EFFECT OF COLCHICINE AND VINBLASTINE ON THE COUPLING OF INSULIN BINDING AND INSULIN ACTION IN FAT CELLS

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1. Introduction

The mode of transmission of the insulin signal from the receptor to effector systems in the plasma membrane is not well understood. Hypotheses on this process include receptor movement [1], direct receptor–receptor [2] and receptor–effector coupling [3], phosphorylation of membrane proteins [4,5], oxidation of membrane proteins [6,7] and the possible involvement of insulin degradation products [8]. We have shown that the action of insulin on cAMP-levels and 3-*O*-methyl-glucose transport in fat cells exhibits a common initial lag-phase of 40–50 s. Binding studies have shown that this delay in the onset of insulin action reflects a step which occurs after the initial binding of insulin to its receptor [9,10]. A similar lag-phase of insulin action was shown [11] in a study of 3-*O*-methylglucose exchange in fat cells. Our findings on the lag-phase of the insulin action on glucose transport have been confirmed [12]. For further characterisation of the unknown coupling mechanism we studied the effect of antimicrotubular agents on the lag-phase.

2. Materials and methods

Fed male Wistar rats (180–200 g) were used. Fat cells were prepared according to [13]. All incubations were performed in Krebs-Ringer–Hepes buffer containing 2.5 g/dl crystalline bovine albumin (Behring Werke AG, Marburg).

2.1. *Binding of ^{125}I-labeled insulin*

Fat cells (4.5–5.5 × 10^5/ml) were incubated in polyethylene vials under constant magnetic stirring at 37°C. ^{125}I-Labeled insulin (Novo Co., Copenhagen) was added at 100 μU/ml and 250 μU/ml. After intervals between 10 s and 4 min, 400 μl aliquots were transferred to polyethylene centrifuge tubes which were fitted with an adaptor into the rotor of a high-speed table centrifuge (Eppendorf, Model 3004).

Separation of cells was performed by centrifugation at 10 000 × *g* for 1 min through dinonylphtalate [14]. The binding reaction was considered to be terminated at the start of centrifugation. In experiments with colchicine and vinblastine, cells (5 × 10^6/ml) were preincubated for 1 h at 37°C with 1, 2.5 and 5 mM colchicine and 5 mM vinblastine (Sigma Co.), control cells were incubated with buffer.

2.2. *Determination of 3-O-methylglucose transport*

Cells (5 × 10^6/ml) were equilibrated at 37°C. Insulin (100 μU/ml) or buffer was added, the suspension was agitated and incubated for 20 s to 6 min. At the time points indicated 100 μl samples were drawn together with 200 μl 3-*O*-methylglucose (final conc. 500 μmol/l, 0.1 μCi 3-*O*-[1-^{14}C]methylglucose, Radiochemical Centre, Amersham, as tracer) into a mixing pipet (Gilson medical Electronics, France). After 10 s, cells and medium were separated by centrifugation through dinonylphtalate. The cell layer was dissolved in 200 μl diphenylethylamine and counted in 10 ml scintillation fluid (Szintigel, Roth Co., Karlsruhe). The extracellular concentration of 3-*O*-methylglucose in the pellet was extrapolated from the kinetics of the initial uptake of 3-*O*-methylglucose. Initial uptake kinetics with the first time points at 2, 5, 10, 15 and 20 s were established for each experi-

ment. The values for extracellular 3-*O*-methylglucose thus obtained correspond to calculations of the extracellular waterspace from [^{14}C]insulin determinations. In experiments with colchicine and vinblastine the cells were prepared as above.

3. Results

Preincubation of cells with 1 mM colchicine for 1 h has no influence on the initial rate of ^{125}I-labeled insulin binding (insert fig.1); after preincubation with colchicine and vinblastine (5 mM) the initial rate of binding is increased by 20–30% (insert fig.1,2).

