Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3969–3972, June 1981
Physiological Sciences

Adipogenic and anti-adipogenic factors in the pituitary and other organs

(adipose conversion/adipogenic factor/fibroblast growth factor/platelet-derived growth factor)

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Contributed by Howard Green, February 17, 1981

ABSTRACT **Adipose conversion of cultured 3T3 cells is known to depend on an adipogenic factor present in serum. In the presence of this factor, extracts of different organs were found to inhibit the adipose conversion. The most active extracts were derived from brain, uterus, and pituitary, but other organs also possessed appreciable activity. Fibroblast growth factor partially purified from both brain and pituitary was much more active in suppressing adipose conversion than were crude extracts of the corresponding organs. Purified platelet-derived growth factor was also an effective inhibitor. Of all the tissue extracts tested, only pituitary possessed, in addition to the inhibitory activity, an adipogenic factor similar to that demonstrated previously in serum. This was revealed at concentrations of extract too low for the inhibitory factor to be effective. Under these conditions the pituitary extract had a specific adipogenic activity orders of magnitude higher than that of serum. We suggest that the adipogenic factor of serum may originate in the pituitary.**

When growth of susceptible 3T3 cells is slowed or arrested, they are able to convert to adipocytes (1, 2). In this process, the cells develop abundant lipogenic and lipolytic enzymes (3–9), become sensitive to hormonal control (10, 11), and accumulate triglyceride. When injected into animals, preadipose 3T3 cells give rise to typical adipose tissue (12).

In order to undergo adipose conversion, 3T3 cells require the presence of an adipogenic factor. This factor is present in serum of humans and most animals but is virtually absent from cat serum (13). Using cat serum for cell growth and maintenance, it is possible to assay the adipogenic factor by measuring the increased glycerophosphate dehydrogenase activity developing in the cells (14, 15).

We have now examined extracts of different bovine organs for adipogenic activity and found it only in the pituitary. On the other hand, inhibitory activity, associated with certain mitogenic polypeptides, was more widely distributed.

MATERIALS AND METHODS

Cell Culture Conditions. Stocks of 3T3-F442A cells were inoculated in the Dulbecco–Vogt modification of Eagle's medium supplemented initially with 10% calf serum and then refed with medium containing 5% cat serum. For experiments, exponentially growing cultures were disaggregated with 0.1% trypsin/1 mM EDTA, and cells were inoculated into 35-mm or 96-well microtest Falcon dishes at about 500 cells per cm^2. On the following day, the serum supplement was changed to fetal bovine serum or cat serum at 5% and insulin was added to a concentration of 5 μg/ml.

Assay of Adipose Conversion by Glycerophosphate Dehydrogenase Activity. After cultures in 35-mm dishes had been confluent for some time and adipose conversion was evident, the cells were washed with neutral isotonic phosphate buffer and then harvested in 0.3–0.5 ml of 25 mM Tris·HCl/1 mM EDTA, pH 7.5, by using a rubber policeman. The cells were sonically disintegrated for 3 sec at 30 W with the microtip of a Branson model 185 sonifier. The suspension was centrifuged at 10,000 × *g* for 5 min at 4°C, and the supernatant was stored at −70°C. Cultures in microtest plates were treated identically, except that the cells were sonicated directly in the wells in 0.1 ml of Tris buffer. Assay of activity of glycerophosphate dehydrogenase (EC 1.1.1.8) was carried out by measuring oxidation of NADH at 340 nm (14, 16). Protein concentration was determined by using Coomassie brilliant blue G250 (17).

Tissue Extracts. Frozen or lyophilized bovine tissues were obtained from Pel-Freeze. Frozen tissues were added to 3 vol of cold isotonic phosphate buffer and homogenized either with a Sorvall Omni-mixer or by sonic disintegration. The homogenate was stirred at 4°C for 2 hr and then centrifuged for 60 min at 20,000 × *g*. If not clear, the supernatant was further centrifuged at 100,000 × *g* for 20 min. The supernatant was sterilized by filtration and stored at −70°C. Lyophilized pituitary glands had been separated into anterior and posterior lobes by the supplier. Whether the intermediate lobe had separated with the posterior lobe alone or with anterior as well could not be ascertained. The glands were either hydrated and then extracted like other tissues or were powdered and dissolved in phosphate buffer by sonic disintegration.

