STUDIES IN NEUROSECRETION IX. POSSIBILITY OF OCCURRENCE OF A SODIUM-REGULATING HORMONE IN THE CAUDAL NEUROSECRETORY SYSTEM OF TELEOSTS

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The problem of osmoregulation in teleosts has been extensively studied by a number of workers to the effect that the basic plan of adaptive water and salt balance is figured out in its outline (Krogh, 1939; Baldwin, 1949; Black, 1951; Gaunt and Birnie, 1951; Fontaine, 1953, etc.). Several lines of evidences are available for physiological interpretation of observed osmotic phenomena in terms of the branchial, renal and/or dermal activities, but, despite much efforts hitherto made, the pattern of endocrine co-ordination responsible for the control of the gates for salt and water is yet only superficially understood.

On the basis of recognition of the significant morphological characters of the caudal neurosecretory system in the terminal region of the spinal cord in various kinds of freshwater as well as marine teleosts (Enami, 1955; Enami and Imai, 1955, 1956 a, b), work has been directed to the inquiry of the role played by this possible endocrine source. The most recent experiments concerning the variability of the spinal neurosecretion in the loach, Misgurnus anguillicaudatus, are much demonstrable of the primary dependence of the phenomenon on prevailing osmotic relations between the fish body and the medium water (Enami, 1956). Such observation stimulated an attempt to investigate a possibility of production by the said neurosecretion of a certain hormonic principle concerned with the teleostean osmoregulation. Present report is rather of a preliminary nature, showing the outcomes of some of the experiments in vivo composing a part of such attempt.

MATERIALS AND METHODS

The small cyprinodont medaka, Oryzias latipes, was employed as the experimental animal in the present study. Of the available forms of the fish, use was made exclusively of the red variety which, on account of its higher tolerance to various kinds of experimental treatments, had been learned preferable to the black wild type. Preliminary trials of the measurement of total body content of Na and K with groups of fishes in wide range of the dry weight, which had been allowed to acclimate to the laboratory freshwater environment, had shown that the visceral contents are the main source of large-scaled individual variation, and that fairly uniform values are obtainable with animals, from which the viscera are wholly removed off. Also it had been learned that viscid extracts result from large-sized specimens enriched with fatty substances, which are unfavorable for the practice of flame photometry. Taking into account such observations, the present study was carried out in the following way: A total of more than one
thousand small-sized animals (50–200 mg in wet weight) that had been kept without food in freshwater from a well in the laboratory for a week, were divided into a number of control and experimental lots of 8–15 individuals each, and, after respective experimentation, the fish body as a whole was rapidly rinsed with jet of re-distilled water to be blotted dry on filter paper. Then the viscera were removed off and the interior of the visceral cavity was wiped up. On a torsion balance such processed body of the fish was weighed to the nearest tenth of milligram, and the fish body was hung on a hook by the opercular aperture to be dried to a constant weight at 105°C. After weighing the dried specimen, each sample was homogenized with 3 or 4 ml of re-distilled water, and supernatant fluid of the centrifugate was used for the determination of total body Na and K by flame photometry with Beckman spectrophotometer model DU.

Throughout the present experiments a Ringer recommended by Yamamoto (1949) for Oryzias was employed as physiological saline. Experimental salt load was applied by immersion of the fish to this Ringer, or, when more profound effect was required, to Herbst’s artificial sea-water diluted to half the concentration. As the donor of homogenate or extract of the spinal cord to be tested upon Oryzias, the common eel, Anguilla japonica, weighing approximately 150 g was used. Isolated spinal cord was cut into pieces representing respectively the terminal portion including the bulk of the caudal neurosecretory system (Enami, 1955), the anterior portion located just above the anal opening, and the portion located midway between the two regions. Aliquots of respective portions obtained in the same wet weight per individual of the eel were homogenized with known quantities of the fish Ringer to be directly injected to test animals. In some of the experiments, crude Ringer extracts were prepared by centrifugation. Injection was made intraperitoneally by means of a micrometer syringe at a dose of 1/400 ml, regardless of size-variety of the recipients. Since the homogenates or extracts contained slightly less amounts of Na and K per unit volume of fluid as compared with plain Ringer solution which was injected to control animals for comparative data, observed values of total body Na and K at the end of each injection experiment were corrected on the basis of the ionic concentrations in injected volume of respective fluids.

