Induction of Rabbit Casein Synthesis in Organ Culture by Tilapia Prolactin and Growth Hormone

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Highly purified tilapia (Sarotherodon mossambicus) prolactin and growth hormone added to culture medium of rabbit mammary gland explants specifically stimulated casein synthesis. The synthesized casein was quantified by an immunoprecipitation and further identified by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. In addition, the lactogenic activity of the fish hormones was determined by measuring the accumulation of β-casein mRNA using a 3H-labeled DNA complementary to the β-casein mRNA as a probe in a molecular hybridization. Moreover, tilapia prolactin was able to compete slightly with [125I]prolactin for the binding to rabbit mammary gland prolactin receptor. The lactogenic activities of the tilapia prolactin and growth hormone estimated by the rabbit casein assay were about 10 and 100 times lower, respectively, than the activity of ovine prolactin, which was used as a reference.

Prolactin and growth hormone have been isolated in highly purified form from tilapia (Sarotherodon mossambicus) (Farmer et al., 1976, 1977). These hormones proved to be involved in ionic balance and growth, respectively. The criterion used for the isolation of prolactin was based on its capacity to control ionic flux in tilapia (Doneen, 1976). Unlike mammalian prolactin, tilapia prolactin was shown to be devoid of lactogenic activity as judged by a casein assay using mouse mammary organ culture (Doneen, 1976). Similarly, extracts of fish pituitaries were shown to be unable to initiate milk synthesis when added to culture medium (Nicoll et al., 1966) or when injected into rabbit (Chadwick, 1966a, b). This suggested that the activity of prolactin on ionic balance (Bern, 1975) appeared in evolution long before the lactogenic activity (Nicoll et al., 1966). In a recent report, it was shown that extracts from salmon pituitaries were capable of competing with ovine prolactin for the binding to rabbit mammary gland receptor (Prunet et al., 1977). It was further demonstrated that various fractions extracted from salmon pituitaries exhibited a lactogenic activity as judged by their capacity to initiate casein and lactose synthesis in rabbit mammary gland organ culture (Prunet et al., 1979). These observations prompted us to reassess the question of the lactogenic activity of tilapia prolactin and growth hormone using the rabbit casein assay.

MATERIAL AND METHODS

Tilapia prolactin and growth hormone were fractions purified earlier (Farmer et al., 1976, 1977). Ovine prolactin was provided by the National Institute of Health (NIH-PS 13) and bovine growth hormone was from Pentex.

Rabbit mammary gland organ cultures were carried out essentially as previously described (Prunet et al., 1979). Casein synthesis was evaluated by an immunoprecipitation of the protein synthesized for 3 hr in the presence of 14C-amino acids at the end of the cultures (Prunet et al., 1979). Triton X-100 (1%) and sodium dodecyl sulfate (0.5%) were added to the incubate during the immunoprecipitation to reduce blanks (Dimitriadis, 1979). Polyacrylamide gel electrophoresis and hybridization of the [3H]cDNA probe with ca-
sein mRNA were carried out also as depicted in an earlier work (Prunet et al., 1979) with slight modifications: the cDNA probe was synthesized using partially purified rabbit β-casein mRNA instead of total casein mRNA and hybridizations were performed in 5-μl incubates (Teyssot and Houdebine, 1980).

The radioreceptor assay was conducted as previously described (Prunet et al., 1977): the labeled hormone was hGH (which is inherently a prolactin) and the membrane fraction was crude microsomes extracted from the mammary gland of a lactating rabbit treated for 36 hr with CB 154.

RESULTS

Initiation of casein synthesis. Ovine prolactin added to culture medium in the presence of insulin and cortisol exhibited a lactogenic activity as a function of its concentration (Fig. 1). The maximum activity was reached with 100 ng/ml. Higher concentrations and especially 20 μg/ml were less efficient, an observation which may be at least partly explained by the fact that prolactin receptor is down-regulated by prolactin itself and that acute prolactin treatments reduce greatly the number of prolactin receptor, leading to a possible desensitization of the mammary cell (Djiane et al., 1979). Tilapia prolactin and growth hormone both exhibited lactogenic activity also, however, the potencies were 10 and 100 times lower than that of ovine PRL (Fig. 1). By contrast, bovine growth hormone was essentially inactive, 0.7% relative to ovine PRL.

