK may suggest that protein had also increased in the liver of the hormone-treated eel.

It is well known that glucocorticoids induces several enzymes which participate in amino acids degradation and gluconeogenesis in mammals.¹⁷⁻²³⁾ Thus, several workers reported an elevation of liver GPT activity¹⁷⁻²¹⁾ and increase^{17,19,21)} as well as no change¹⁸⁾ of liver GOT activity in hydrocortisone-treated rats. As for fish, however, STORER²⁴⁾ is the only worker who reported the increase of liver GPT activity in the hydrocortisone-treated goldfish. In the present study, hydrocortisone administration produced a marked elevation of liver GOT activity but no change in liver GPT activity. In mammals, alanine is known to play an important role in endogenous amino acid metabolism²⁵⁾ and to be one of the most important precursors for gluconeogenesis.^{26,27)} In this point of view, the increase of liver GOT activity in hydrocortisone-treated eel may suggest some significance of aspartate in amino acid metabolism of the eel.

WEBER *et al.*^{22,23)} has described that FDP, known to be one of the key gluconeogenic enzymes,^{22,28)} is induced by glucocorticoids in rats. Also, a similar change was confirmed in the hydrocortisone-treated eel in the present study. In this case, however, it is to be mentioned that the activity of PFK, one of the key glycolytic enzyme,²²⁾ pronouncedly increased in the hydrocortisone-treated eel. This fact seems to indicate that the effect of glucocorticoids is not restricted to gluconeogenic aspect but more overall on the metabolism of the eel. INGLE²⁹⁾ first suggested that adrenocortical hormone do not have obligatory action upon the metabolic process but rather they support the capacity of the

Table 2. Comparison of metabolic and histological changes between fasted, alloxanized, and hydrocortisone-treated eels

	Fasting	Alloxan	Hydrocortisone
Blood glucose	±	+	+
Plasma amino-N	+	+	±
Liver glycogen	- ~ +	- ~ +	+
Liver GOT	+	±	+
Liver GPT	+	±	±
Liver FDP		±	+
Liver PFK		±	+
Activity of Langerhans' islet	-	-	+
Number of skin goblet and clavate	-	-	±

+ Increase - Decrease ± No change

tissue to attain peak rate of metabolic process, and many workers⁸⁾ seems to have supported this idea accepted as "permissive action".

Metabolic and histological changes in the fasted,¹⁾ alloxanized,²⁾ and hydrocortisone-administered eels are compared in Table 2. It is clear that gluconeogenesis is activated in eels in all these conditions. However, the metabolism of hydrocortisone-treated eel differed from the alloxanized one in the behavior of plasma amino nitrogen, liver glycogen, and liver enzymes together with some histological features of islet tissue and skin. The fact clearly shows that gluconeogenesis induced by hydrocortisone and alloxan are different in their metabolic process.

In the fasted eel as well as the hydrocortisone-treated eel, liver GOT activity showed an apparent increase. However, elevation of this enzyme activity in the fasted eel could not be attributable only to the action of glucocorticoids. Further discussion on the enzyme induction awaits future study.

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Explanation of Plate I

Fig. 1. A normal islet from a control eel. The B cells are heavily granulated and have distinct cell contour. G-P fixation, AF stain, $\times 700$.

Fig. 2. An islet from a hydrocortisone-treated eel 10 days after the initial injection. The B cells show degranulation of moderate degree and indistinct cell contour with slightly enlarged nuclei. G-P fixation, AF stain, $\times 700$.