ADENYLATE CYCLASE OF HUMAN FAT CELL GHOSTS

Effects of the vasoactive intestinal polypeptide (VIP) on the enzyme system of preadolescent and adult fat cells

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1. Introduction

An intriguing feature of cAMP-mediated lipolysis is its species specificity [1]. In rat adipose tissue catecholamines and several peptide hormones such as adrenocorticotropic hormone (ACTH), glucagon, secretin, thyroid stimulating hormone (TSH) and the vasoactive intestinal polypeptide (VI) are active stimulators of lipolysis [2,3] whereas only catecholamines and parathyroid hormone have been shown to be effective in adipocytes of adult human beings [4–6]. However, the responsiveness of rat adipose tissue towards peptide hormones such as glucagon and ACTH appears to decline very early in life [7,8] suggesting that at least part of the differences which have been noted between adipose tissue of human beings and of other species may be a result of the use of fat tissue of adults.

The effects of lipolytic hormones are mediated via the membrane-bound adenylate cyclase [2]. Studies on the rat fat cell enzyme revealed that this effector system of hormone action reflects the responsiveness of the intact tissue including changes of hormone sensitivity which are related to cell size or age [7–9]. In this report it is shown that the vasoactive intestinal polypeptide (VIP) is capable of activating the human fat cell adenylate cyclase. The stimulatory effect of this hormone is statistically significant only in membranes from children, but not in fat tissue of adults (50–70 years).

2. Materials and methods

Biopsies of preadolescent adipose tissue (~0.5 g) were obtained from 7 boys (9–12 years) which underwent herniotomia and from 2 girls (12 years) undergoing hip surgery. Tissue specimens from adults (~3 g) were from 6 male and 7 female subjects (50–70 years) which were operated for gall stones, duodenal ulcer, hernia inguinalis (males), or underwent breast surgery or hysterectomy (women). All subjects were operated on after an overnight fast. Aesthesia was induced with a short acting barbiturate and maintained with halothane, nitrous oxide and oxygen. The biopsies were usually obtained after the skin incision.

Experimental procedures were essentially the same as in [6]. Fat cells and fat cell ghosts were prepared according to [10]. The adenylate cyclase activity was determined by the method in [11] at 30°C. The assay mixture contained 25 mmol/l Tris–HCl (pH 8.0), 5 mmol/l $MgCl_2$, 20 mmol/l creatine phosphate, 100 U/ml creatine phosphokinase, 1 mmol/l 3′,5′-cyclic AMP, 0.01 mmol/l GTP and 1 mmol/l [α-^{32}P]-ATP (40–50 cpm/pmol). The reaction was initiated by addition of 1–20 μg ghost protein and was terminated by addition of 0.1 ml stopping solution composed of 2% (w/v) lauryl sulfate, 1 mmol/l cAMP and 40 mmol/l ATP. cAMP was purified by column chromatography with Dowex AG-50 W-X4 and neutral alumina [11]. The protein content of the samples

was determined by the Lowry method [12]. Statistical analysis was by the Wilcoxon test.

[α-^{32}P]ATP (2–6 Ci/mmol) and [^{3}H]AMP (27 Ci/mmol) were purchased from The Radiochemical Centre, Amersham; epinephrine-bitartrate was from Merck AG, Darmstadt; enyzmes, coenzymes and nucleotides were from Boehringer Mannheim; synthetic ACTH and glucagon (insulin-free) were from Ciba-Geigy, Wehrbaden, and Eli Lilly, Bad Homburg, respectively; synthetic aminoterminal 1–34 PTH fragment (PTH; D 0414, 3820 U/mg) was from Beckman Instruments, Fullerton, Ca; VIP (highly purified, porcine) was purchased from Fa. Päsel, Frankfurt.

3. Results

Table 1 shows the effects of epinephrine (0.5 mmol/l) and of the peptide hormones ACTH, PTH, glucagon and VIP (10 μg/ml each) on the adenylate cyclase of preadolescent and adult adipose tissue. Basal enzyme activities in membranes of children tended to be somewhat higher than those of adult fat cells. This difference was not statistically significant, however.