Inactivation of Inhibitory Factors by Acidification. Crude tissue extracts (protein ≈ 1 mg/ml) and purified pituitary fibroblast growth factor (FGF) (10 μg/ml) were mixed with an equal volume of 0.6 M acetic acid, kept at 37°C for 30 min, and then neutralized with 0.6 M NaOH. As some precipitate formed, the suspension was centrifuged for 10 min at 10,000 × *g* and then sterilized by filtration. Appropriate dilutions were tested for ability to support adipose conversion, with untreated extract used as control.

RESULTS

Presence of an Inhibitor of Adipose Conversion in Organ Extracts. Extracts were prepared from frozen bovine organs and tested for adipogenic activity on 3T3-F44A cells. Cat serum was used as serum supplement because it contains very little adipogenic factor. Initially, no adipogenic activity was found, and

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Abbreviations: FGF, fibroblast growth factor; I_{90}, concentration of material required to decrease glycerophosphate dehydrogenase activity by 90%; PDGF, platelet-derived growth factor.

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Table 1. Effect of organ extracts on adipose conversion

Tissue	Inhibitory activity* Range tested, μg/ml	Inhibitory activity* I_{90}, μg/ml	Adipogenic activity† Range tested, μg/ml	Adipogenic activity† Max. activity
Brain	0.2–100	3.3	0.003–200	1.6
Uterus	0.3–142	9.0	0.004–300	2.7
Pituitary:				
Posterior	1.5–215	20.0	0.001–10	>400
Anterior	2.0–250	28.0	0.01–8	>1000
Hypothalamus	0.9–115	27.0	0.1–230	2.9
Adrenal	0.3–160	33.0	0.005–300	5.4
Spleen	0.4–211	37.0	0.07–400	2.8
Submaxillary gland	0.4–190	43.0	0.008–380	6.0
Pancreas	0.5–250	48.0	0.024–500	2.6
Liver	0.6–280	66.0	0.03–500	1.5
Kidney	0.7–360	93.0	0.08–700	2.5
Ovary	1.6–215	94.0	0.036–430	3.0

* Inhibitory activity is expressed as I_{90}, the concentration of extract protein that decreases cellular glycerophosphate dehydrogenase activity by 90%. For assay of inhibitory factor, organ extract was added to culture medium supplemented with 5% fetal calf serum and insulin at 5 μg/ml. Cells were extracted for measurement of enzyme activity 8–9 days after reaching confluence.

† Adipogenic activity was assayed by adding organ extract to cultures incubated in medium supplemented with 5% cat serum and insulin at 5 μg/ml. Cells were harvested 16 days after reaching confluence. Controls to which no extract was added gave 1–5 units of enzyme activity per mg of cell protein. Activity is expressed as units of glycerophosphate dehydrogenase activity per mg of cell protein.

the extracts were then tested for inhibitory activity by using medium enriched with 5% fetal bovine serum and insulin at 5 μg/ml, a supplement supporting extensive adipose conversion. Organ extracts were added at various concentrations beginning when the cells were still preadipose, several days before the cultures became confluent. Eight or 9 days after cultures became confluent, the cells were homogenized and the activity of glycerophosphate dehydrogenase was measured. An increase in activity depends on the concentration of adipogenic factor over a certain range and is a sensitive and precise measure of adipose conversion (14, 15). The levels of enzyme activity showed good correlation with the number of adipose cell clusters visible in the cultures.

Extracts of numerous organs inhibited the adipose conversion (Table 1). The most active were those of brain, uterus, and pituitary; these extracts inhibited adipose conversion by 90% at protein concentrations of 3–30 μg/ml. Extracts of other organs were active at higher concentrations.

