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SHORT COMMUNICATIONS

APPLICATION OF A RADIORECEPTOR ASSAY FOR LACTOGENIC ACTIVITY TO THE DETECTION OF FISH PROLACTIN-LIKE HORMONE

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Fish prolactin bioassays lack specificity (Sage & Bern, 1972), have poor sensitivity, are difficult to use (Ensor & Ball, 1968; Clarke, 1973) and contradictory conclusions have been drawn with them. Nicoll & Bern (1968) obtained no lactogenic effect of teleost pituitary gland in the pigeon-crop and the rabbit mammary gland tests, but Chadwick (1966) demonstrated the occurrence of a mammotrophic effect. Other workers have used heterologous radioimmunoassay (RIA) systems (McKeown & Van Overbeeke, 1972); these RIA systems do not seem to cross-react specifically with fish prolactin (Nicoll, 1975). We present a new approach using rabbit mammary gland prolactin receptors.

The technique used was a modification of the radioreceptor assay (RRA) for lactogenic hormones (Shiu, Kelly & Friesen, 1973). At the beginning of lactation the rabbit was injected with bromocryptine (CB 154, Sandoz) 36, 24, and 12 h before being killed, in order to reduce saturation of the receptors by endogenous prolactin. Mammary gland membrane proteins (400 µg) were incubated in the presence of 10^5 c.p.m. 125 I-labelled ovine prolactin for 6 h at 20 °C. Ovine prolactin NIH PS7 (24 i.u./mg) was used as the Standard. About 40% of the total labelled prolactin was specifically bound in the absence of any lactogenic hormone. Aspecific binding was about 5–7% of the total radioactivity added.

Fig. 1. Competition curves on rabbit mammary gland receptors between 125 I-labelled ovine prolactin and (★) salmon (female) gonadotrophin; (△) salmon (male and female) hypothalamic extract; (■) salmon (male) pituitary extract; (○) salmon (female) pituitary extract; (▲) ovine prolactin (NIH PS7 24 i.u./mg). Solid lines: µg of total protein/ml; broken lines: ng. The control value (100%) corresponds to the total labelled prolactin bound in the absence of unlabelled hormone.

The pituitary and hypothalamic extracts were prepared by homogenizing lyophilized salmon (*Oncorhynchus tshawytscha*) tissues in 25 mM-Tris-HCl, pH 7.6, 10 mM-MgCl₂ buffer. The extracts were centrifuged (5000g, 20 min, 4 °C) before use.

The results (Fig. 1) show that the extracts of male and female salmon pituitary glands were able to compete with the ovine prolactin on the rabbit mammary gland receptors. There was no difference between the male and female preparation. Actually the sensitivity of the technique allowed determination of prolactin activity in the range equivalent to 1–10 ng ovine prolactin; under these conditions 1 mg protein extracted from salmon pituitary glands contained the equivalent of 46 µg ovine prolactin. Similar assays with hypothalamic extracts and highly purified salmon gonadotrophin (B. Breton & P. Prunet, unpublished results) according to Idler's technique (Idler, Bazar & Hwang, 1975) do not show any competitive reaction. The results suggest the hypothesis of a specific binding of prolactin-like material in the salmon pituitary extracts. These preliminary results do not demonstrate exact parallelism between the displacement curves of ovine prolactin and the fish pituitary extracts; thus the values obtained do not represent an exact quantification of prolactin-like hormone present in the salmon hypophysis. But the apparent absence of effects due to protein concentration alone demonstrate a specific binding between rabbit mammary gland receptors and fish prolactin; this allows us to propose the use of the system for the detection of fish prolactin-like hormone.

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