Whereas these compounds either do not affect or accelerate the hormone–receptor interaction, they cause a marked prolongation of the lag phase, followed by a slower activation rate of the glucose transport system. Figure 1 shows the time course of insulin action on methylglucose transport in colchicine-treated and control cells. The onset of insulin action is in control cells after 40 s, in colchicine-treated cells after 65–120 s, depending on the concentration of colchicine. The basal methylglucose uptake (25.3 ± 2.6 pmol/10^5 cells × 10 s, n = 13) is not influenced by 1 mM colchicine and 5 mM vinblastine, and somewhat decreased at higher colchicine concentrations. The maximal ratio of stimulation is unaltered in cells

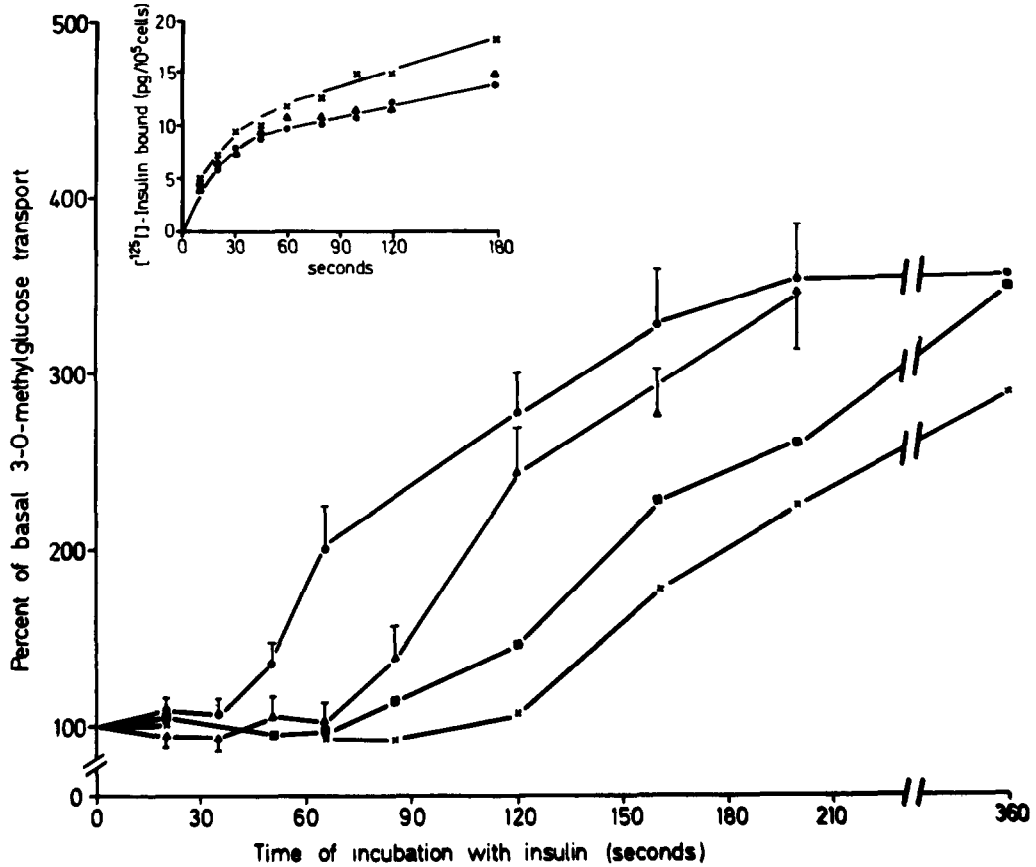

Fig.1. Effect of colchicine, 1 mM (△——△), 2.5 mM (□——□) and 5 mM (x——x) on the time course of insulin action on glucose transport; control (○——○). The values are the mean of 5–9 experiments ± SEM for control and colchicine 1 mM, and the mean of 3 experiments for colchicine 2.5 and 5 mM. Insert: Effect of colchicine, 1 mM (△——△) and 5 mM (x——x) on the initial kinetics of ^{125}I-labeled insulin binding; control (○——○).

