Effect of Lipids on Growth Hormone Synthesis by Isolated Pituitaries (35720)

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(Introduced by A. Lopez-S)

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Previous studies from our laboratory have indicated a suppressive effect of lipids on growth hormone secretion. Both intravenously administered soybean oil emulsion (Intralipid) and sodium octanoate have been shown to inhibit insulin-induced plasma growth hormone elevations in Rhesus monkeys (1). More recent observations from our laboratory have shown that oral soybean oil emulsion (Lipomul) plus parenteral heparin inhibits the plasma growth hormone elevations in man caused by insulin and arginine (2). Ketones which are formed from FFA and are more readily utilized by the central nervous system do not have an inhibitory effect on growth hormone secretion (1). In an attempt to elucidate the mechanism of suppression of growth hormone secretion by lipids, a study of the effect of fatty acids on growth hormone synthesis by the isolated pituitary was undertaken.

Materials and Methods. The materials and experimental procedures used were the same as those previously described by the authors (3). Male Holtzman rats weighing 180 g were fed Purina rat chow and kept in our animal room at 26° and 12 hr of darkness for 3 days prior to decapitation. The posterior pituitary was removed and the anterior pituitary was placed in Krebs–Ringer bicarbonate (KRB) media (0.2 ml), at pH 7.4 containing 360 μg of glucose and 0.1 μCi of leucine-^{14}C. The experimental media contained either sodium palmitate or sodium octanoate at 1.5 and 3 mM concentrations. In the palmitate experiments, the fatty acid was conjugated to bovine serum albumin (FFA poor) according to the method of Evans and co-workers (4) and the control flasks for these experiments contained 4% bovine serum albumin without palmitate. The FFA concentration of the media was confirmed by direct measurement.

The completely submerged anterior pituitary was flushed with 95% O_2 and 5% CO_2 and its container sealed and incubated at 37.5° for 3 hr. It was then removed, rinsed, and homogenized. The homogenates were

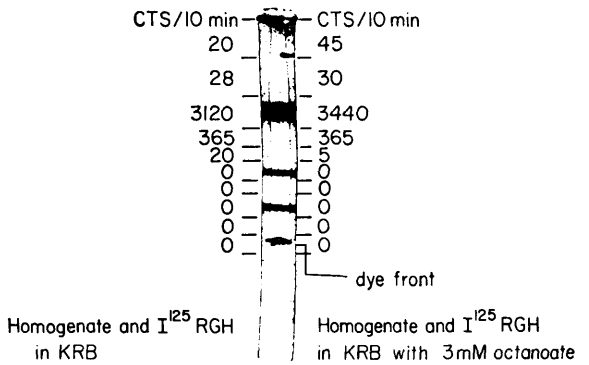

Fig. 1. Distribution of radioactivity in disc electrophoretic pattern when ^{125}I labelled rat growth hormone and rat pituitary homogenate are mixed in sample gel with and without 3 mM sodium octanoate.

TABLE I. Effect of Sodium Octanoate on Incorporation of Leucine-^{14}C into Growth Hormone of Isolated Rat Pituitaries.

Number of pituitaries	Medium	Incorporation into pituitary GH (dpm/pituitary)	Released into media as GH (dpm/pituitary)
5	KRB	27614 ± 3372[a]	591 ± 109
5	KRB–1.5 m*M* Octanoate	12800 ± 424	1054 ± 170
5	KRB–3 m*M* Octanoate	1814 ± 127	1452 ± 167

[a] Mean ± SEM.

fractionated by disc electrophoresis, stained and destained. The growth hormone (GH) bands were clearly visible and were cut out and counted in a liquid scintillation counter. In postincubation media not containing albumin, the incorporation of leucine-^{14}C into media growth hormone ("release") was assessed by adding homogenized carrier anterior pituitary to the media and subjecting it to the same procedures as for the experimental anterior pituitary homogenates.

Results and Discussion. The first figure shows a typical disc electrophoretic pattern of a pituitary homogenate. The heavy top band is the growth hormone containing band followed by the albumin and prolactin bands. The identity of the growth hormone containing band was established in our experiments by testing migration of ^{125}I labeled rat growth hormone. All but a small amount of the radioactivity as represented by the numbers on the left was detected in the top band. Further, the presence of sodium octanoate did not interfere with the migration of either the stained band or the labelled rat growth hormone as indicated by the numbers on the right.

The data in Table I indicate that sodium octanoate added to the incubation media at 1.5 and 3 m*M* concentrations inhibits the incorporation of leucine-^{14}C into pituitary growth hormone. The decreased radioactivity of the pituitary growth hormone band in the presence of octanoate can not be attributed solely to release of growth hormone.

As octanoate is not a constituent of the mammalian FFA pool and as this eight-carbon fatty acid has been shown to uncouple oxidative phosphorylation (5) and cause hemolysis (1), an attempt to study the effect of a more physiological fatty acid such as palmitate on growth hormone synthesis was made. In order to solubilize palmitate, it was necessary to add 4% bovine serum albumin (FFA poor) to the media (4). Unfortunately, the high concentration of albumin produced a quenching effect on scintillation counting of the media and prevented adequate study of "release". However, palmitate of 1.5 and 3 m*M* concentrations did not significantly inhibit the incorporation of leucine-^{14}C into pituitary growth hormone (Table II). As Buse and co-workers (6) had shown that the inhibitory effect of palmitate on protein synthesis by isolated rat diaphragm is observed only in the presence of insulin, further experiments with the addition of insulin were performed. As seen in the

TABLE II. Effect of Sodium Palmitate With or Without Insulin on Incorporation of Leucine-^{14}C into Growth Hormone of Isolated Rat Pituitaries.

Number of pituitaries	Medium[a]	Incorporation into pituitary GH (dpm/pituitary)
6	KRB–4% Albumin	16468 ± 1991[b]
6	KRB–1.5 m*M* Na Palmitate	16570 ± 1551
6	KRB–3 m*M* Na Palmitate	17373 ± 1806
6	KRB–Insulin–4% Albumin	20574 ± 1074
6	KRB–Insulin–1.5 m*M* Palmitate	20545 ± 1314
6	KRB–Insulin–3 m*M* Palmitate	18093 ± 2399

[a] All media were made up with 4% bovine serum albumin (FFA poor). Insulin was added to the media at a concentration of 100 mU/ml in experiments with insulin.

[b] Mean ± SEM.

bottom half of Table II, palmitate even in the presence of insulin, failed to alter leucine-^{14}C incorporation into pituitary growth hormone.

Summary. The effect of octanoate and palmitate on growth hormone synthesis by isolated rat pituitaries was studied. Octanoate had an impressive inhibitory effect on the incorporation of leucine-^{14}C into pituitary growth hormone. The more physiological fatty acid palmitate however, had no effect on incorporation of leucine-^{14}C into pituitary growth hormone. This latter finding suggests that the *in vivo* inhibitory effect of lipids on growth hormone secretion can not be explained by a direct inhibitory effect on pituitary growth hormone synthesis.

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