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Immunological Cross-Reaction between Pituitary Gonadotropin from North Atlantic Fish

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Pituitary extracts of 14 species of fish from the North Atlantic were tested in rainbow trout and carp gonadotropin radioimmunoassays (RIA). The poor cross-reaction and low gonadotropin content measured in the pituitary extracts indicate that RIA used for trout and carp gonadotropins are unsuitable for these species. The degree of specificity in the two systems does not reflect the phylogenetic relationships of the tested species. It is suggested that fish gonadotropins are species specific so that a sensitive RIA must be developed for each family investigated.

A species specificity between fish and mammalian gonadotropins was suggested by work on goldfish spermatogenesis (Billard *et al.*, 1970; Billard and Escaffre, 1973). Specificity among some groups of freshwater fish has also been indicated by other work on gonadotropins (Burzawa-Gérard and Fontaine, 1972; Fontaine *et al.*, 1972; Breton *et al.*, 1973). However during the final stages of gametogenesis (ovulation and spermiation) the species specificity appears to be less pronounced since human chorionic gonadotropin or heterologous pituitary preparations are potent treatments (cf. reviews by Pickford and Atz, 1957; Barnabé and René, 1972; Kuo *et al.*, 1973; Shehadeh *et al.*, 1973; De Vlaming, 1974).

In the present study the immunological cross-reaction between pituitary preparations of a variety of fish species was tested in order to extend the investigation of species specificity and to examine the possibility of gonadotropin measurement in other species using carp and trout radioimmunoassay (RIA) systems already available (Breton *et al.*, 1971; Breton and Billard, 1977).

MATERIALS AND METHODS

Preparation of the pituitary. Pituitaries were collected in March 1973 during a cruise aboard the MAFF Research Vessel CIROLANA in the North Sea and Barents Sea from both sexes of the species listed in Table 1.

Pituitaries were collected from freshly caught fish, immediately plunged into cold acetone (−10°), and then homogenized in the same medium. The pituitary material was dried and then homogenized in a glass-Teflon homogenizer at 5 mg ml^{-1} in 0.025 *M* veronal buffer pH 8.6, containing 2.5% human serum albumin. Extraction was allowed to proceed overnight at 4° with gentle stirring. The mixture was then centrifuged at 5000*g* at 4° for 20 min. Dilution of the supernatant gave the following set of concentrations: 1, 0.75, 0.25, 0.075, 0.05, and 0.025 mg of acetonic powder ml^{-1}.

RIA systems. All the pituitary extracts were tested by competition in the two RIA systems using purified fish gonadotropins.

The carp system was used in the conditions previously reported (Breton *et al.*, 1973).

The trout system used a gonadotropin purified from rainbow trout (t-GTH) (Breton *et al.*, 1976) and an antiserum prepared in guinea pig against the salmon gonadotropin S_G-G100 (Donaldson *et al.*, 1972). The initial concentration of the antibody was 1 to 30,000. t-GTH was iodinated (^{125}I Radiochemical Centre, Amersham, England) by the method described by Greenwood *et al.* (1963). Incubation and competition were as described for the carp system (Breton *et al.*, 1971).

Presentation of the results in Tables 2 and 3. Results for each immunological cross-reaction are expressed by the relationship logit $B/B_0 = f$ (log dose). The slope of the curve, the coefficient R for the linearity, and the t-GTH or c-GTH equivalent were also calculated for each species. Regression curves were compared by considering their residual variances (dispersion around the regression line), slopes, and zero intercepts according to the Snedecor and Cochran (1967) method.

RESULTS

In Tables 2 and 3 the species are classified according to the parallelism between

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TABLE 1

Order	Family	Species	
Teleosts			
Heterosomata	Pleuronectidae	Plaice	*Pleuronectes platessa*
		Dab	*Limanda limanda*
		Long rough dab	*Hippoglossoides platessoides*
		Halibut	*Hippoglossus hippoglossus*
Anacanthini	Macrouridae	Grenadier	*Coryphaenoides rupestris*
	Gadidae	Cod	*Gadus morhua*
		Haddock	*Melanogrammus aeglefinus*
Isospondyli	Osmeridae	Smelt	*Osmerus eperlanus*
		Capelin	*Mallotus villosus*
	Clupeidae	Herring	*Clupea harengus*
Scleroparei	Scorpaenidae	Redfish	*Sebastes marinus marinus*
	Cyclopteridae	Lumpsucker	*Cyclopterus lumpus*
Percomorphi	Anarhichadae	Catfish	*Anarhichas minor*
Elasmobranchs			
Selachii	Rajidae	Starry ray	*Raja radiata*

the slope of their competitive inhibition curve and those obtained with the specific hormone used in each system. The slope varied from −0.87 to −0.20 with the trout system and from −1.37 to −0.18 with the carp system. There were no significant differences between the coefficients of linearity for the various inhibition curves.

The immunological specificity was rarely the same in the two systems. Grenadier, cod, and lumpsucker showed marked differences. However the specificity of smelt and ray was particularly low in both systems.

