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# IN VITRO EFFECTS OF THEOPHYLLINE ON INSULIN RECEPTORS IN ADIPOCYTES: CORRELATION WITH THE LIPOLYTIC ACTION OF THE AGENT

H. J. Steinfelder and H. G. Joost

Institute of Pharmacology and Toxicology,
University of Göttingen, Robert-Koch-Str. 40,
D-3400 Göttingen, Federal Republic of Germany

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SUMMARY: To test the hypothesis that insulin receptors may be modulated by metabolic events within the target cells, we investigated the relation between lipolysis and insulin binding in isolated fat cells of the rat. The stimulation of lipolysis by theophylline was associated with a significant decrease of insulin binding. The concentration-response curves suggested a close correlation of both effects of theophylline. Binding curves revealed a decrease of the affinity rather than a reduction of the number of insulin receptors. Methylpyrazolecarboxylic acid, an antilipolytic agent, antagonized the effects of theophylline on both lipolysis and insulin binding.

## INTRODUCTION

The primary step of insulin action is its binding to a specific membrane receptor site. Several agents and conditions which alter the response to insulin have previously been shown to modulate insulin receptors. It is generally accepted, therefore, that the affinity as well as the number of specific binding sites may regulate the effects of insulin on cellular metabolism. Our present study is based on the hypothesis that also the inverse relationship might exist: the modulation of insulin receptors by the cell metabolism.

At present only few data are available to support this hypothesis. In cultured liver cells, agents that produce insulin-like effects without combining with the receptor site alter the binding of insulin in parallel with their metabolic effects (1). Further, the "down-regulation" mechanism, a receptor loss in-

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duced by insulin itself, was recently reported to be prevented by nicotinamide, presumably at a postreceptor site (2).

In the fat cell, insulin predominantly regulates the synthesis and degradation of triglycerides. Therefore, to test the hypothesis that metabolic events controlled by insulin may modulate insulin receptors, we investigated the effects of both stimulated (theophylline) and inhibited (5-methylpyrazole-3-carboxylic acid) lipolysis on insulin binding in isolated fat cells of the rat.

## MATERIALS AND METHODS

Male Wistar rats, body weight 200 - 280 g, were used throughout. Fat cells were prepared according to Rodbell (3), and all incubations were carried out in Krebs-Ringer phosphate buffer containing 40 mg/ml defatted bovine albumin (Cohn's fraction V, obtained from Serva, Heidelberg). Cell suspensions ($3-5 \cdot 10^5$ cells/ml) were incubated in polystyrene vials with the indicated concentrations of theophylline or methylpyrazolecarboxylic acid in a shaking waterbath at 37°C. The incubation was stopped after 1 hour, and an aliquot of the medium was deproteinized with 10 % trichloroacetic acid. The glycerol content of the supernatant was determined according to Lambert and Neish (4).

Aliquots (200 µl) of the remaining cell suspension were transferred to polypropylene tubes, and [$^{125}$I]insulin (Behringwerke, Frankfurt) and unlabelled pork insulin were added. Samples containing $10^{-5}$ M insulin were prepared in order to assess non-specific binding. The cell suspension was allowed to equilibrate at 22° for 45 min, and the cells were separated from the medium by the "oil-flotation" method (5). The top layer containing the fat cells was removed and cell-associated radioactivity was determined.

In order to analyse insulin binding a Scatchard plot (6) was constructed and binding parameters were determined on the basis of a two site model (7). In addition, an average-affinity plot was drawn to consider the alterations of insulin binding according to the cooperative-binding-sites model of DeMeyts (8).

## RESULTS

Theophylline (1 mM) stimulated the rate of lipolysis as reflected by the 30-fold increase in glycerol release from the fat cells. Simultaneously the specifically bound insulin was reduced by 30 % (Fig. 1). When 5-methylpyrazole-3-carboxylic acid was added (0.1 mM), the stimulatory effect of theophylline on

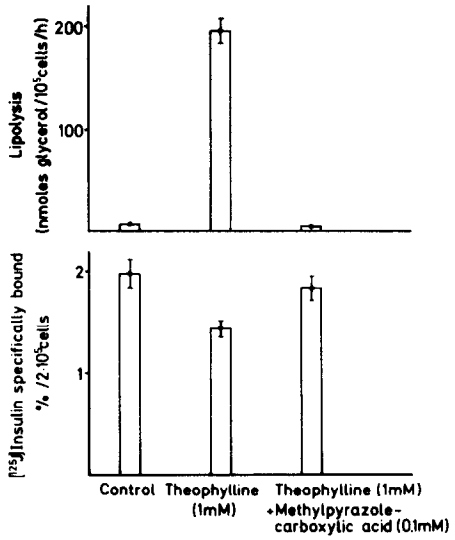


Figure 1 Effects of theophylline and methylpyrazolecarboxylic acid on lipolysis and specific insulin binding in fat cells. Cells were preincubated for 1 hour at 37° with the indicated concentration of agents, and lipolysis was determined. Insulin binding was measured after an equilibration period of 45 min at 22° in the presence of 0.15 nM [$^{125}$I]insulin. Data were corrected for nonspecific binding (radioactivity not displaceable by $10^{-5}$M unlabelled insulin). Values represent means ± SE of 8 experiments.

lipolysis disappeared, and insulin binding was reversed towards normal. Theophylline did not interfere with the binding assay, since the agent, as well as having failed to affect lipolysis, also failed to affect insulin binding when the incubation was carried out at 4° (data not shown).

Fig. 2 shows the concentration dependency of the observed effects. The triglyceride hydrolysis closely paralleled the decrease in insulin binding: half maximal effects were produced by 1.4 mM (lipolysis) and 2.1 mM (insulin binding) theophylline. When lipolysis was stimulated maximally, insulin binding decreased by 60 %.