Nature of the Inhibitory Activity. In an attempt to identify the inhibitory agent, we tested a number of known hormones. None was inhibitory (footnote to Table 2). However, marked inhibitory activity was present in an impure luteinizing hormone preparation (NIH-LH) obtained from the National Institutes of Health. At 10 μg/ml, equivalent to 0.09 unit/ml, this preparation inhibited adipose conversion by 90%. Because NIH-LH is known to contain FGF as a contaminant (18), we tested purified FGF (19, 20) kindly made available by D. Gospodarowicz, R. Bradshaw, and P. Kelley. Between concentrations of 0.03 and 1 μg/ml, FGF of either brain or pituitary origin was able to prevent adipose conversion in a concentration-dependent manner (Fig. 1). The I_{90} of the different preparations was less than 0.18 μg/ml (Table 2).

The mitogenic activity of FGF is known to be inactivated by acid (20) but not by reducing agents (23). Crude extracts of brain and posterior pituitary as well as purified pituitary FGF were treated with these agents and then assayed for their ability to inhibit adipose conversion (Table 3). In all three cases, inhibitory activity was much diminished by treatment with 0.3 M acetic acid for 30 min at 37°C but was unaffected by 0.1% mercaptoethanol.

Other polypeptides mitogenic for 3T3 cells were also tested for their ability to inhibit adipose conversion. Platelet-derived growth factor (PDGF) (24) was found to be effective in preventing adipose conversion (Table 2). Consistent with this finding, preliminary experiments have indicated that human serum derived from plasma is much more adipogenic than human serum derived from whole blood.

In contrast to FGF and PDGF, epidermal growth factor (25)

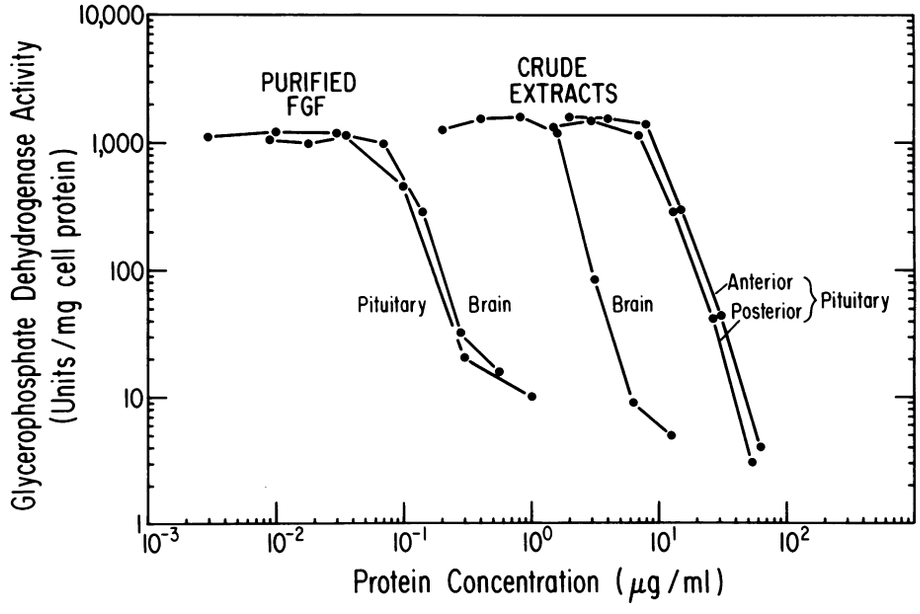

FIG. 1. The inhibitory effect of pituitary and brain extracts and of purified FGF on adipose conversion. Crude extracts and purified FGF derived from corresponding organs were added to culture medium supplemented with fetal bovine serum. Cultures were harvested for enzyme assay about 8 days after reaching confluence. The FGF was 50- to 500-fold more active than the crude extracts in suppressing adipose conversion. The preparation of brain FGF was that of R. Bradshaw; the pituitary FGF was that of P. Kelley.

