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REEVALUATION OF LIPOLYTIC ACTIVITY OF GROWTH HORMONE IN RABBIT ADIPOCYTES

Bruno Barenton [1], Véronique Batifol [1], Yves Combarnous [2], Jean-Paul Dulor [1], Philippe Durand [2] and André Vezinhet [1]

[1] Institut National de la Recherche Agronomique
ENSA - Station de Physiologie Animale, 9, place P. Viala - 34060 Montpellier, France

[2] Station de Physiologie de la Reproduction, 37380 Nouzilly, France

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SUMMARY The lipolytic activities of porcine pituitary fractions and purified growth hormone (GH) from human (h), porcine (p), ovine (o) and rabbit (Rb) origin as well as ovine placental lactogen (oPL), were compared to that of ACTH on rabbit adipocytes. All the GH preparations and oPL were equivalent in inhibiting the binding of labelled oGH to liver plasma membranes from pregnant rabbits. ACTH, and to a lesser extent porcine pituitary fractions and hGH, stimulated free fatty acid production by isolated adipocytes. The sensitivity of the adipocytes to these factors was increased when adenosine deaminase was added to the incubation medium. But, RbGH, pGH, oGH and oPL had no effect. We conclude that GH is not directly involved in the control of lipolysis in rabbit adipocytes and that the effect of hGH is rather due to a contamination of this preparation by other pituitary factors.

The existence of binding sites specific for human growth hormone (hGH) in rat adipocytes argues for a direct effect of this hormone on the adipose tissue (1). hGH has been assumed to be involved in the control of most of the metabolic activities of that tissue. These include glucose and leucine oxydation, lipolytic, antilipolytic (2), lipogenic (3) and antilipogenic (4) activities. However, the control of lipolysis by hGH in the rat is controversial (5-8). Even the use of bacteria-derived hGH has given conflicting results (5,7).

In rabbit adipocytes, hGH has also been shown to stimulate the rate of lipolysis; this effect being associated with an accumulation of cAMP (9). Likewise, earlier reports from our group claimed that porcine and rabbit GH,

ABBREVIATIONS

RbGH, pGH, oGH and hGH = rabbit, porcine, ovine and human growth hormone; oPL = ovine placental lactogen; ACTH = adrenocorticotrophic hormone, P_{40} and S_{40} = pellet and supernatant after 40% ammonium sulfate precipitation of porcine pituitary fractions; FFA = free fatty acids, IC_{50} = half maximum inhibiting concentration; ED_{50} = half maximum stimulating of effective dose; Rmax = maximum response; cAMP = cyclic adenosine monophosphate.

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but not that from ovine and bovine species, exert a lipolytic effect in rabbit adipocytes (10,11). However in all these studies, the doses of GH employed were supra-physiological. In addition, the hormonal preparations presently used have a higher degree of purity than those previously available. Therefore, we have reevaluated the lipolytic effect of GH from different species in rabbit adipocytes incubated in vitro.

MATERIALS AND METHODS

Hormones: Purified rabbit GH (RbGH) was prepared according to the method described by Ellis et al (12). Human GH (hGH; 1 IU/mg), porcine GH (pGH ; 1.5 IU/mg) and ovine GH (OGH-NIH-S_5 ; 1 IU/mg; and oGH - I_3) were kindly supplied by Dr. F. Dray (Paris - France), Dr. A. Wilhelmi (Atlanta - U.S.A.) and the NIADDK respectively - Synthetic $ACTH_{1-24}$ (Synacthen) was purchased from Ciba (Rueil-Malmaison - France). Ovine placental lactogen (oPL) was donated by Dr. J. Martal (Jouy-en-Josas - France). It was used according to its GH activity expressed as equivalent of oGH-NIH-S_5 in the following binding assay.

The supernatant (S_{40}) and the pellet (P_{40}) lipolytic fractions were obtained after 40% ammonium sulfate precipitation of a porcine pituitary extract (pH 9.5) in which the gonadotrophins were previously extracted at pH 5. These fractions were dialysed, lypophilized and weighed. All the hormonal preparations were dissolved in 10 mM $NaHCO_3$ before use.

