STIMULATION OF LIPOLYSIS AND CYCLIC AMP ACCUMULATION IN RABBIT FAT CELLS BY HUMAN GROWTH HORMONE*

by

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SUMMARY. Human growth hormone (HGH) has been found to produce a rapid stimulation of lipolysis in isolated rabbit fat cells but not in rat adipocytes. The species specificity exhibited by HGH and other evidence strongly suggest that the lipolytic action is an intrinsic property of HGH and not due to contamination with other pituitary lipolytic agents. The stimulation of lipolysis by HGH appears to be mediated by cyclic AMP since the effect of the hormone on both cyclic AMP accumulation and glycerol release are potentiated by theophylline.

INTRODUCTION

Among the pituitary hormones adrenocorticotropin (ACTH), thyrotropin and the gonadotropins are known to stimulate lipolysis rapidly in isolated rat fat cells (1-3); growth hormone, however, requires a lag period of at least 1 hr to produce the effect (4). This slow action of growth hormone is potentiated by glucocorticoids and prevented by inhibitors of RNA and protein synthesis which do not block the effect of the fast acting lipolytic agents (4, 5). Fain and Saperstein (5) concluded that any rapid stimulation of lipolysis in isolated adipose tissue due to higher concentrations of growth hormone must be attributed to contamination with other pituitary hormones like ACTH or TSH. Li et al. (6) examined the action of HGH on isolated fat pads of rat, rabbit and

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guinea pig and found that rabbit adipose tissue was the most responsive. We report herein the action of HGH on fat cells isolated from rat and rabbit adipose tissues. Whereas high concentrations of HGH failed to produce a rapid stimulation of lipolysis in rat fat cells, the hormone was found to be a potent, fast acting lipolytic agent in rabbit fat cells.

MATERIALS AND METHODS

Collagenase (CLS grade) and lima bean trypsin inhibitor were purchased from Worthington Biochemical Corporation. ^{3}H-cyclic AMP was obtained from Schwarz/Mann. Bovine serum albumin (fraction V, Armour) was purified by acidification to pH 3.6 and dialysis against a suspension of charcoal (Norit) in distilled water at 4°.

HGH was isolated by previously published procedures (7, 8) and further purified by applying on a column of carboxymethyl cellulose equilibrated with 0.01 M NH_4Ac (pH6.8) and eluting with the same buffer. The hormone, which came through unadsorbed, was dialyzed extensively against 0.1 M tris-HCl (pH 8.4), concentrated in a Diaflo cell using a PM-10 membrane (Amicon) and chromatographed on Sephadex G-100 equilibrated with the same buffer as previously described (9). The main fraction which emerged as a monomer (V_e/V_o, 1.97) was dialyzed extensively and lyophilized. ACTH and prolactin were prepared from sheep pituitaries by published methods (10, 11).

Fat cells were isolated from the perirenal fat pads of male New Zealand white rabbits (2 Kg) and the epididymal fat pads of Sprague-Dawley rats (160-180 g) by digestion with collagenase according to the procedure of Rodbell (1). The cells were dispersed in Krebs-Ringer bicarbonate buffer containing 4% bovine serum albumin and 0.1% lima

bean trypsin inhibitor to yield a suspension containing 40-50 mg cells by dry weight per ml. Aliquots of 0.9 ml of the cell suspension were incubated at 37° with 0.1 ml of hormone or water for varying periods up to 1 hr in the case of rabbit fat cells and 2 hr in the case of rat fat cells. At the end of the incubation glycerol released into the medium was determined by the procedure of Vaughan (12). For the determination of cyclic AMP, the incubation was stopped by the addition of 1 ml of 10% trichloroacetic acid. The supernatant was extracted with ether (5 times) to remove the trichloroacetic acid and lyophilized. Cyclic AMP was measured by the method of Gilman (13). All incubations were performed in triplicate.

RESULTS AND DISCUSSION

The effects of HGH on the stimulation of glycerol release in rat and rabbit fat cells are compared in Table I. It is evident that HGH is inactive in rat fat cells at a concentration of 4.5×10^{-6} M but highly active in rabbit fat cells at this concentration. For comparison, the effects of ACTH and prolactin are included. ACTH is very active in both species but prolactin is inactive.

