

Restoration by insulin of the responsiveness of stimulated adipocytes to adenosine

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The stimulation of adipocyte-cyclase by isoproterenol decreases its sensitivity to adenosine, with, as a consequence, a decrease in its antilipolytic effect. The presence of insulin under conditions where its action on the phosphodiesterase activity is impaired, restores the responsiveness of adenylate-cyclase and of lipolysis to adenosine.

Adipocyte

Adenylate-cyclase

Lipolysis

Adenosine

Insulin

1. INTRODUCTION

Insulin, prostaglandin E_2 and adenosine are physiological inhibitors of lipolysis in rat fat cells [1]. Lipolysis is controlled by at least two feedback loops. The first is proximal to the origin of the cascade and is mediated by adenosine, a degradation product of cyclic AMP, whereas the second is more distal and is mediated by the prostanoids (prostaglandin E_2 and prostacyclin) which are synthesized from arachidonate, one of the end-products of lipolysis [2]. Both loops interact with adenylate-cyclase, the first enzymic system of the cascade. Although the physiological role of the adenosine-dependent inhibition is recognized [3], that of the prostanoids is not clearly established [4,5].

We showed that the sensitivity of adenylate-cyclase to prostaglandin E_1 decreases by one order of magnitude (IC_{50} increasing from 20 to 200 nM), when the enzyme is stimulated by a β -adrenergic agonist [6]. Under these conditions, the concentration of prostaglandins, which can be estimated at 3 nM for PGE_2 , is too low to exert a feedback control on lipolysis [7]. Insulin is a hormonal inhibitor of lipolysis, which stimulates phosphodiesterase [8] and inhibits adenylate-cyclase activities [9,10]. We showed that the latter effect is not direct and

involves the participation of endogenous prostanoids whose activity is decoupled [6]. The purpose of this study is to investigate whether the sensitivity of adenylate-cyclase to adenosine is also decreased during adrenergic stimulation, and if insulin restores the sensitivity in the absence of endogenous prostaglandins. This hypothesis is supported by the fact that prostanoids and adenosine share a common site of action at the adenylate-cyclase level [11]. Our results show that this is the case, and provide further information on the mode of action of insulin.

2. MATERIALS AND METHODS

Crystalline bovine B-grade insulin (INS) (25.5 IU/mg) and collagenase (200 IU/mg) were purchased from Calbiochem and Worthington Biochemicals, respectively. D,L-isoproterenol-HCl (IPNE) (lot 1-5627) and bovine serum albumin fraction V (made fatty acid-free as in [12]) were from Sigma. N^6 -(phenylisopropyl) adenosine (PIA) and adenosine-deaminase (ADA) were from Boehringer. 3-(3,4-dimethoxybenzyl)-2-imidazolidinone (RO 7-2956) was kindly donated by Hoffman-La-Roche (Basel). Indomethacin (INDO) was provided by Merck, Sharp and Dohme. Male Wistar rats (180 g) from Animalabo (Paris) were

kept at room temperature and given free access to food and water. They were decapitated and the epididymal fat pads were removed as quickly as possible, rinsed in ice-cold saline (NaCl 0.9%, w/v). The fat pads were cut into small fragments (80–100 mg), and distributed among vials containing Krebs-Ringer bicarbonate buffer 0.1 M (pH 7.4) with half the recommended (Ca^{2+}) (1.3 mM) and 4% (w/v) fatty acid-free albumin. White fat cells were isolated by collagenase digestion as in [13].

Hormones, enzymes and drugs were made up freshly in buffer to the required final concentrations. RO 7-2956 and indomethacin were dissolved in a minimal volume of dimethyl sulfoxide which has no effect on lipolysis or on cyclic AMP production at the concentrations used. N^6 -(phenylisopropyl) adenosine was dissolved in a water-ethanol mixture, the final concentration of ethanol being less than 10^{-5} M. When indicated, the cells were first preincubated for 20 min at 37°C with indomethacin alone and then placed in a fresh medium containing indomethacin, the appropriate hormone, enzyme and drugs. Incubations lasted 1 h for studies on lipolysis (free fatty acid release) or 6 min when the production of cyclic AMP was studied.

Free fatty acids were extracted as in [14] and determined as in [15]. Triglycerides were extracted as in [16]. Aliquots of total lipid extracts were saponified in KOH 4% (w/v) in 95% (v/v) ethanol for 30 min at 60°C . Glycerol was measured as in [17]. Cyclic AMP was assayed by the radio-immunological method as in [18] except that bound ligand was separated from the free ligand by polyethylene glycol precipitation (P. Mayeux, unpublished).

The statistical differences were calculated by the Student's *t*-test. The mean values $\pm$ standard error of the mean (SEM), are given.