The specificity of the immunoprecipitation technique was checked by an electrophoresis of the immunoprecipitated material on acrylamide gel in denaturing conditions. The patterns of Fig. 2 clearly indicate that the immunoprecipitates contained fractions which migrated identically with the casein marker when either ovine or tilapia prolactin, or tilapia growth hormone were present in the culture medium.

Accumulation of β-casein mRNA. Previous work has established that prolactin injected into rabbits or added to culture medium was responsible for an accumulation of casein mRNA in the mammary cell which roughly correlated with the rate of casein synthesis (Houdebine, 1976; Devinoy et al., 1978). Results shown in Fig. 3 indicate that tilapia prolactin and growth hormone, like ovine prolactin, induced an accumulation of β-casein mRNA in relation with their capacity to stimulate casein syn-

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**Fig. 1.** Induction of casein synthesis under the influence of hormones added to culture medium. Results are expressed as the percentage of the labeled mammary proteins synthesized during the last 3 hr of the culture and immunoprecipitated with the anti-casein as a function of the concentration of hormones in the medium. (+) Ovine prolactin; (⊙) tilapia prolactin; (□) tilapia growth hormone; (▵) bovine growth hormone.
thesis. This further demonstrates that the fish hormones act similarly to mammalian prolactin on the mammary cell.

**Binding of tilapia prolactin to rabbit mammary gland prolactin receptor.** Salmon prolactin proved to compete specifically with ovine prolactin for binding to rabbit mammary gland receptors (Prunet et al., 1977). Since both tilapia and salmon prolactin have lactogenic activity, the tilapia hormone should also be able to bind to this prolactin receptor. Results shown in Fig. 4 indicate that this is indeed the case. However, the fish hormone appeared to be much less efficient that the ovine prolactin in competing with the labeled ligand. The observation that the binding of the fish hormones was not strictly related to their lactogenic activity may be due to the fact that in the radioreceptor assay, the fish prolactin was in competition with a mammalian prolactin whereas in the casein assay tilapia prolactin was acting alone. This suggests that tilapia prolactin has a lower affinity than ovine prolactin for the mammary receptor.

**DISCUSSION**

The data of the present report clearly indicate that tilapia prolactin is endowed with significant lactogenic activity. The fact that the salmon pituitary also contains a factor capable of inducing casein synthesis in rabbits suggests that this property might be general among teleosts and that lactogenic activity is present in the prolactin molecule earlier in evolution than previously suggested (Nicoll et al., 1966). The lower activity of the fish hormone as compared to ovine PRL may well be due to species. This finding may also suggest that full lactogenic potency of the prolactin molecule was gained only progressively.

Tilapia GH also showed significant lactogenic activity, 10% relative to tilapia PRL. By radioimmunoassay tilapia GH has only a 0.05% contamination with tilapia PRL (Nicoll et al., 1981). Because of this result and the high degree of purity of the tilapia GH (Farmer et al., 1976), we are convinced that the lactogenic activity is intrinsic and not due to PRL contamination. This is not a surprising finding. Growth
hormone and prolactin genes are known to have a common ancestor (Cooke et al., 1980). These closely related molecules have previously been shown to have "cross-intrinsic" activities, particularly when tested in distantly related heterologous assays. Tilapia PRL showed greater cross-reactivity in a mammalian GH RIA than did tilapia GH (Farmer et al., 1977). Human GH is well known for its intrinsic lactogenic activity.

The results reported herein are con-
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tradicory with previous work in which it was shown that tilapia prolactin was devoid of lactogenic activity (Farmer et al., 1977). The precise reason for this discrepancy is not known. However, it should be considered that the rabbit mammary gland is known to be very sensitive to prolactin, since it can be stimulated even in the absence of glucocorticoids (Devinoy et al., 1978). The lactogenic activity of tilapia PRL may have escaped detection in the less sensitive mouse casein assay.

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REFERENCES