EXPERIMENTS

Disorder of Na regulation in animals with operated spinal cord

That the integrity of the terminal portion of the spinal cord including the caudal neurosecretory system is closely related to the normal physiological control of sodium balance in Oryzias was observed in a series of experiments on animals, whose spinal cord was transected at the level of the anterior extremity of the dorsal fin or excised out at its caudal region for the range of 6 hindmost vertebrae (Fig. 1). The organization of the neurosecretory system in the terminal spinal cord of this species of fish was already dealt with by Enami and Imai (1955).

Fig. 1. X-ray photo of Oryzias showing sites of operations on the spinal cord
In animals subjected to either of the operations and directly returned to freshwater, death ensued on a large scale, attaining to as high as 50% of mortality in the postoperative 24 hrs. Though small fractions of specimens were found alive even at the end of 30 days, most of the remainders that tolerated the initial stress succumbed gradually in the course of the following 7 days. Such an observation is in line with that of Tuge and Hanzawa (1937) stating of the failure of *Oryzias* to survive in freshwater after spinal transection. In all of the present instances death was preceded by signs of edema and asthenia, and most remarkably by roughened appearance of the skin surface. However, fishes were able to survive without any loss for at least 10 days, when they were transferred to fish
Ringer following respective operations. With such animals, several experiments were made in order to observe changes in the tissue ionic balance relative to salt concentrations in the medium water. Fig. 2 represents the result of an experiment, in which operated animals together with unoperated controls were repeatedly subjected to different salt loads. The animals, after having been kept in Ringer for 2 days following transection or removal of the terminal spinal cord, were transferred to 1/2 sea-water for 6 hrs. and then returned to Ringer for one day to be put into freshwater for subsequent 2 days. They were further transferred from freshwater to Ringer for one day and onto 1/2 sea-water thereafter. In this way immersion of the fishes to the three fluids for respective periods was repeated 3 times, and at the end of 12 hrs. of the final freshwater immersion the animals were taken out for measurement of water content and total Na and K. While all of the unoperated controls as well as the animals with transected spinal cord survived the whole periods of the experimental treatments, approximately 60 % fish succumbed in the group with the terminal spinal cord removed, death occurring exclusively in freshwater in consequence of transfer from the salty media.

It was learned that water content of the 3 lots of animals showed but slight difference from each other (78.87±0.84 % for the group of spinal transection, 79.51±0.84 % for the group with terminal spinal cord removed, 79.82±2.08 % for the control group), and total body K expressed in terms of dry weight did not represent any obvious tendency of mutual divergence. In contrast, Na was found most liable to change according to the operations. That is, amounts of Na expressed in terms of dry weight in the operated animals were pointing to relatively higher levels than those in the controls, though quantitative treatment of the differences was difficult on account of large-scaled variation in relatively small number of individuals. Differences in Na content between the operated and the control lots of animals were especially pronounced in small-sized animals, total Na being at much higher levels in small operated animals as compared with that in larger ones which approximated to that in each of large control animals. Because of large loss of animals, mainly of small ones, in the group with the terminal spinal cord removed, it was impossible to figure out which of the 2 operated groups was of relatively high Na content. However, in view of that exceedingly high values of Na (104.30 mEq and 87.47 mEq per 100 g dry weight) were obtained with 2 dying animals in the group of terminal cord removal (13.6 mg and 17.0 mg in dry weight respectively), which were taken out of freshwater in the final immersion at the onset of asthenia, it might be conjectured that Na would have been more concentrated in such kind of operated fish than in animals with transected spinal cord.

Such a line of observation was substantiated by other experiments showing that marked loss of control of Na transport characterized both of the animals with the terminal spinal cord removed and those with the cord transected at the level cephalad to the site of the caudal neurosecretory system.

Decrease of Na in response to injection of homogenate or extract of terminal spinal cord

Several experiments were made with the aim to see if there were any indications for the presence in the terminal region of the spinal cord of certain humoral
principle that is responsible for the control of Na metabolism. In one of the experiments, result of which is shown by Fig. 3, homogenates of the 3 different portions of _Anguilla_ spinal cord mentioned above were tested upon _Oryzias_ that had been kept in Ringer for 2 days following removal of the terminal spinal cord and were transferred to freshwater upon injection to be taken out 6 hrs. later. Homogenates were prepared from 10 specimens of the eel to be of the same
Data resulting from the experiment were significantly demonstrable of a decrease of Na in animals subjected to injection of the homogenate of the terminal spinal cord. As compared with the values in the controls injected with plain Ringer solution, those of the animals which received either the homogenate of the anterior piece of the spinal cord or that of the middle piece did not show any marked differences. In line with the results stated in the foregoing accounts, effect of injection of the terminal cord homogenate was observed in most significant measure with small-sized animals, data for larger ones being approximated by those of other groups of different treatments. Water content of the animals was 79.60±1.24% for the controls, 80.06±1.84% for the group injected with the anterior cord homogenate, 80.31±1.53% for the group injected with the middle cord homogenate, and 81.62±2.98% for the group injected with the terminal cord homogenate respectively, mutual differences being not so significant as to render definitive information. Also, no apparent deviation from the control values was observable with respect to total body K in each of the homogenate-injected groups.