Hormone Assays: The binding ability of the porcine pituitary fractions and hormonal preparations were compared using crude liver plasma membranes from pregnant rabbit as described by Gerasimo et al (13). The iodination of oGH-I_3 was achieved using [^{125}I]-Na (Amersham - GB) according to the Iodogen (Pierce) method (14).

ACTH contamination was estimated on 1, 10 and 100 µg of each GH preparation. It was measured by radioimmunoassay in terms of $ACTH_{1-24}$. This was achieved using [^{125}I] $ACTH_{1-24}$ labelled by the chloramine T method (15) and an anti-human ACTH antibody purchased from Wellcome (Beckenham -GB). Bound and free hormones were separated using Dextran-coated charcoal precipitation.

Lipolysis: The experiments were performed on 50 day-old New Zealand rabbits. After 24 hours of fasting, the animals were sacrified and the fat pad from the neck region was quickly removed. About 3-4 grams of tissue were dissociated by collagenase (Worthington) digestion according to the method of Rodbell (16) as previously described (10). The incubation was carried out at 37°C for 3 hours using 5 x 10^5 fat cells in 2 ml glucose-free Krebs-Ringer aluminum bicarbonate buffer (pH 7.4) containing the hormones and 100 mIU/ml adenosine deaminase (EC 3.5.44) (Sigma). Free fatty acid (FFA) production was titrated by the method of Dole and Meinertz (17). Each sample was run in duplicate and was followed by an albumin blank. The results are expressed as µEquivalent of palmitate for 3 hours of incubation and for 5 x 10^5 cells.

RESULTS

Figure 1 shows that oGH-S_5 and pGH had comparable ability to inhibit the binding of [^{125}I]oGH-I_3 to liver plasma membranes from pregnant rabbit. Similar competition curves were observed for hGH and RbGH (not shown). For the four hormones the IC_{50} was between 3 and 4 ng per tube. The IC_{50} for porcine pituitary fractions P_{40} and S_{40} were 20 ng and 4 µg, respectively.

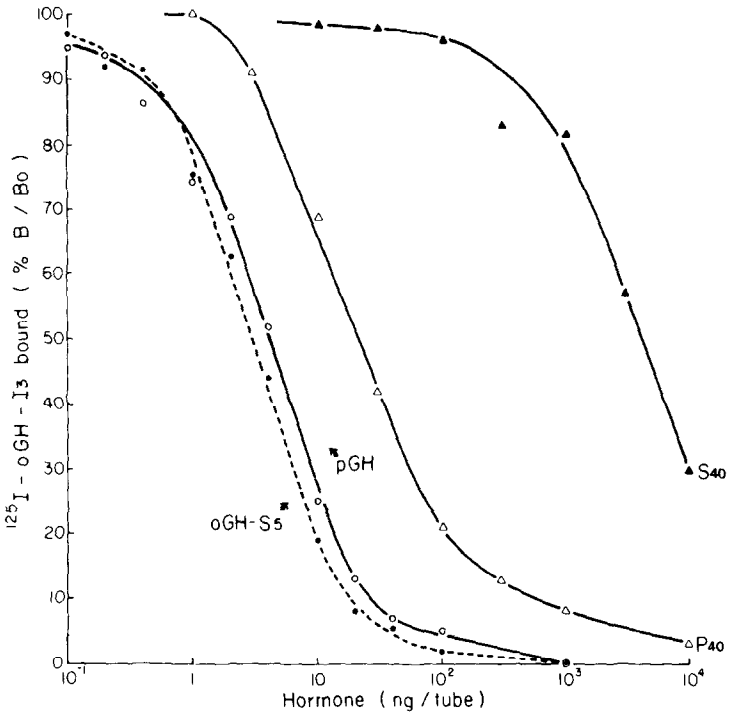

Figure 1 Competitive inhibition of [^{125}I]oGH-I$_3$ binding to crude liver plasma membranes from pregnant rabbit by unlabelled oGH-S5 (●--●), pGH (o—o) and porcine pituitary fractions S_{40} (▲-▲) and P_{40} (△-△) (see Materials and Methods Section)

This indicated that these fractions contained 20% for P_{40} and 0.1% for S_{40} of pGH binding activity (Table 1).