The degree of specificity in the systems did not closely reflect the phylogenetic re-

TABLE 2
TROUT RIA SYSTEM

Alkaline pituitary extract from	Coefficient of correlation R	Slope of the curve logit $(B/B_0) = f$ (log dose)	Equivalent t-GTH ng mg^{-1}
Pure t-GTH	−0.998	−0.925	
♀ immature rainbow trout	−0.995	−0.915	5954.0
♀ mature rainbow trout	−0.995	−0.886	66880.0
Cod	−0.989	−0.869	0.8
Lumpsucker	−0.998	−0.774	210.9
Halibut	−0.978	−0.716	3.0
Plaice	−0.998	−0.714	9.6
Herring	−1.057	−0.662	5.6
Haddock	−0.992	−0.648	2.5
Redfish	−0.994	−0.585	6.9
Catfish	−0.954	−0.586	8.7
Dab	−0.993	−0.536	2.6
Long rough dab	−0.981	−0.463	2.9
Capelin	−0.987	−0.434	17.6
Starry ray	−0.979	−0.316	2.1
Smelt	−0.899	−0.292	4.9
Grenadier	−0.913	−0.197	1.0

TABLE 3
CARP RIA SYSTEM

Alkaline pituitary extract from	Coefficient of correlation R	Slope of the curve logit $(B/B_0) = f$ (log dose)	Equivalent c-GTH ng mg^{-1}
Pure c-GTH	−0.995	−0.973	
Carp	−0.992	−0.969	14200.0
Grenadier	−0.974	−1.374	3.6
Dab	−0.960	−1.156	1.2
Plaice	−0.974	−1.002	5.0
Herring	−0.984	−1.000	1.1
Long rough dab	−0.997	−0.965	1.6
Halibut	−0.996	−0.931	2.0
Haddock	−0.905	−0.896	0.5
Capelin	−0.996	−0.790	2.6
Redfish	−0.974	−0.721	3.2
Cod	−0.807	−0.659	1.2
Catfish	−0.985	−0.654	2.3
Lumpsucker	−0.995	−0.618	2.8
Starry ray	−0.924	−0.461	4.0
Smelt	−0.961	−0.183	2.5

lationships of the tested species. For example, although the pleuronectids were grouped in the carp system they were in two separated groups in the trout system. The osmerids were closer when tested against trout than against carp. The gadids were not grouped in either system.

Although the herring, smelt, and capelin are in the same order as the trout their phylogenetic relationship is not more apparent in the trout system than it is in the carp system.

Pituitary contents expressed in equivalents of c-GTH and t-GTH were very low in all the species tested in comparison with the homologous extracts. The trout system seems to be less specific than the carp but more sensitive. The high t-GTH equivalent for the lumpsucker in the trout system is not easily explained.

DISCUSSION

The high immunological species specificity for pituitary gonadotropins shown in this study indicates that a RIA to measure pituitary and plasma gonadotropin content using a carp or trout system is not suitable for the species tested.

Similar results have been obtained by Tan and Dodd (1978) using a heterologous RIA incorporating anti-salmon GTH and carp GTH as standards. In their system specificity was enhanced for some of the species examined, but there was no indication of the sensitivity of the assay.

Our results have been obtained from total pituitary extracts in which other components are able to destroy the immunoreactivity of the GTH. This was clearly demonstrated for growth hormone by Hayashida *et al.* (1975) and Farner *et al.* (1976). Crude pituitary extracts from nonmammalian species show varying degrees of nonparallelism with rat GH standard, but purified GH from the same species exhibits steeper curves which may be completely parallel. However, the experiments of Tan and Dodd (1978), and Breton and Burzawa-Gerard (1972), using crude pituitary extracts, in which parallelism with salmon and carp GTH standards was shown only for species within the respective family argues against this hypothesis.

Alternatively, the presence of proteolytic enzymes in the crude pituitary extracts or the reproductive condition of the donors

might account for the low values of pituitary GTH in our assays. In trout the pituitary content of GTH is 11 times greater in mature females than in immature fish (Table 2). However, the majority of the fish sampled were mature and either spawning or just spent and it is known for some species (trout, carp, and tench) that pituitary GTH content is still high in the spent condition (Breton *et al.*, 1976). It is more probable that the low values result from the high specificity of the antibodies employed, which cross-react well only with the antigens to which they have been raised.

The pronounced species specificity in the immunoreactive activity of gonadotropin throws doubt on the validity of RIA using a homologous system which differs from the GTH under assay. Although an immunological cross-reaction of 10% gives a positive reaction by immunofluorescence with antisera against o-LH and c-GTH in some species (Billard *et al.*, 1971; Goos *et al.*, 1976), it does not give specific displacement curves in a carp RIA system (Breton *et al.*, 1973) and it cannot be used for RIA, contrary to the suggestion of Goos *et al.* (1976).

It appears that some of the pituitary powders tested contained some common antigenic determinants. However these do not appear to be closely related to the phylogeny of the donor species. These common sites could be sufficient for a positive reaction in immunofluorescent studies, but not adequate for quantitative measurements. To provide a sensitive RIA system it will be necessary to obtain gonadotropin of at least each family, and probably of each genus, under investigation. These results confirm that the GTH of each fish species has a unique structure as has been demonstrated in other classes of vertebrates.

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