Scatchard-plots of the binding data derived from a replacement curve are shown in Fig. 3. As the slope of the curvilinear plot changed, whereas the abscissa intercept did not change, the receptor affinity rather than the receptor number was decreased by theophylline. Accordingly, the analysis of the

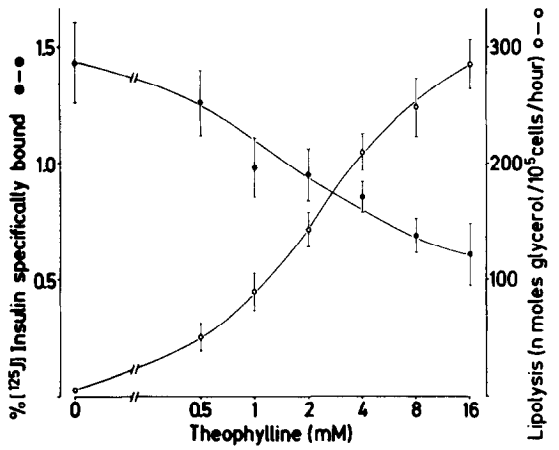


Figure 2 Concentration dependency of the effects of theophylline on lipolysis (open circles) and specific insulin binding (filled circles). The experimental conditions were identical with those shown in Fig.1. Data represent means ± SE of 9 experiments.

data (7) on the basis of a two-site model revealed that the affinities of both binding sites were reduced compared to a negligible reduction in receptor number. Furthermore, identical results were obtained by the analysis based on the assumption of a single class of binding sites with negative cooperativity

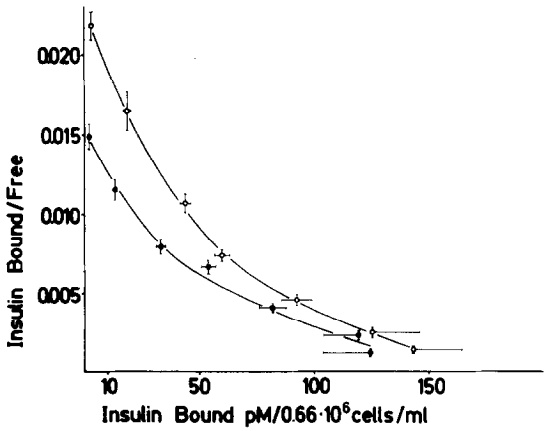


Figure 3 Scatchard plot of a displacement curve. Cells preincubated for 1 hour at 37° with 1 mM theophylline were equilibrated at 22° with 0.15 nM [$^{125}$I] insulin and increasing amounts of unlabelled insulin. The control curve is symbolized by the open circles. Data represent means ± SE of 12 experiments. Binding parameters were obtained on the basis of a two site model. Dissociation constants: 1.75 and 20 nM (control), 2.4 and 24.1 nM (theophylline). Receptor numbers: 25000 and 128000/cell (control), 22000 and 120000/cell (theophylline).

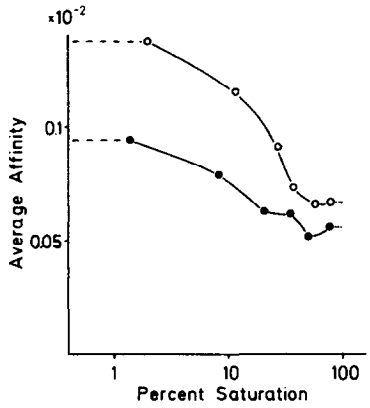


Figure 4 Average affinity profile of the data from Fig. 3. The average affinity was calculated according to DeMeyts (8) and plotted as a function of the percent receptor occupation. The control curve is symbolized by open circles.

(average affinity profile, Fig. 4). The plot shows that receptor affinity (which decreases with increasing insulin concentrations) was reduced in adipocytes treated with theophylline.

## DISCUSSION

The stimulation of lipolysis by theophylline for one hour prior to the binding assay resulted in a dose-dependent reduction of specific insulin binding. The effect apparently depended on a metabolic effect induced by theophylline, since the agent failed to affect insulin binding when the incubation was carried out at $4^{o}$.

The observed effect was closely related to the rate of lipolysis. Further, methylpyrazolecarboxylic acid reversed the effects of theophylline an both lipolysis and insulin binding. The close correlation of lipolysis to insulin binding suggests that some step or metabolite of the lipolysis may account for the observed effect.

The stimulation of lipolysis is associated with elevated levels of cellular cyclic-AMP, and may be paralleled by a reduction of the ATP content in the adipocyte (9,10). Agents which reduce the cellular ATP content have previously been reported

to decrease insulin binding in rat adipocytes (11,12). Thus the possibility cannot be excluded that the cellular ATP content may mediate the effect of a stimulated lipolysis on insulin receptors, as was observed in the present study. A participation of cyclic-AMP, however, seems less likely. The long-term elevation of cyclic-AMP levels by a phosphodiesterase inhibitor in cultured fibroblasts (13) gave rise to an increase in insulin receptor number without any alteration in affinity. The short-term elevation of cyclic-AMP levels, in contrast, failed to affect insulin binding (14).

Several agents mimicking the effects of insulin have recently been reported to reduce insulin binding in cultured liver cells (1), an effect similar to the receptor loss induced by exposure of cells to insulin itself. Since these agents did not combine with the insulin receptor site, a step of the cell metabolism distal from the receptor is thought to account for the effect. Unlike this so-called "down regulation" of receptors by insulin, the decrease in insulin binding observed in the present study reflected rather an impaired affinity than a reduced number of insulin receptors. Thus apparently not only the number of receptor sites on the cell membrane accessible to insulin but also their affinity may be controlled by the cell metabolism.

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