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Insulin-like effect of dichloroacetic acid on hexose transport in Swiss 3T3 cells[1]

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Summary. Hexose transport in Swiss 3T3 cells was increased by treatment with dichloroacetic acid as well as by treatment with insulin. Neither extra- nor intracellular Ca^{2+} was found to be involved in their stimulatory action. On the other hand, the removal of intracellular Mg^{2+} resulted in a loss of the stimulation. These results suggest that dichloroacetic acid stimulates the hexose transport in Mg^{2+}-dependent manner, similar to that of insulin.
Key words. Hexose transport; dichloroacetic acid; insulin; divalent cation; mouse fibroblast.

Dichloroacetic acid is known as a specific activator of pyruvate dehydrogenase which inhibits the transformation of the enzyme into the inactive phosphorylated enzyme[2], and as an inhibitor of the phosphorylation of several mitochondrial proteins[3]. Insulin has also been shown to stimulate pyruvate dehydrogenase activity by activating pyruvate dehydrogenase phosphatase[4,5]. From this point of view, these agents appear to show a similar effect on this enzyme.

The stimulation of hexose transport is one of the main actions of insulin, and the analysis of the mechanism of this phenomenon has been carried out extensively. Recently, it has been reported that insulin stimulates hexose transport by translocation of the carrier from intracellular membranes to plasma membranes[6,7]. Moreover, Mg^{2+}, rather than Ca^{2+} was found to be essential for the stimulatory action of insulin on hexose transport in muscle[8], adipocytes[9] and cardiocytes[10].

In the work described in this paper we examined the effect of dichloroacetic acid on hexose transport in Swiss 3T3 cells and compared it to the effect of insulin. The results show that dichloroacetic acid has an insulin-like stimulatory effect on the hexose transport and that Mg^{2+} may be involved in the transmission of the signal of this compound which induces hexose transport in Swiss 3T3 cells.

Materials and methods. Cell culture. Swiss 3T3 cells were prepared by plating 3×10^5 cells/dish (35 mm in diameter) in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. Cells were grown in a plastic tissue plate in a humidified CO_2 incubator at 37 °C. After 3 days, cultures were used for experiments.

Measurement of hexose uptake. The uptake of 3-O-methylglucose by Swiss 3T3 cells was measured as described previously[11]. Cells were rinsed with 2 ml of phosphate-buffered saline. The uptake was initiated by addition of 1 ml of phosphate-buffered saline containing 4 μM 3-O-methylglucose (0.1 μCi/ml). After the cells had been incubated for 2 min at room temperature, uptake was stopped by washing the cells with ice-cold phosphate-buffered saline. The attached cells were dissolved in 1 ml of 0.1 N NaOH/0.1% sodium dodecyl sulfate solution, and aliquots of the lysate were taken for assay of radioactivity and determination of protein concentrations[12].

Table 1. Effect of dichloroacetic acid and its derivatives on hexose transport

Addition	3-O-Methylglucose uptake (pmol/mg protein/min)
None (control)	2.70 ± 0.12
Monochloroacetic acid (250 μM)	3.06 ± 0.47
Dichloroacetic acid (250 μM)	6.09 ± 0.60*
Trichloroacetic acid (250 μM)	3.81 ± 0.18
Dichloroacetamide (250 μM)	4.40 ± 0.38

Cells were washed and the medium was replaced with Dulbecco's modified Eagle medium containing each agent. After 2 h, the cultures were used for uptake assay as described in 'Materials and methods'. The data are expressed as mean ± SE (n=3). *$p < 0.01$ (vs control value).

Materials. [^{3}H]-3-O-Methylglucose (5 Ci/mmol) and [^{3}H]-L-glucose (10.7 Ci/mmol) were purchased from New England Nuclear. A23187 was obtained from Calbiochem and insulin was from Sigma Chemical Co. Inc. All other chemicals were obtained from commercial sources and were either of reagent grade or the highest purity available.

Results. Dichloroacetic acid stimulated the hexose transport activity of Swiss 3T3 cells (table 1). The stimulatory effect of dichloroacetic acid was time- and dose-dependent, and showed half-maxima at 40 min and 100 μM, respectively. The effect of dichloroacetic acid was completely reversed by washing of the cells (data not shown). Mono- or trichloroacetic acid at 250 μM concentration did not stimulate hexose transport significantly. Dichloroacetamide stimulated hexose transport less effectively than dichloroacetic acid at the same concentration.

Table 2 shows the effect of extra- and intracellular divalent cations on basal, dichloroacetic acid- and insulin-stimulated hexose transport. Removal of extracellular Ca^{2+} and Mg^{2+} by treatment of cells with ethylenediaminetetraacetic acid (EDTA) had no effect on the basal transport or on the transport stimulated by dichloroacetic acid or insulin. When EDTA and A23187 were added to the culture medium to remove both intra- and extracellular Ca^{2+} and Mg^{2+}, the stimulatory effect of dichloroacetic acid or insulin on hexose transport was completely suppressed. In order to exclude the possibility that the inhibition of dichloroacetic acid- or insulin-induced stimulation of hexose transport by EDTA plus A23187 may be caused by their general cytotoxicity, the effect of the restoration of Ca^{2+} or Mg^{2+} was examined. The inhibitory effect of these agents was removed by restoration of Mg^{2+} to the culture medium, On the other hand, it was difficult to draw conclusions as to whether the restoration of Ca^{2+} was effective in reversing the inhibition, since the basal uptake of 3-O-methylglucose was elevated by increased cytosolic Ca^{2+}.