TABLE I

COMPARISON OF HORMONES AND PORCINE PITUITARY FRACTIONS LIPOLYTIC ACTIVITIES TO THEIR RELATIVE GH BINDING AND CONTAMINATION BY ACTH

	Relative GH Binding Activity(a)	Immunoreactive ACTH (pg/µg)	Relative lipolytic activity (b)
Rb GH	1	1.3	1
O GH-S5	1	0.4	0
p GH	1	20	0
h GH	1	29	45
P_{40}	0.2	235	50
S_{40}	0.001	246	200

(a) binding relative to RbGH
(b) lipolytic activity relative to 50 µg/ml RbGH, in the presence of 100 mIU/ml adenosine deaminase

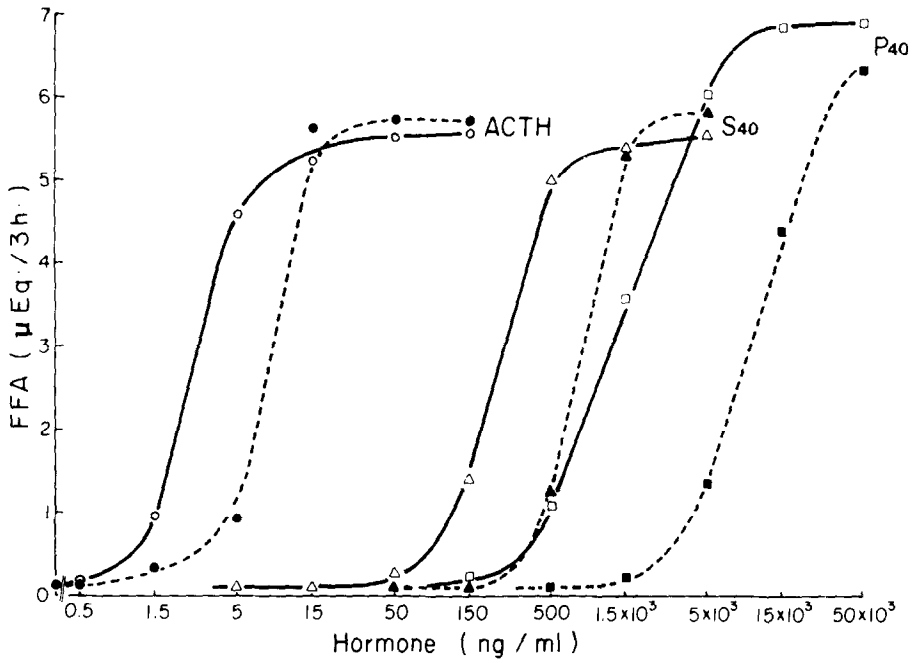

Figure 2 Dose-response curve of the lipolytic effect of $ACTH_{1-24}$ (o,●) and porcine pituitary fractions S_{40} (△,▲) and P_{40} (□,■) incubated with 5 x 10^5 rabbit adipocytes in presence (——) or absence (- - -) of 100 mIU/ml adenosine deaminase.

Under ACTH stimulation, the maximal FFA production (Rmax) was 5.7 µEq/3h/5 x 10^5 adipocytes (Fig. 2). The addition of adenosine deaminase in the incubation medium did not change the Rmax but the ED_{50} was reduced from 8 to 3 ng/ml. The ED_{50} for porcine pituitary fractions S_{40} and P_{40} were respectively 0.8 and 10 µg/ml in absence and 0.25 and 1.5 µg/ml in presence of adenosine deaminase (Fig. 2). The Rmax for S_{40} and P_{40} was comparable to that for ACTH, although it increased to 7 µEq/3h for P_{40} in presence of adenosine deaminase.

Purified pGH, oGH and oPL had negligeable effects on FFA production at a dose as high as 50 µg/ml in presence or absence of adenosine deaminase (Fig. 3). The same dose of RbGH stimulated the production of 3 µEq FFA/3h but only in presence of adenosine deaminase (Fig. 3). The Rmax to hGH was 4.7 µEq/3h and its ED_{50} was reduced from 10 to 1 µg/ml when adenosine deaminase was added.

