Short Paper

Adipogenic Activity of Fish Growth Hormones on Mouse Preadipose 3T3 Cells

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Several laboratories succeeded in the isolation of growth hormone (GH) complementary deoxyribonucleic acid from several species of fishes and recombinant GH was produced by genetically engineered bacteria.¹⁻³ As a result, a massive supply of fish GHs is available for fish culture as well as of human GH (hGH) for the treatment of dwarfism in the human. The industrial use of GH for fish culture is noteworthy.

Recently, Morikawa *et al.*⁴ found that under proper culture conditions quiescent 3T3-F442A preadipose cells⁵ derived from a Swiss mouse undergo differentiation into adipocytes by the addition of GH to the culture medium. This adipogenic property was specific to mammalian GHs, including hGH.⁶ This fact indicates the possibility that 3T3 preadipocytes can be used as standard cells for assaying the activity of GHs from species other than mammals and various variants of hGH derived by means of mutagenesis.⁷ In the present paper, the activities of the GHs from salmon and eel were compared with the activities of mammalian GHs, using 3T3 preadipose cells.

The purification of recombinant hGH was described previously.⁸ Recombinant salmon GH (sGH) and eel GH (eGH) were purified as described by Sugimoto *et al.*^{9,10} Figure 1 shows the adipogenic activities of sGH and eGH, compared with ovine GH (oGH). The hormone concentration required for half the maximal response

elicited by oGH was in the range of 20–50 ng/ml and hGH also showed a similar dose response curve (data not shown).^{4,9} The effective concentration of sGH was about 15 times greater than that of oGH, although its maximal response was similar to that of oGH. On the contrary, eGH was only slightly active at 25 µg/ml. We confirmed that the recombinant hormones showed similar activities in radioreceptor assays in the trout and eel with those of natural hormones.^{11,12} Since eGH showed little activity at concentrations up to 5 µg/ml, it seems that the binding activity of eGH to the mouse GH receptor is small. In the separate experiments (data not shown), the adipogenic activity of oGH (50 ng/ml) in the presence of various concentrations of eGH was examined. The addition of a low concentration of eGH (10 ng/ml) inhibited the oGH activity by 20–25%, but no further competitive inhibition or additive activity was detected with increasing concentrations of eGH up to 25 µg/ml. This suggests that the binding affinity of eGH to the GH receptor of 3T3 cells may be much weaker than that of oGH. Furthermore, the sGH and eGH incubated with the media containing 10% fetal bovine serum for 2 weeks had the same adipogenic activities (data not shown), indicating that those fish GHs were not degraded by serum proteases. We also examined the adipogenic activity of natural salmon prolactin. It did not induce the adipose conversion of 3T3 cells at 25 µg/ml (data not shown), suggesting that the adipogenic activity is specific to GH even in salmon. These results showed that Swiss mouse 3T3 cells can possibly be used to determine the biological activity of GHs from some lower vertebrates including fishes, although they are less sensitive to fish GHs than mammalian GHs.

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Fig. 1. Adipogenic activity of oGH and fish GHs. Preparations of oGH (circle), sGH (square), and (triangle) were assayed as described previously.^{4,9} Individual points represent the mean $\pm$ SE of duplicate determinations.

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