The Urophysial Hydrosmotic Factor of Fishes. III. Survey of Fish Caudal Spinal Cord Regions for Hydrosmotic Activity[1] (36652)

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Osmoregulatory function has frequently been associated with the caudal neurosecretory system of fishes and its neurohemal organ in teleosts, the urophysis (1, 2). This has led to a search for neurohypophyial-like activity using the isolated amphibian urinary bladder (3, 4, and unpublished research of Ferguson and Bern), an assay preparation responsive to neurophypophysial hormones. In only one instance to date, however, have the results been positive (5). Neurohypophysial-like activity was found to be present in *Gillichthys mirabilis* urophyses (5). The other attempts using three other teleost species resulted in essentially negative results. The question then arises as to whether the urophysical hydrosmotic factor, which is probably arginine vasotocin (6), is also present in other species.

Materials and Methods. Sixteen species of fish were used in this study. Caudal spinal cord and urophysis extracts were prepared as previously described (7). The acetone-dried material was homogenized in a hand glass homogenizer with 0.25% acetic acid, heated in a boiling water bath for 3 min, and centrifuged. The supernatant was assayed for hydrosmotic activity on the isolated urinary bladder of the toad *Bufo marinus* from Hawaii according to the method of Bentley (8, 9), using acetone-dried *Gillichthys* urophysis as reference standard (10). Activity was expressed in mU per mg acetone-dried weight, where 1 mU is equal to the hydrosmotic avtivity of 1 μg of acetone-dried *Gillichthys* urophysis (10). When little or no activity was detected with 50 μg per ml of incubating fluid (as little as 1 mU of standard causes a clear response), this was recorded as less than 20 mU per mg ($<$ 20 in Table I).

Results. Of the 10 teleost species examined, only two showed urophysial activity comparable to that of *Gillichthys* (Table I). Urophysial preparations of the milkfish (*Chanos chanos*) were approximately equal in potency to *Gillichthys* material, whereas those of the rainbow trout (*Salmo gairdneri*) were 2.5 times more active. The other species showed little or no consistent activity at a concentration of 50 μg per ml of bath fluid. Habitat (seawater vs freshwater) was not correlated with the presence or absence of activity. Extracts of abdominal spinal cord of *Gillichthys* (5) and of trout were inactive. None of the nonteleostean fishes showed significant activity.

Discussion. The urophysial hydrosmotic factor found in *Gillichtys mirabilis* (5) is apparently not present in detectable quantities in some teleost species of fishes. This would explain the failure of previous attempts to demonstrate a neurophypophysial-like activity on the isolated amphibian urinary bladder. Urophyses of two species (*Tilapia mossambica*—3; *Mugil cephalus*—unpublished experiments of D. R. Ferguson & H. A. Bern) used in earlier investigations were found to have little or no hydrosmotic activity in the present study, even using the very sensitive bladders of Hawaiian *Bufo marinus*.

The results of this survey have further

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TABLE I. Survey of Fishes for "Urophysial" Hydrosmotic Factor.

Species[a]	Activity (mU)[b] per mg dry weight				
	Preparations				
	1	2	3	4	Mean
Hagfish (*Polistotrema stouti*), SW	66	<20	<20	<20	<31
Leopard shark (*Triakis semifasciata*), SW	<20	<20	<20	<20	<20
Pacific angel shark (*Squatina californica*), SW	<20	<20	<20	<20	<20
Starry skate (*Raja stellata*), SW	<20	<20	<20	<20	<20
Bat stingray (*Myliobatis californicus*), SW	<20	<20	<20		<20
Bowfin (*Amia calva*), FW	<20	<20			<20
Milkfish (*Chanos chanos*), SW	617	843	1100	1000	890
Rainbow trout (*Salmo gairdneri*), FW	2630	2900	1600	2950	2520
Brown bullhead (*Ictalurus nebulosus*), FW	<20	<20	<20	<20	<20
Striped bass (*Roccus saxatilis*), FW	<20	<20	<20		<20
White croaker (*Genyonemus lineatus*), SW	<20	<20	<20	<20	<20
Tilapia mossambica, FW	<20	<20	28	93	<40
Tilapia mossambica, SW	55	<20			<37
Pile perch (*Rhacochilus vacca*), SW	<20	<20	<20		<20
Striped mullet (*Mugil cephalus*), FW	<20				<20
Striped mullet (*Mugil cephalus*), SW	27				27
English sole (*Parophrys vetulus*), SW	<20	<20	<20	<20	<20
Starry flounder (*Platichthys stellatus*), FW	<20	<20	<20		<20
Starry flounder (*Platichthys stellatus*), SW	<20	<20	<20		<20
Longjawed mudsucker (*Gillichthys mirabilis*), SW	Standard: 1000				

[a] FW = freshwater habitat; SW = seawater habitat.

[b] 1 mU = hydrosmotic activity of 1 μg of acetone-dried *Gillichthys* urophysial powder.

distinguished the hydrosmotic factor from another urophysial principle—urotensin II, the smooth muscle-contracting substance, which has different properties (7). Urophyses of species used in the present study have all been found to contain urotensin II (11), with the exception of the hagfish.

If the urophysial hydrosmotic factor is indeed arginine vasotocin (6), its presence in the caudal neurosecretory system of some fishes but not of others might reflect varying neurohypophysial content of this octapeptide. A comparable survey of neurohypophysial hydrosmotic activity could be informative. If species with high urophysial activity were found to have relatively low neurohypophysial activity, this would suggest a possible role for the caudal neurosecretory system supplementary to that of the cranial neurosecretory system.

Summary. Sixteen species of fishes were examined for urophysial hydrosomotic activity comparable to that present in the caudal neurosecretory system of the globiid teleost *Gillichthys mirabilis*. Only two teleosts (milkfish and rainbow trout) showed hydrosmotic activity comparable with that of *Gillichthys*; the other species showed essentially no detectable activity on the isolated urinary bladder of the Hawaiian toad *Budo marinus*.

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