3. RESULTS

PIA is a very powerful inhibitor of lipolysis effective also in the basal state. Lipolysis stimulated up to 400% when phosphodiesterase was inhibited with 1 mM RO 7.2956, was almost completely suppressed with 0.1 μM PIA (fig.1). In contrast, when lipolysis was stimulated with 1 μM IPNE in the presence or absence of 1 mM RO 7.2956, PIA was

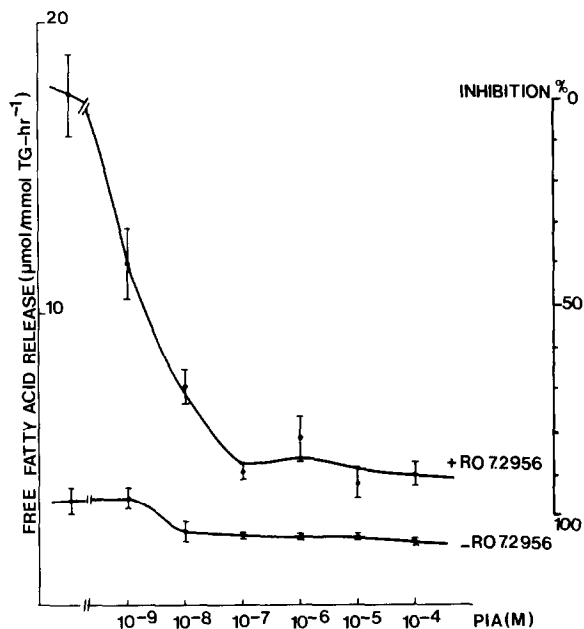


Fig.1. Effect of PIA on lipolysis in adipose tissue, in the absence of IPNE, in the presence or absence of RO 7.2956 (10^{-3} M). The mean values $\pm$ SEM of 5 assays of free fatty acid release are presented after 1 h of incubation. The inhibition % due to PIA is indicated on the right ordinate.

less effective up to μM (fig.2). The inhibition varied between 43% and 67% according to the experiments.

In these experiments the action of PIA was superimposed to the action of endogenous adenosine and prostanoids. In order to study the action of PIA by itself on lipolysis stimulated by RO 7.2956 plus IPNE supplemented or not with INS, endogenous adenosine was destroyed with adenosine deaminase (1 $\mu\text{g}/\text{ml}$) and the formation of prostanoids was prevented with INDO (20 $\mu\text{g}/\text{ml}$). Under these conditions, the following results were observed (fig.3):

- After the destruction of endogenous adenosine and the prevention of the synthesis of prostanoids, insulin lost its antilipolytic activity;
- The addition of increasing concentrations of PIA decreased the lipolysis intensity, the inhibitory effect reaching 50% for μM PIA;
- When PIA was applied in the presence of INS, inactive by itself, the slope of the inhibition curve was increased and μM PIA produced a

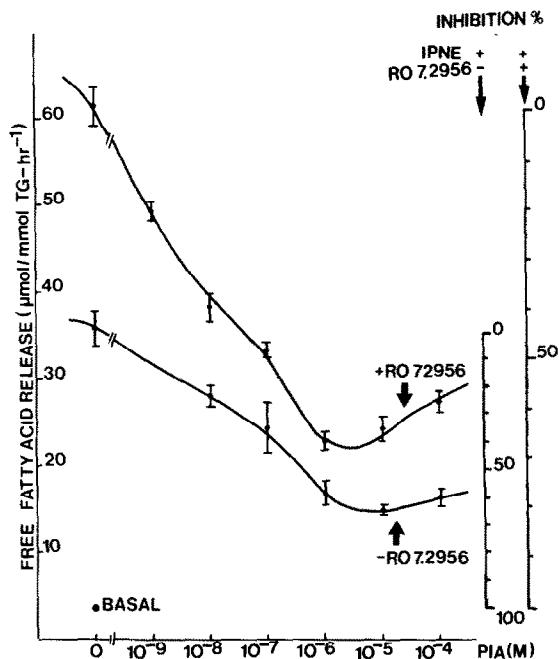


Fig. 2. Effect of PIA on lipolysis in adipose tissue, in the presence of IPNE (10^{-6} M), in the presence or absence of RO 7.2956 (10^{-3} M). The mean values $\pm$ SEM of 5 assays of free fatty acid release are presented after 1 h of incubation. The inhibition % due to PIA is indicated on the right ordinate.

75% decrease in free fatty acid release; i.e., a 50% enhancement of the own action of PIA. This effect of INS involved adenylate-cyclase, since RO 7.2956, at concentrations raised to $1 \mu\text{M}$, prevented whatever action at the phosphodiesterase level [19].

Similar results were obtained on the production of cAMP (fig. 4): in the absence of PIA and after suppression of the endogenous regulators (adenosine and prostanoids) INS did not decrease the cAMP accumulation after 6 min incubation. An almost complete inhibition of cyclic AMP accumulation was obtained at $1 \mu\text{M}$ PIA in the absence of INS, whereas the same maximal inhibition was reached at $0.1 \mu\text{M}$ PIA in the presence of INS. Here again, the slope of the curve was increased in the presence of INS.