The species specificity exhibited by HGH strongly suggests that the lipolytic action of HGH in rabbit fat cells is intrinsic and not due to contamination with other pituitary hormones. The activity of HGH in rabbit fat cells cannot be due to ACTH contamination since ACTH is active in both species. Thyrotropin and the gonadotropins stimulate lipolysis in rat fat cells but are inactive in rabbit adipocytes (2). Hence, the activity of HGH in rabbit fat cells cannot be ascribed to contamination with the glycoprotein hormones. Prolactin is completely inactive in both species

TABLE I

STIMULATION OF LIPOLYSIS IN RAT AND RABBIT FAT CELLS BY HGH, ACTH AND PROLACTIN

Hormone	Concentration ($\times 10^{-6}$M)	Glycerol production rat[a]	rabbit[b]
None	----	0.9 ± 0.04[c]	1.6 ± 0.2
HGH	1.13	0.9 ± 0.1	6.5 ± 0.3
HGH	4.50	1.3 ± 0.1	24.1 ± 0.6
ACTH	0.03	24.3 ± 0.2	22.9 ± 0.5
Prolactin	4.50	0.44 ± 0.2	2.2 ± 0.1

[a] μmoles glycerol/g cells/2 hr

[b] μmoles glycerol/g cells/hr

[c] mean ± S. E.

and can be ruled out. α- and β- melanocyte stimulating hormones are the only peptides of the pituitary gland which are able to stimulate lipolysis in rabbit but not in rat fat pads (14). HGH was found to be devoid of any melanocyte stimulating activity at concentrations as high as 1×10^{-5} M. The failure to find melanocyte stimulating activity in HGH indicates that contamination with melanotropins can also be eliminated as the source of the lipolytic activity of HGH. Thus, it may be concluded that the rapid stimulation of lipolysis in rabbit fat cells by HGH is an intrinsic property of the hormone.

In view of the role of cyclic AMP in the stimulation of lipolysis by

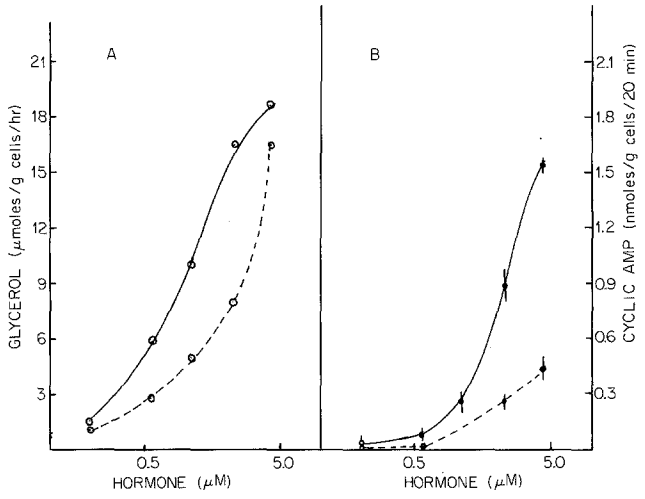

Fig. 1. Effect of theophylline on the stimulation of lipolysis (A) and cyclic AMP accumulation (B) in isolated rabbit fat cells by HGH. Theophylline was present at a concentration of 3.2×10^{-5}M. The basal rate of cyclic AMP accumulation in the absence and presence of theophylline was 0.4 and 1.6 pmoles/g cells 20 min respectively. The basal rates of glycerol release were 1.6 and 1.8 μmoles/g cells/hr in the presence and absence of theophylline, respectively; ---, absence of theophylline; ——, presence of theophylline.

fast acting lipolytic agents (3), the effect of HGH on cyclic AMP accumulation and glycerol release was examined in the presence and absence of theophylline. From Figure 1A, it is apparent that theophylline potentiates the lipolytic action of HGH in rabbit fat cells. HGH stimulates the accumulation of cyclic AMP significantly and this response to HGH is also potentiated by theophylline (Figure 1B). The concentration of theophylline employed (3.2×10^{-5}M) had no effect on the basal rates of cyclic AMP generation or glycerol release. These results suggest that HGH stimulates lipolysis in isolated rabbit fat cells by mechanisms similar to those found for the action of other fast acting lipolytic agents like ACTH.

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