NOTE

EFFECTS OF HYPOPHYSECTOMY AND UROPHYSECTOMY ON WATER AND SODIUM TRANSPORT IN ISOLATED INTESTINE AND GILLS OF JAPANESE EEL (*ANGUILLA JAPONICA*)

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SYNOPSIS

Effects of hypophysectomy and/or urophysectomy on the water transport in isolated intestines and on the sodium transport in isolated gills of sea-water adapted eels were examined. When isolated intestine was incubated in Ringer's solution, the net water flux across the intestinal wall of the eels, adapted to sea water, was greater than that of fresh-water eels. This increase in water flux in the intestines of sea-water eels was not observed when the hypophysis or the hypophysis and urophysis were removed together, but urophysectomy alone produced no significant change in the water flux. It is likely, therefore, that the hypophysis is involved in the observed increase in water flux. On the other hand, when isolated gills of the sea-water eels were incubated in sea water, the rate of penetration of sodium from surrounding sea water was smaller than that of the gills of fresh-water eels. Hypophysectomy and/or urophysectomy had little effect on this adaptation process of the isolated gills.

Euryhaline fishes, upon being transferred from fresh to sea water, are believed to lose water through the body surface and in turn compensate for the loss by drinking sea water. The excess monovalent ions absorbed in this manner would be excreted mainly through the gills. Although the above principles are generally accepted, principally due to Smith's work (1930), quantitative confirmation is rather scanty. In a previous paper, it was shown that in isolated intestine of cultured eels a large amount of water passes through the intestine of sea-water adapted eels than fresh-water eels (Utida *et al.*, 1967). It has also shown that when isolated gills of eels

adapted to sea water were incubated in sea water, the gills maintained a constant level of sodium. In contrast, a considerable amount of sodium penetrated the isolated gills of fresh-water eels during the same incubation (Bellamy, 1961; Kamiya, 1967).

The purpose of this investigation was to determine whether the hypophysis or the urophysis is involved in the process of such adaptation of intestines and gills of Japanese eel, *Anguilla japonica*.

MATERIALS AND METHODS

Fish

In April, 1966, forty eels, weighing 130–150g, were obtained from a fish pond in Yaizu, Shizuoka pref. and were maintained in a fresh water aquarium at 20°C for at least one week before use. Then, these eels were divided into 8 groups. Five intact

Received for publication January 20, 1967.

This investigation was supported in part by a Grant in Aid for Fundamental Scientific Research from the Ministry of Education, Japan.

eels of the first group were left in fresh water. The eels of the other 7 groups, that is, intact, hypophysectomized, sham - hypophysectomized, hypo - urophysectomized, sham - hypo-urophysectomized, urophysectomized and sham- urophysectomized eels were transferred to sea water (35‰) and kept for 24 hrs. Then transport studies of the intestines and gills were carried out.

Hypophysectomy

Eels were lightly anaesthetized with 0.5% tricaine-methane sulphonate (MS-222, Sandoz) and placed ventral side upward on an operating board. After incising the buccal epithelium the parasphenoid bone was exposed and drilled with a No. 44 dental carborandum point. The pituitary was exposed after a small incision of the meninges and was sucked with a glass pipette. Sham-hypophysectomy was performed similarly except that the bone was drilled but the pituitary was not sucked. These fish were transferred to sea water 7 days after the operation.

Urophysectomy

The caudal neurosecretory system of the eel is distributed in the spinal cord over the last 6 or 7 vertebrae (Enami, 1955). In order to expose the whole neurosecretory system, an incision of about 1.5 cm was made through the skin and muscle layers and the last 7 caudal vertebral centra and their neural arches were removed. Then small pieces of spinal cord including the urophysis were removed with forceps. Following extirpation of the spinal cord, the skin and muscle layers were sutured. Sham-operation was performed in the same manner except that the urophysis was left intact. All these fish were transferred to sea water on the second day of operation.

Hypo-urophysectomy

Eels were urophysectomized 5 days after removal of the pituitary, and transferred to sea water 2 days after urophysectomy. All the operations were carried out as described above.

Water transport in isolated intestine

Eels were killed by decapitation and after opening the abdomen the intestine was excised. The excised segment which was about 7 or 8 cm in

length, was washed several times with Ringer solution (NaCl, 145 mM; KCl, 3 mM; CaCl₂, 5 mM; MgCl₂, 4 mM/l, pH 7.4 with 0.01 M Tris buffer). One end of the intestine was tied with cotton thread and it was filled with the solution (usually 0.5–1 ml) and then the other end was sealed with the thread. This sac was lightly blotted on filter paper, weighed and transferred to a incubation vessel containing 40 ml of the solution. After aerated for 3 mins. with O₂, the vessel was incubated for 60 mins. by shaking at 20°C. The water movement was expressed as a percentage of water lost from the mucosal solution.

Movement of sodium in isolated gills

Gills were removed from the same eel as used for the water transport study of intestine and incubated in sea water for 60 mins. Determination of sodium content in the gills was carried out principally according to the method of Bellamy (1961).