Results similar to that of this injection-experiment were obtained with test animals that had been kept in Ringer or 1/2 sea-water prior to injection. Tab. 1 summarizes the conditions responsible for Na-decrease, which indicate that the effect of terminal cord homogenate or extract was primarily related to preadaptation of test animals to isotonic or hypertonic media.

Table 1. Experimental treatments responsible for Na-decrease as the result of injection of homogenate or extract of the terminal spinal cord of the eel

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Injection</th>
<th>Treatment after Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kept in Ringer for 3 days after spinal transaction</td>
<td>Homogenate, 10 TSC*/0.5 ml Ringer</td>
<td>Transferred to freshwater for 2 hrs.</td>
</tr>
<tr>
<td>Kept in Ringer and 1/2 sea-water for 2 days and 12 hrs. respectively after removal of terminal spinal cord</td>
<td>Extract, 46 TSC/2 ml Ringer</td>
<td>Returned to 1/2 sea-water for 3 hrs.</td>
</tr>
<tr>
<td>Kept in Ringer for 2 days after removal of terminal spinal cord</td>
<td>Extract, 65 TSC/2 ml Ringer</td>
<td>Returned to Ringer for 15 min.</td>
</tr>
<tr>
<td>Kept in 1/2 sea-water for 3 days after removal of terminal spinal cord</td>
<td>Extract, 50 TSC/2 ml Ringer</td>
<td>Transferred to Ringer for 3 hrs.</td>
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* Terminal spinal cord comprising the bulk of the caudal neurosecretory system

Increase of Na following injection of homogenate or extract of terminal spinal cord

In the course of the present study it came out that homogenates or extracts of the terminal spinal cord of the eel were sometimes responsible for the increase of total body Na, an effect which was contradictory to the Na-decreasing effect in the foregoing instances. Fig. 4 shows the result of an experiment, in which test animals with the terminal spinal cord removed had been allowed to acclimate to freshwater for 2 days in advance to injection of an extract of the terminal spinal cord of the eel (34.5 mg from 39 fishes/2 ml Ringer), and were transferred to 1/2 sea-water for 3 hrs. after injection. In contrast to nearly comparable values of
both water (74.90 ± 1.36 % for controls, 75.48 ± 4.04 % for experimental) and K contents in control and experimental lots of animals, Na content in many of the latter animals was observed at higher levels than that in the former ones.

Similar evidences pointing to Na-increasing effect of homogenate or extract of the terminal spinal cord of the eel were obtained under the conditions summarized in Table 2.

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**Fig. 4.** An example of Na-increase as the result of injection of a crude extract of terminal spinal cord of the eel. Open circle, fish injected with Ringer; solid circle, fish injected with an extract of the caudal piece of the spinal cord. Explanation of the experiment is in text.

**Table 2.** Experimental treatments responsible for Na-increase as the result of injection of homogenate or extract of the terminal spinal cord of the eel

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Injection</th>
<th>Treatment after Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kept in freshwater for 8 days after spinal transection</td>
<td>Extract, 33 TSC*/2 ml Ringer</td>
<td>Returned to freshwater for 1 hr.</td>
</tr>
<tr>
<td>Kept in freshwater for 2 days after removal of terminal spinal cord</td>
<td>Homogenate, 10 TSC/0.3 ml Ringer</td>
<td>Returned to freshwater for 6 hrs.</td>
</tr>
<tr>
<td>Kept in freshwater for 2 days after removal of terminal spinal cord</td>
<td>Homogenate, 5 TSC/0.2 ml Ringer</td>
<td>Transferred to Ringer for 3 hrs.</td>
</tr>
</tbody>
</table>