4. DISCUSSION

Our previous studies [6] were designed to assign the respective role of prostaglandins and of insulin

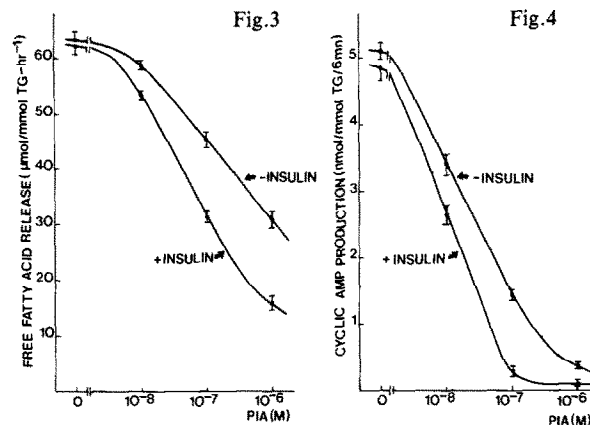


Fig. 3. Effect of phenylisopropyladenosine (PIA) and insulin ($100 \mu\text{units/ml}$) on lipolysis in adipocytes stimulated by isoproterenol (10^{-6} M) in the presence of RO 7.2956 (10^{-3} M), indomethacin ($20 \mu\text{g/ml}$) and adenosine deaminase ($1 \mu\text{g/ml}$). The mean values $\pm$ SEM of 6 assays of free fatty acid release are presented after 1 h of incubation.

Fig. 4. Effect of phenylisopropyladenosine (PIA) and insulin ($100 \mu\text{units/ml}$) on cyclic AMP accumulation by adipocytes stimulated by isoproterenol (10^{-6} M), in the presence of RO 7.2956 (10^{-3} M), indomethacin ($20 \mu\text{g/ml}$) and adenosine deaminase ($1 \mu\text{g/ml}$). The mean values $\pm$ SEM of 3 assays of cyclic AMP accumulated after 6 min are presented.

in antilipolysis at the adenylate-cyclase level. Phosphodiesterase activity was completely inhibited by 3-isobutyl, 1-methylxanthine which also prevented adenosine production from cAMP. Moreover, 3-isobutyl, 1-methylxanthine presented the advantage to compete with adenosine at the R receptor level [20]. Prostaglandin synthesis was impaired by phenelzine, which did not inhibit (as indomethacin did) the prostaglandin transport system [21], allowing the uptake and the action of exogenous prostaglandin. Under these conditions, in the presence of insulin, 10-times less PGE_1 was needed to induce a similar effect than in its absence, the dose-response curve being shifted to the left.

The aim of these experiments was to test if the efficiency of adenosine, as an inhibitor, would be decreased, when the adenylate-cyclase was stimulated, and whether insulin could restore this efficiency. It was compulsory to replace 3-isobutyl, 1-methylxanthine by a good inhibitor of phosphodiesterase activity, such as RO 7.2956, which would not interfere at the adenosine receptor level

[22]. Endogenous adenosine, whose actual concentration was not determined, was destroyed by adenosine deaminase. The biosynthesis of prostanoids was prevented by indomethacin, a conventional inhibitor of prostaglandin synthetase. PIA was used as a substitute for adenosine: its own inhibitory action on cyclic AMP production and on free fatty acid release was concentration-dependent. Insulin which was inactive by itself at the adenylate-cyclase level was able to increase by 50% the efficiency of PIA on cyclic AMP accumulation and on lipolysis. At this stage of our experimentation, the following conclusions can be drawn:

- (i) Insulin decreases lipolysis [19] and cyclic AMP accumulation [9] under conditions where phosphodiesterase is completely inhibited and adenylate-cyclase stimulated. This action, due to an inhibition of adenylate-cyclase activity, is not seen when the endogenous production of prostanoids and adenosine are suppressed ([6] and this paper).
- (ii) The sensitivity of stimulated adenylate-cyclase to its feedback inhibitors is lower than in the basal state.
- (iii) Insulin restores the inhibitory potency of adenosine by an increase of its efficiency upon adenylate-cyclase (this paper) and of prostanoids by an increase of their apparent affinity for the enzyme [6].
- (iv) Insulin exerts its action at the same site as prostanoids and adenosine, cholera toxin treatment preventing the action of the three inhibitors [11]. The nature of the mediator of this insulin action is unknown.

Our results are compatible with those previously reported in [23] and [24] recognizing the compulsory character of the presence of adenosine in the expression of the antilipolytic action of insulin. Nevertheless, their interpretation was opposite, they spoke about the potentiation by adenosine of insulin action. This concept, which seems tenable in the cases of glucose transport and lipid synthesis [25] is not appropriate in the case of the control of adenylate-cyclase activity.

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