Immunoreactive ACTH was detectable in all the GH preparations and porcine pituitary fractions (Table 1). S_{40} and P_{40} contained about 10 fold more ACTH

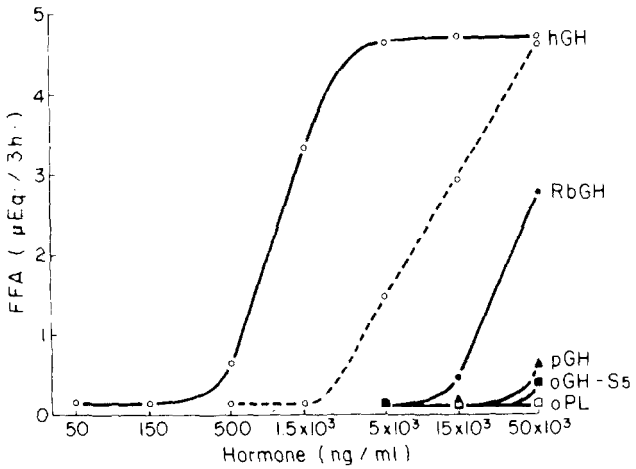

Figure 3 Dose-response curve of the lipolytic effect of hGH (○), RbGH (●), pGH (▲), oGH-S_5 (■) and oPL (□) incubated with 5 x 10^5 rabbit adipocytes in presence (—) or absence (- - -) of 100 mIU/ml adenosine deaminase.

than purified hGH or pGH but Rb GH and oGH contained only negligeable traces of ACTH. The amount of ACTH in P_{40}, S_{40} and hGH at the ED_{50} was 0.35, 0.06 and 0.03 ng/ml, respectively.

DISCUSSION

The GHs used in this study have been chosen because they had similar binding activity on rabbit liver membranes. Thus, we could expect a comparable binding on putative GH receptors in fat cells, even if no evidence for such receptor sites has been demonstrated in the rabbit. These results taken together show that physiological doses of GH do not exert any lipolytic effect on rabbit adipocytes incubated in vitro. This was particularly clear for pGH, oGH, oPL and the homologous RbGH. These data are in opposition with our previous findings where pGH and RbGH were reported to have a lipolytic activity (10,11). This discrepancy may be accounted for a higher degree of purity and possibly a different method of purification of the hormones now available. Furthermore, the previous studies were performed using single and very high doses of GH.

The lipolytic activity of hGH is consistent with that described in rabbit (9) or rat (8) fat cells. Meanwhile we cannot agree that it can be attributed to an intrinsic effect of the molecule because the doses required are not

compatible with physiological concentrations of GH. Moreover repurified or bacteria-derived hGH has no effect on lipolysis in absence of dexamethasone (6,7). The cause of the small degree of stimulation of lipolysis by low doses of hGH associated with dexamethasone remains unexplained (5). It may well be a particularity of the epididymal fat pad of the rat (18). But no evidence has been brought that hGH could act through the activation of the hormone-sensitive triglyceride-lipase, as has been shown for the potent lipolytic hormone ACTH (19-20).

Two observations suggest that the lipolytic activity found in porcine pituitary fractions and hGH is due to a contamination by one or several lipolytic agents. Firstly, the lipolytic power of partially purified pGH is lost in the highly purified hormone. Secondly, the sensitivity of the adipocytes to these hormonal preparations is increased in presence of adenosine deaminase as for ACTH. Adenosine acts as an inhibitory modulator of cAMP accumulation and decreases lipolytic activity of fat cells to hormonal stimulation (20,21). This is prevented by the addition of adenosine deaminase in the incubation medium which leads to an apparent increase in the sensitivity of the adipocytes to lipolytic hormones action (20,21). Of course, this is limited to the hormones which act through the adenylate system but there is no evidence that GH action involves stimulation of this system. In fact, it has even been shown to decrease the adenylate cyclase activity in hypophysectomized rats (22). But, except for hGH, the effect of adenosine deaminase was greatly reduced when purified GHs were used suggesting that the cAMP-dependent lipolytic activity was removed. The amount of ACTH present in porcine pituitary fractions and in hGH does not explain all their lipolytic potency. Therefore, it is likely that other pituitary contaminants contribute to GH apparent lipolytic activity.

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