* Terminal spinal cord comprising the bulk of the caudal neurosecretory system
These observations may indicate that preadaptation of test animals to freshwater environment was primarily related to the Na-increasing effect of homogenate or extract in question. As stated above, significant decrease of Na took place as the result of injection of the homogenate or extract of the terminal spinal cord of the eel, when the recipients had been kept in salty media. Whether such divergent effects of the same kind of injection were due to the presence of different kinds of hormonal principles in the terminal region of the spinal cord, or they were representing the outcomes of transformation of the activity of a single salt hormone, is left to be determined in the future, but it appears safe to presume under the present circumstances that a single hormone is working, which would be responsible for but one-way activation of certain target organ(s), where the mechanism of excretion and/or uptake of Na could be located. Possibly such target organ(s) would behave differently according to occasional demand for excretion or uptake of Na, that is raised by prevailing osmotic relationship between the medium water and the fish body. The observed significance of preadaptation of test animals with respect to the qualitative effect of expected hormonic principle seems to imply that the mode of work of target organ(s) set up in the course of preadaptation would persist for certain period of time, say at least 6 hrs., in which period the effects of injections were observed, even after the animals are transferred to different medium water.

It is to be added here that the expected Na-regulating hormone appears to exert influence relatively rapidly, since Na-decreasing effect was observable at the end of 15 min. and Na-increasing effect was detectable at the end of 60 min. after injection.

COMMENTS AND CONSIDERATIONS

It is not the scope of this paper to go into detailed discussion, a few considerations being made together with brief comments on some of the works related to the possibility of endocrine control of salt balance in teleosts.

In view of the presently observed significance of the integrity of the caudal portion of the spinal cord in the adaptive regulation of Na transport, the work of Drilhon (1942) might be accepted to be the most interesting. She rendered a brief account of apparent loss of tolerance to concentrated salty medium in the carp, Cyprinus carpio, whose spinal cord was surgically destructed as a whole. In contrast to that normal carp could survive in a medium of high salt concentration \((d=0.90^\circ C)\), operated animal is stated to have succumbed directly after transfer to a water of \(d=0.20^\circ C\). Nevertheless, the operation does not seem to have profoundly affected the viability of the fish for periods of 20–30 postoperative days, as far as the fish was kept in freshwater, no marked change being observed as regards the blood serum content of Na and Cl at the end of 20 days. These observations look like contradictory against those in the present work with Oryzias with the spinal cord operated on at its caudal aspect, which show some large-scaled mortality in freshwater and better survival in Ringer \((d=approximately\ 0.4^\circ C)\) as well as in more concentrated \(1/2\) artificial sea-water \((d=approximately\ 0.9^\circ C)\). However, since some fractions of the operated Oryzias could survive for at least
30 days in freshwater, and because long-term observation on the viability in concentrated salt water is lacking in the present study, any definitive saying about the seemingly inconsistent outcomes mentioned would be premature. Maybe the difference of postoperative viability in respective species of fishes in salty media is due to difference of basic physiological capacity to withstand external hypertonicity, since it has long been known that intact *Oryzias* is able to acclimate to much high salt concentration, even to the one as high as 62.59% (Tateishi and Yamashita, 1956), while *Cyprinus* has been stated to be a strictly freshwater animal (Vars, 1934; Drilhon, 1942). Unfortunately no comparable data are available in Drilhon’s paper, but the present observation indicates that the cause of death is certainly related to the disorder of the mechanism of osmoregulation in fishes after destruction of the integrity of the terminal spinal cord.

Of the recent works dealing with the problem of endocrine control of water and salt balance in teleosts, those of Burden (1956), Arvy et al. (1954, 1955, 1956), etc. appear most important. Results of these authors are respectively demonstrable of functioning of osmoregulation of certain principle(s) from the pituitary, though the way of approach and the conclusion attained are different. Burden has suggested the presence of principle(s) in the pituitary of *Fundulus heteroclitus* and *Perca fluvescens*, which is/are in all the possibility not identical with either of the known posterior-lobe principles, ACTH, GH and TSH. His reasoning is based on observations on the loss of viability of hypophyssectomized *Fundulus* upon transfer from sea-water to freshwater, and on success of replacement therapy with pituitary homogenates. Serum chloride determination shows that, while hypophysectomized fish kept in sea-water maintains nearly normal level, the fish dying in freshwater is deprived of over 50% of normal content. It is of much interest that preadaptation of the fish to freshwater prior to hypophysectomy is stated to have prolonged survival in some measure in the postoperative freshwater environment, an effect suggesting the durability of pre-existing pattern of osmoregulation. As mentioned above, similar line of observation was made in the course of the present work, which shows that injection of homogenate or extract of the caudal portion of the eel’s spinal cord was responded by mutually opposite effects, depending on whether the recipient *Oryzias* had been kept in advance in salt water or in freshwater. Possibly the long-claimed difference of Na metabolism in salt- and freshwater environments (Krogh, 1939; Black, 1951; Fontaine, 1953, etc.) is reflected on the effects of hypophyssectomy in *Fundulus* or of administration of the spinal principle to *Oryzias* in such a way that the mechanism of Na-excretion is predominantly maintained in fishes preadapted to salt water and the mechanism of Na-uptake persists in animals preadapted to freshwater. In this connection, the so-called chloride cell and the mucous cell in the gill attract special attention, since the histological elements of the gill are believed to be of dual character, working so as to expel Na in salt water and to absorb Na in freshwater, though further extended studies are required to determine which of the generally conceded gates for Na transport, viz., the gill, the kidney and the integument, is representing the target organ of the hormone postulated to occur in the caudal neurosecretory system.

