INTERACTION BETWEEN INSULIN RECEPTORS AND GLUCOSE TRANSPORT: EFFECT OF PROSTAGLANDIN E_2.

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SUMMARY

The effect of prostaglandin E_2 on the adipocyte glucose transport system was measured. Prostaglandin E_2 enhanced insulin's effects to accelerate adipocyte glucose transport without increasing insulin binding. However, prostaglandin E_2 did not increase transport in the absence of insulin, and is thus not an insulin-like agent. This augmenting effect appeared specific for the insulin stimulatory process since prostaglandin E_2 did not enhance the ability of "insulin-like" agents to increase glucose transport. These results suggest that prostaglandin E_2 may play a role in the interaction between insulin receptors and the glucose transport system.

INTRODUCTION

Prostaglandins are a group of endogenously synthesized intracellular compounds which are known to mediate a variety of cell functions (1), and in the current studies, the relationship between prostaglandin E_2[1] and insulin action was assessed. The results indicate that PGE_2 potentiates insulin's cellular effects on glucose transport, and suggest a role for PGE_2 in mediating the interaction between occupied insulin receptors and the glucose transport system of isolated adipocytes.

MATERIALS AND METHODS

Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of the Eli Lilly Co. Bovine serum albumin (fraction V) was purchased from Armour and Co., collagenase from Worthington Biochemicals, and 2-deoxy-1-{^{14}C}-glucose and {^{14}C}-inulin from New England Nuclear.

Preparation of Isolated Adipocytes: Male Sprague-Dawley rats weighing 120-150 gms were used for all experiments. All studies were performed in the morning on animals who had free access to standard rat chow. Animals were stunned by a blow to the head, decapitated, and epididymal fat pads removed. Isolated fat cells were prepared by shaking at 37 C for 60 minutes in Krebs-Ringer bicarbonate buffer containing collagenase (3 mg/ml) and albumin (40 mg/ml), ac-

[1]Abbreviation: prostaglandin (PG)

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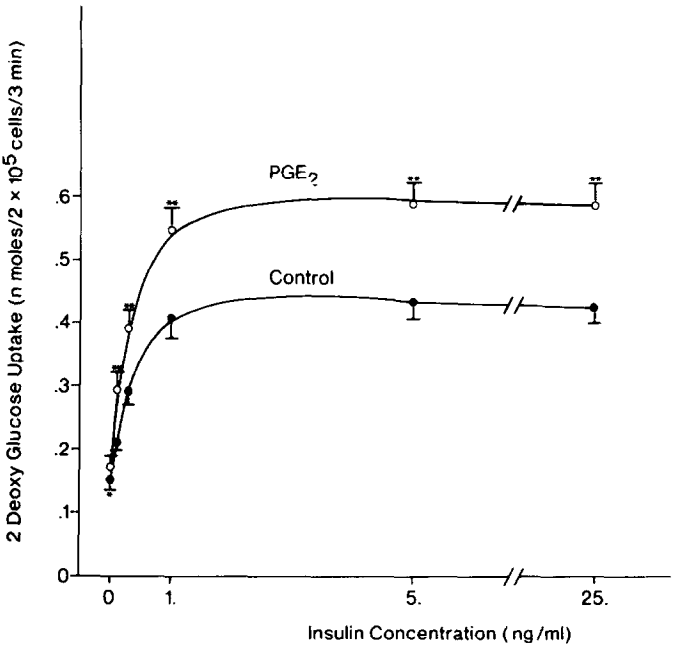

Fig. 1 Effect of PGE_2 on the insulin-glucose transport dose response curve. Glucose transport was assessed by measuring the initial rate of uptake (3 min) of the non-metabolyzable sugar 2-deoxy glucose (0.125mM) as previously described (4,5). Under these experimental conditions (see methods), essentially all of the intracellular 2-deoxy glucose is in the phosphorylated form (4,7,8), and therefore, significant efflux of free 2-deoxy glucose cannot occur. Thus, this provides an accurate measure of glucose transport activity (influx). Closed circles (●) represent rates of uptake in the absence of PGE_2, and open circles (○) represent rates of uptake in the presence of 10^{-7}M PGE_2. Data are the mean of 10 separate paired experiments, and p values are obtained by use of the paired t test. Two asterisks (**) indicates $p < .01$, one asterisk (*) indicates lack of significant differences.

cording to the method of Rodbell (2). Adipocyte counts are performed according to a modification of method III of Hirsch and Gallian (3), as previously described (4).