Table 2. Effect of mitogenic polypeptides on adipose conversion

Agent tested	Range tested, μg/ml	I_{90}, μg/ml
FGF derived from:		
Brain	0.0045–0.554	0.18
Pituitary	0.001–1	0.16
NIH-LH	0.5–10 (0.005–0.09 unit/ml)	10
Pure LH	0.001–1 (0.01–10 units/ml)	No activity
PDGF:		
Partially purified by heat treatment*	0.002–33	3.6
Partially purified by using CM-Sephadex†	0.1–6	3.7
Highly purified‡	0.0005–0.064	0.04
Epidermal growth factor	0.002–0.5	Virtually no activity

Hormones tested over a 3000-fold concentration range and found not to inhibit adipose conversion: α- and β-endorphin, methionine-enkephalin, leucine-enkephalin, luteinizing-hormone releasing hormone, thyrotropin-releasing hormone, somatostatin, adrenocorticotropic hormone, follicle-stimulating hormone, thyroid-stimulating hormone, growth hormone, *vasopressin*, *oxytocin*, aldosterone, *17β-estradiol*, *hydrocortisone*, progesterone, and *testosterone*. None of the above was adipogenic in themselves, but those in italics enhanced the adipose conversion induced by fetal calf serum.

* Kindly supplied by Charles Stiles and James Smith, Sidney Farber Cancer Institute (21).

† Kindly supplied by Russell Ross and Elaine Raines, University of Washington (22).

‡ Kindly supplied by Russell Ross and Elaine Raines (unpublished method).

had practically no effect on the adipose conversion at concentrations up to 100 times greater than necessary to produce detectable cell proliferation. Inhibitory effects on adipose conversion evidently are not exerted by all mitogenic polypeptides.

Presence of Adipogenic Activity in the Pituitary. Extracts of the same tissues were again tested for the presence of an adipogenic factor analogous to that described previously in bovine serum. Because of the inhibitory activity present in these extracts, adipogenic activity, if any, might be evident only at a concentration of extract too low for the inhibitory factor to block adipose conversion. For this reason, each extract was tested over a broad concentration range and the cultures were maintained for 16 days after reaching confluence. Cat serum was used as serum supplement. Extracts were added to the medium beginning 2 days after inoculation of cells and were renewed at each medium change.

Of all the tissues tested, only the pituitary possessed obvious adipogenic activity (Table 1). The activity of posterior pituitary extract increased over a protein concentration range of 50 ng/ml to 1 μg/ml (Fig. 2). At higher concentrations (>3 μg/ml) the activity declined, probably because in this range the inhibitory factor becomes effective (see Fig. 1). The adipogenic activity was about 500 times higher than that of fetal bovine serum. Like the adipogenic factor of serum, the pituitary adipogenic factor was nondialyzable, was stable at 4°C, and did not induce lipid accumulation or increased glycerophosphate dehydrogenase activity in 3T3-C2 cells (Fig. 2). Later experiments showed that extracts of anterior pituitary were active at a protein concentration less than 10 ng/ml, corresponding to a specific activity 10–100 times higher than that of posterior lobe extract. It may be significant that adipose conversion induced by pituitary extract developed more slowly than that induced by fetal bovine serum.

Acidification of posterior pituitary extract with 0.3 M acetic acid removed inhibitory activity (Table 3) and was found to increase the adipogenic activity of a high concentration of extract. Even though acidification of other organ extracts (brain, uterus, hypothalamus, spleen, and submaxillary gland) also greatly decreased their inhibitory activity, no adipogenic activity was revealed.

Table 3. Loss of inhibitory activity after acidification

Treatment of inhibitory factor prior to addition	Glycerophosphate dehydrogenase activity, units/mg cell protein: Brain extract	Posterior pituitary extract	Pituitary FGF
None	92	3	106
0.1% 2-mercaptoethanol	110	7	83
0.3 M acetic acid	530	482	1454
Control (no inhibitory factor)	941	941	1160

Inhibitory factor was added, to a concentration equal to I_{90}, to medium supplemented with fetal bovine serum (5%) and insulin (5 μg/ml). Enzyme activity was determined 8 or 9 days after the cultures became confluent.