That the neurohypophyseal control through the agency of the antidiuretic
factor (vasopressin) or similar hormonic principle would represent the most basic mechanism in the physiological co-ordination of osmotic regulation in fishes has been claimed by several authors, a view which parallels the one amply substantiated in the field of mammalian osmoregulation. Though preparations of mammalian neurohypophyseal hormones and also breis or extracts of teleostean neuro-intermediate lobe of the pituitary appear to have no significant effect upon teleostean osmoregulation (Fontaine and Raffy, 1950; Càllamand et al., 1951; Burden, 1956), the view is revived in the light of morphological evidences of adaptive change of the hypothalamo-hypophyseal neurosecretory system in response to externally applied salt load. As a matter of fact, Arvy et al. (1954, 1955) observed marked signs of hyperactivity of the preoptic neurons of the system mentioned, being preceded by a depletion of pre-existing secretory material in the neurohypophysis, after transfer of Phoxinus and Anguilla from freshwater to hypertonic media. The same is the case for Enami and Imai (unpublished observation) working on Misgurnus. It appears of interest that, in Misgurnus, osmotic stimuli are responded by the hypothalamic and the spinal neurosecretory systems differently in the following ways: Hypertonic media brought about a hyperactive state of the former, but, as was already reported (Enami, 1956), influenced upon the latter so as to suppress both production and release of secretory material. Injection of hypertonic fluid was not responded by any apparent change of the hypothalamic neurosecretion, but called forth in the spinal neurosecretion a depletion of secretory material in the urohypophysis, the depot organ of the caudal neurosecretory system, followed by unusual hyper-secretion in the neuron somata. Such observations would imply a possibility of antagonism between the two neurosecretory systems in relation to the physiological control of salt balance in teleosts, and, despite some negative results mentioned above of the effect of posterior-lobe preparations and extracts, the morphological findings by Arvy et al. stimulate further analytical studies. It is needless to say that the line of consideration here advocated does not reject the possibility of working of other endocrine sources such as the thyroid (Gorbman and Berg, 1955; Fontaine, 1956; Arvy et al., 1956) and the adrenal (Keys and Bateman, 1932; Chavin, 1954) for the maintenance of a steady state of hormone balance in teleostean osmoregulation in general.

SUMMARY

1. A possibility of occurrence of a hormonic principle representing presumed neurosecretory control of salt balance in teleosts was examined with the euryhaline cyprinodont Oryzias latipes with positive results.

2. Removal of the caudal aspect of the spinal cord comprising the bulk of organization of the caudal neurosecretory system or transection of the cord at the level cephalad to the site of the system in question induced serious disorder in the adaptive control of Na. No significant change resulted from the operations in total body content of K, while water moved in some measure, largely independently of total Na.

3. Injection of homogenate or crude waterly extract of the caudal portion of the spinal cord of the eel, Anguilla japonica, likewise called forth either depression
or elevation of total Na level of the test *Oryzias*, from which the caudal portion of the spinal cord had been removed off. Such diversified effects of injection were learned to be dependent upon the history of preadaptation of the test animal; decrease of Na took place in the fish that had been kept in isotonic or hypertonic media, while increase of Na resulted in the fish that had been kept in freshwater prior to injection.

4. A view was tentatively proposed that the expected hormone of the caudal neurosecretory system would be responsible for one-way activation of certain target organ(s) which, as the gate(s) for excretion and uptake of Na, might adaptively change the mode of work.

5. Brief considerations were made concerning mainly a possibility of interaction between the caudal neurosecretory system and the widely known hypothalamo-hypophyseal system in the control of salt balance in teleosts in general.

REFERENCES