Epinephrine and parathyroid hormone were active stimulators of the adenylate cyclase of both collectives. The epinephrine-induced activation averaged 400% in both collectives; parathyroid hormone caused a >2-fold increase of enzyme activity in both samples. As reported previously and shown in table 1, ACTH and glucagon had no stimulatory effects on the adenylate cyclase of adult fat tissue. These latter hormones were ineffective in membranes from children too. The effects of VIP were less predictable. In adult adipose tissue a substantial activation (>1.5-fold increase of enzyme activity) was observed in only 5 out of 13 individual experiments. In addition, the guanine nucleotide analogue 5'-guanylyl-imidodiphosphate (GMP(PNP)), which has been reported to permit expression of hormone sensitivity of the human fat cell adenylate cyclase [13] under certain conditions, had no influence on the expression of VIP sensitivity when tested at 0.1 mmol/l in 4 individual preparations of adult adipose tissue (not shown).

As opposed to adult fat tissue VIP (10 μg/ml) was as effective in stimulating the adenylate cyclase in membranes from children as parathyroid hormone (table 1). On average this hormone caused a 2.5-fold

Table 1
Effects of epinephrine, ACTH, PTH, glucagon and VIP on the fat cell adenylate cyclase of children (9–12 years) and of adults (50–70 years)[a]

Additions[b]	Adenylate cyclase activity (nmol cAMP.mg protein^{-1}.15 min^{-1})	
	Children	Adults
None	1.7 ± 0.2	1.1 ± 0.2
Epinephrine	9.6 ± 1.1[c]	6.5 ± 0.5[c]
ACTH	1.8 ± 0.3	1.2 ± 0.2
Glucagon	1.9 ± 0.3	1.1 ± 0.2
PTH	3.9 ± 0.4[c]	3.0 ± 0.3[c]
VIP	4.5 ± 0.4[c]	1.4 ± 0.2

[a] Values are mean ± SEM of 9 (children) and 13 (adults) separate experiments, respectively, each carried out in triplicate

[b] Epinephrine was 0.5 mmol/l; the peptide hormones were 10 μg/ml each

[c] Significantly higher than the corresponding basal adenylate activities ($p \leq 0.05$)

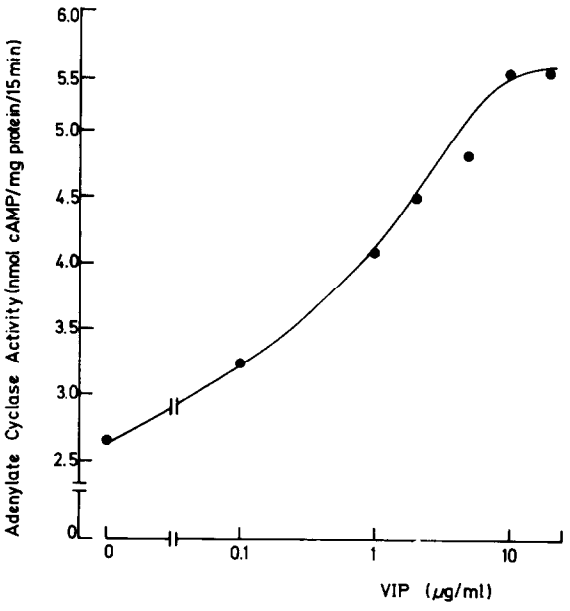

Fig.1. Dose–response curve for VIP in membranes of an 11 year male child. Values are means of triplicate determinations.

increase of enzyme activity in preadolescent preparations.

The stimulatory effect of VIP was dose dependent (fig.1). Maximal effects were observed at 10 μg VIP/ml. Half-maximal effects occurred at ~1 μg hormone/ml.

4. Discussion

Rat adipose tissue shows a decline of the responsiveness towards certain peptide hormones such as glucagon and ACTH which is reflected by a decrease of the hormone-responsiveness of the fat cell adenylate cyclase [7–9]. This impairment of hormone sensitivity appears to be closely linked, if not causually related to age. Because of the smallness of tissue specimens usually obtainable from children only few reports have been published about age-associated changes of human adipose tissue towards lipolytic hormones. Our observation that ACTH and glucagon failed to activate the adenylate cyclase of preadolescent adipose tissue is consistent with metabolic studies showing that glucagon is not lipolytic in children [14] and that a lipolytic effect of ACTH, if existent at all, might be restricted to a narrow perinatal interval [15].

To our knowledge, our investigation is the first to show that vasoactive intestinal polypeptide is capable of activating the human fat cell adenylate cyclase. In addition, by demonstrating that the stimulatory effect induced by VIP is statistically significant only in membranes of preadolescent adipose tissue, but not in preparations from adults (50–70 years), this study suggests, that the human fat cell adenylate cyclase, like the rat fat cell enzyme, is subject to age-associated changes of hormone-responsiveness. It is, therefore, likely that part of the differences which have been noted between adipose tissue of human beings and of other species might result from the use of adipose tissue from adults.

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