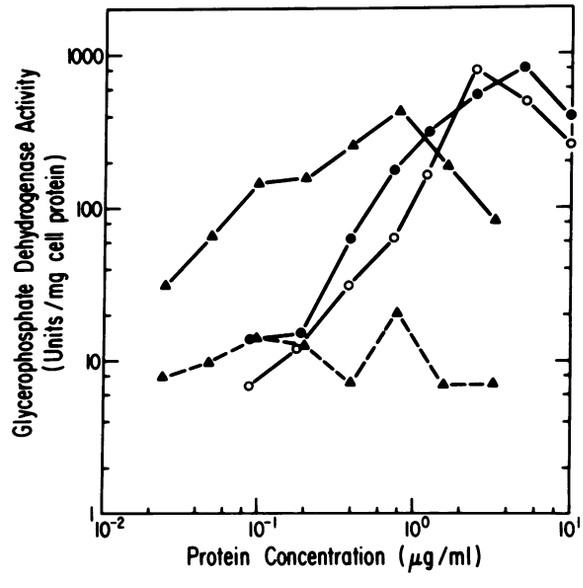

FIG. 2. The presence of adipogenic factor in the pituitary. Extracts of bovine posterior pituitary gland were added to culture medium supplemented with cat serum, which possesses practically no adipogenic activity. The cultures were harvested for enzyme assay about 15 days after reaching confluence. Solid lines, effects on 3T3-F442A cells; open circles, fresh extract; solid circles, same extract dialyzed; triangles, different dialyzed extract stored at 5°C for 6 months; broken line, absence of effect on 3T3-C2 cells, which are virtually insusceptible to adipose conversion.

DISCUSSION

The simple presence in organ extracts of activity inhibitory to adipose conversion does not necessarily mean that these organs have a role in fat cell formation. Such activity was widely distributed and there was only a difference of about 30-fold between the most active and least active extracts. The inhibitory activity of brain and pituitary extracts was associated with the polypeptide FGF. All preparations of this mitogen are highly impure (26), but a similar activity was associated with a highly purified platelet mitogen prepared by R. Ross and E. Raines (unpublished method). Both FGF and PDGF are known to be mitogenic for 3T3 cells (19, 27–29); however, in medium richly supplemented with serum they were able to inhibit adipose conversion with very little, if any, effect on cell multiplication. Epidermal growth factor, also mitogenic for 3T3 cells (30), did not inhibit adipose conversion even at a concentration 100 times higher than necessary to induce proliferation. The ability of FGF and PDGF to inhibit adipose conversion is therefore not shared by all mitogenic polypeptides.

The inhibitory factor in the pituitary and in FGF preparations probably does not act by preventing interaction of the adipogenic factor with the cells because removal of medium containing both was followed by some adipose conversion. These agents may be useful in studying the initial stages of adipose conversion.

Whether FGF or PDGF exerts any effect on adipose tissues of animals is problematic; but a physiological role for the adipogenic factor of the pituitary seems quite plausible. This organ was the only one in which adipogenic activity was detected; inhibitory factor might have concealed small amounts of adipogenic activity in other organs, but extracts were tested over a broad concentration range and elimination of most of the inhibitory activity by acidification did not reveal adipogenic activity.

We were unable to associate the adipogenic activity with any known pituitary hormone and cannot say whether the adipogenic activity of anterior and posterior lobes resides in the same molecule. The adipogenic factor cannot even be assigned definitively to both anterior and posterior lobes because we do not know the contribution that might be made by the intermediate lobe. Nevertheless, it is reasonable to suppose that the serum adipogenic factor originates from the pituitary.

Recent studies have led to the conclusion that the pituitary contains several polypeptides that promote multiplication of cultured human pre-adipose cells (ref. 31; personal communication). Whether these factors are related to those described here remains to be elucidated.

These investigations were aided by a grant from the National Institute of Arthritis, Metabolism and Digestive Diseases.

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