Discussion. The present study shows that dichloroacetic acid stimulates hexose transport, and that this depends upon Mg^{2+} in similar manner to stimulation by insulin. Mg^{2+} appears to play an important role in the regulation of hexose transport in various cells. In muscle, hexose transport is regulated by an intracellular magnesium pump and stimulated through an increase in

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Table 2. Effect of Ca^{2+} and Mg^{2+} on the hexose transport stimulation induced by dichloroacetic acid and insulin

Additions	3-O-Methylglucose uptake (pmol/mg protein/min) None	Insulin (1 μg/ml)	Dichloroacetic acid (250 μM)
Treatment			
None	2.14 ± 0.27 (100)	5.36 ± 0.53* (250)	4.07 ± 0.50* (190)
EDTA (5 mM)	2.26 ± 0.30 (100)	4.53 ± 0.32* (200)	3.62 ± 0.11* (160)
EDTA (5 mM) + A23187 (50 μM)	2.20 ± 0.28 (100)	2.43 ± 0.35 (110)	2.20 ± 0.19 (100)
EDTA (5 mM) + A23187 (50 μM) + $MgSO_4$ (5 mM)	2.82 ± 0.18 (100)	5.21 ± 0.46* (185)	4.26 ± 0.30* (151)
EDTA (5 mM) + A23187 (50 μM) + $CaCl_2$ (5 mM)	3.53 ± 0.44 (100)	4.10 ± 0.37 (116)	3.74 ± 0.40 (106)

Cells were washed and the medium was replaced with Dulbecco's modified Eagle/medium containing EDTA and /or A23187 and/or $MgSO_4$ and/or $CaCl_2$. After 10 min, dichloroacetic acid or insulin was added and then incubated for 2 h. Then 3-O-methylglucose uptake was assayed as described in 'Materials and methods'. Data are expressed as the mean ± SE (n=3). The data in parentheses indicate % of activity: the value obtained in control experiment was defined as 100%. *$p < 0.05$ (vs control value).

cytoplasmic Mg^{2+} concentration[13]. Recently, Kono et al.[9] reported that Mg^{2+} supports the binding of insulin to its receptor and facilitates the insulin-sensitive hexose transport in adipocytes. Our results indicate that dichloroacetic acid has an insulin-like stimulatory effect on hexose transport in Swiss 3T3 cells, and also indicate an important role of intracellular Mg^{2+} in the regulation of hexose transport induced by dichloroacetic acid or insulin. However, the half-maximal stimulation occurred at 40 min after the treatment with dichloroacetic acid, while it was found only after 5 min with insulin. Since such a difference was also observed in their stimulatory effect on pyruvate dehydrogenase when measured in whole cells, the delay of the affect of dichloroacetic acid on hexose transport system may be caused by a slower transmission of the signal or lower permeability of plasma membrane to the compound. Thus, it was found that dichloroacetic acid has an insulin-like stimulatory effect on hexose transport, although its effect is less than that of insulin.

It has been reported that some membrane proteins are phosphorylated when cells are incubated with lipolytic hormones[14,15], and that insulin reverses the phosphorylation induced by these agents[15,16]. Dichloroacetic acid has also been known to inhibit protein phosphorylation[2,3]. These findings led us to propose that dichloroacetic acid-induced stimulation of hexose transport may be mediated by the inhibition of phosphorylation of some membrane proteins. Investigation of the action of dichloroacetic acid will provide further information on that of insulin.

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Cyclosporin A enhances Streptozocin-induced diabetes in CD-1 mice[1]

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Summary. Cyclosporin A (CYA), when administered to CD-1 mice treated with a subdiabetogenic dose of Streptozocin (STZ), exacerbated the STZ-induced insulitis and elevated the plasma glucose levels, parallel to a reduction of the insulin content of the pancreas. The possible mechanisms of CYA-mediated aggravation of STZ-induced diabetes are discussed.
Key words. Cyclosporin A; cellular immunity; Streptozocin; insulitis; diabetes mellitus.

There are data on autoimmunity to pancreatic B-cells in case of insulin dependent diabetes mellitus (IDDM)[2]. In autopsied patients, evidence of insulitis has been obtained[3], and islet cell antibody (ICA) and/or islet cells surface antibody (ICSA) are detectable in many newly diagnosed patients with IDDM[4,5]. Experimentally, Rossini et al. first reported the role of cellular immunity in spontaneously diabetic BB-rats[6], and accumulating data have suggested that autoimmune mechanisms play a significant role in the development of experimental diabetes mellitus. Consequently, Paik et al. and Nakamura et al. clearly showed the importance of thymic immunity and insulitis in the development of Streptozocin (STZ) induced DM in mice[7,8]. This clinical and experimental evidence suggests that autoimmune mechanisms might play a crucial role in the pathogenesis of IDDM. The possibility that patients with autoimmune related IDDM can be treated with immunosuppressive agents has to be given attention. Indeed, clinical trials using Cyclosporin A (CYA), a specific T-cell suppressant[9], have to some extent been