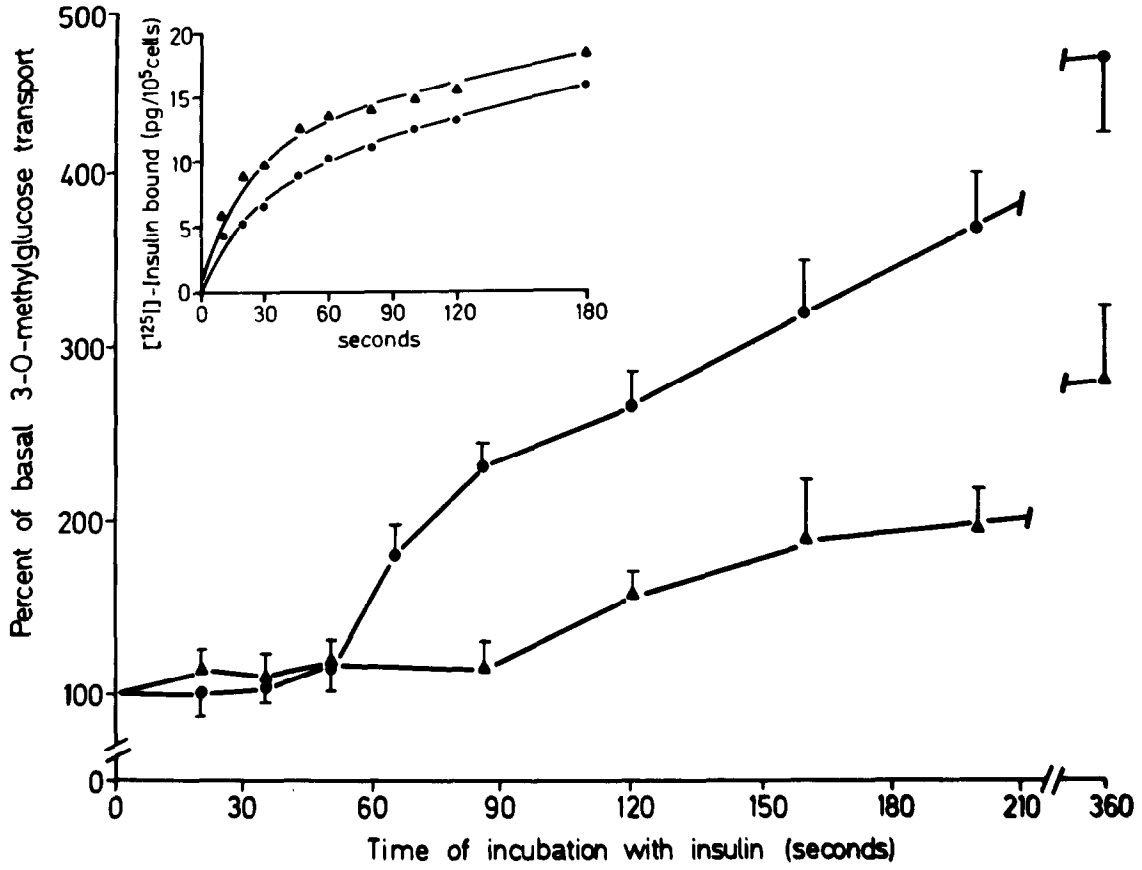

Fig.2. Effect of vinblastine 5 mM (△——△) on the time course of insulin action on glucose transport, control (○——○). The values are the mean of 4 experiments ± SEM. Insert: Effect of vinblastine 5 mM (△——△) on the initial kinetics of ^{125}I-labeled insulin binding, control (○——○).

pretreated with 1 mM and 2.5 mM colchicine. Pretreatment with 5 mM vinblastine has a similar effect (fig.2) as 5 mM colchicine.

4. Discussion

Colchicine and vinblastine have been shown to bind to the microtubular system and to alter its function [15]. The prolongation of the lag phase after pretreatment of fat cells with colchicine and vinblastine suggests an influence of the microtubular system on the transmission of the insulin signal in fat cells. The relatively high concentrations of vinblastine and colchicine used in this study were employed [16,17] when the test systems allowed only short preincubation times.

As shown in the binding studies, the delayed response is not caused by a delayed insulin–receptor coupling as pretreatment with colchicine or vinblastine did not alter or even increase the initial rate of receptor association. This increase of binding in fat cells by colchicine has been described [17]. The unchanged maximal over basal ratio of insulin stimulation after pretreatment with 1 mM and 2.5 mM colchicine suggests that under these conditions only the coupling mechanism between insulin receptor and glucose transport system is altered. This might explain why in earlier studies with fat cells where the metabolic effects of insulin after pretreatment with

antimicrotubular substances were measured at later times after addition of insulin, only small or no influences were seen [18,19].

If one speculates on the mechanism of the transmission of the insulin signal behind the receptor, the present data could probably best be interpreted with a model which assumes the movement and coupling of receptors and effectors in the plasma membrane [1–3]. The microtubular system could preserve a definite membrane topography with a particular distribution of receptors and carriers or other membrane proteins involved in the transmission of the insulin signal. Such a function of the microtubular system on surface receptors and glucose carriers has been shown for different cell types [20–22]. A disturbance of this membrane topography could decrease the probability of a coupling reaction and therefore delay the insulin signal.

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