RESULTS

Effects of hypophysectomy and/or urophysectomy on the water transport of isolated intestines are shown in Figure 1. When intact eels were kept in sea water for 24 hrs., their intestine showed a greater rate of water transport from the mucosal to the serosal side than that of the eels kept in fresh water ($P < 0.01$). However, water transport in the intestines of the hypophysectomized and hypo-urophysectomized eels was significantly lower ($P < 0.02$) than the intact or sham-operated groups kept in sea water. It was nearly equal to the rate of water transport in fresh water eels. In one specimen in which the pars nervosa and a part of the pars intermedia were left, the intestine behaved in a similar way. In contrast to the pale color in the completely hypophysectomized fish, the skin color of this eel remained black. On the other hand, urophysectomy alone did not cause any significant change in the water flux across the intestinal wall.

Table 1 shows the results of measurement of sodium content in the gills before and after

Fig. 1. Effect of hypophysectomy and/or uropysectomy on water transport of eel intestine. Eels were adapted to sea water for 24 hrs., except intact eels in fresh water (FW). Dotted column represent intact eels and shaded and white columns represent operated and sham-operated eels, respectively. Vertical bars indicate standard error of means. Figures in the columns are the number of fish used.

Table 1. Effect of hypophysectomy and/or uropysectomy on sodium transport of eel gills incubated in sea water

Treatment	No. of eels	Sodium content (mequiv./100 mg dry weight)		Net Sodium influx during 1 hr. of incubation
		before incubation	1 hr. after incubation	
Intact (fresh water)	5	31.4±1.24*	49.7±0.85	18.3 ±2.06
Intact (sea water)	5	38.7±1.41	44.5±2.64	5.82±1.35**
Hypophysectomy	3	34.9±2.76	39.2±2.69	4.33±2.82
Sham-hypophysectomy	3	40.8±0.87	47.1±1.64	6.30±1.79
Hypo-uropysectomy	5	33.4±0.49	38.8±2.58	5.50±2.52
Sham-hypo-uropysectomy	4	39.2±1.50	45.9±1.76	6.65±0.34
Uropysectomy	5	38.8±1.31	42.7±1.98	3.92±2.13
Sham-uropysectomy	5	36.4±2.02	44.7±0.32	8.32±1.62

* Mean±standard error.

** Significant compared to intact fresh-water value ($P<0.001$).

incubation in sea water. Initial sodium content of the gills from fresh water eels was slightly lower than those of the sea-water adapted groups, whereas the final content was higher than that of the other groups. This shows that larger amount of sodium ion penetrated from the surrounding sea water into the gills of fresh-water adapted eels than those of the sea-water adapted groups. Hypophysectomy, urophysectomy or hypo-urophysectomy had little effect on this net flux of sodium.

DISCUSSION

From the results of the present measurement of water flux of isolated intestines, it is evident that in comparison with the value for the fresh-water eels, the net water flux from mucosa to serosa increased in the intestine of the eels adapted to sea water for 24 hrs. Since the intestines were bathed on both surfaces with identical Ringer's solution, this seems to indicate that the intestines of the sea-water adapted eels can absorb water more actively from mucosal solution than can intestines of fresh-water eels. Active transport of water was also observed in the intestines of gold fish (Smith, 1964) and marine teleost, *Cottus scorpius* (House and Green, 1965) bathed on both sides by Ringer's solution. On the other hand, increased permeability to water was observed in the isolated intestines from European silver eel adapted to sea water (Sharratt *et al.*, 1964). As shown in Figure 1, the increase in water flux in the intestine of the sea-water eels was not observed when the hypophysis or both the hypophysis and urophysis were removed. However, urophysectomy alone did not cause any significant change in the transport. Therefore, it is highly probable that hypophysis is involved in the adaptation process of water transport.

Using whole animal, several investigators have suggested that adrenocortical or neurohypophyseal hormones are involved in the electrolyte balance of the euryhaline fish loaded with saline or adapted to sea water (Holmes, 1959; Holmes and Butler, 1962; Motais and

Maetz, 1964). It is to be noted here that in the case of incomplete hypophysectomy, the intestine still showed a low, fresh-water level of water transport. Therefore, it seems likely that hormone(s) of the pars distalis is necessary for regulating the rate of water transport in the intestine. Further studies on the nature of the hormone(s) involved are now in progress in our laboratory.

Bellamy (1961) first used an isolated preparation in the transport study of the gills and found that a constant level of sodium was maintained against the passive diffusion gradient in the gills of sea-water adapted eels. This is in good agreement with the present results. Recently, Butler (1966) reported that the content of plasma sodium chloride as well as muscle potassium was significantly higher in hypophysectomized silver eels adapted to sea water than in intact fish, and suggested that elevated tissue electrolyte concentrations in operated eels reflect a decrease in net branchial efflux of sodium chloride. Evidences suggesting the involvement of the urophysis in salt adaptation are also reported (see Bern *et al.*, 1965). However, as regards the roles of the hypophysis and the urophysis in salt adaptation of gills, the positive result could not be obtained in the present *in vitro* study.

ACKNOWLEDGEMENT

The authors are indebted to Miss Hiromi Kondo for technical assistance in water transport study of the intestines.

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