Glucose Transport Studies: Transport studies were performed by previously described methods (4,5). Unless otherwise stated, isolated adipocytes were incubated with 2-deoxy-{^{14}C}-1-D-glucose (specific activity 2mCi/mM) at a concentration of 0.125mM in Krebs-Ringer bicarbonate, pH 7.4, containing BSA (10 mg/ml) at 24 C. This assay measures the total uptake of the radiolabeled 2-deoxy glucose and is based on the principle that while 2-deoxy glucose is transported and phosphorylated by the same process as D-glucose it cannot be further metabolized (6). At the low concentration of this sugar which was used, and at the early time points studied (3 mins), initial rates of uptake are approximated. Thus, under these conditions the activity of the adipocyte glucose transport system can be monitored (4,5,7,8). The assay is terminated at the end of 3 mins by transferring 200 µl aliquots from the assay mixture to plastic microtubes containing 100 µl dinonyl phthalate oil. The tubes are centrifuged for 30 secs

in a Beckman microfuge, and the assay is considered terminated when centrifugation begins. In experiments in which the stimulatory effect of insulin on uptake was measured, the cells were preincubated with insulin for 45 mins at 24 C. The amount of sugar trapped in the extracellular water space of the cell layers was determined using {^{14}C}-inulin according to the method of Gliemann (9). Extracellular water space is measured in each experiment, and all data of sugar uptake are corrected for this factor. The amount of trapped sugar ranged from 2-10% of the total sugar uptake depending on the conditions of incubation. In experiments in which the effects of PGE_2 were studied, the prostaglandin was added to the cells during the collagenase digestion. A further 45 min preincubation was carried out in the presence of PGE_2 prior to the transport study and, thus, the total time of exposure to this agent was 105 mins. It should be noted that after the cells were washed, fresh PGE_2 at the appropriate concentration was added. At the concentrations employed PGE_2 was found to be soluble in aqueous solution. However, when $PGF_{2\alpha}$ was used, this agent was initially dissolved in ethyl alcohol. The final concentration of ethyl alcohol in the transport assay was 0.3% and control experiments showed that this concentration had no effect on 2-deoxy glucose uptake.

RESULTS AND DISCUSSION

When 2-deoxy glucose uptake is measured as a function of insulin concentration, a dose response curve is obtained with 1/2 maximal stimulation at an insulin level of 0.2 ng/ml and maximal stimulation of approximately 300% at 5 ng/ml (Fig. 1). When similar studies are performed in the presence of PGE_2 (10^{-7}M) the dose response curve is greatly enhanced (Fig. 1). Thus, although PGE_2 had no effect in the absence of insulin, at each insulin concentration used the rate of 2-deoxy glucose uptake is significantly increased in the presence of this agent. Indentical results were found over a PGE_2 concentration range of 10^{-4}M - 10^{-10}M, while submaximal effects were seen at 10^{-11}M (Table IIA)[2]. This was true whether the prostaglandin was added at 5 mins before the transport assay, or whether various preincubation schedules (up to 180 mins) were employed. Furthermore, PGE_2 had no effect on insulin binding to adipocytes (data not shown), and, therefore, this agent appears to enhance the interaction (coupling) between insulin receptors and the glucose transport system.

This effect is considerably different than that seen with various insulin-

[2]It should be emphasized that this refers to the external concentration of PGE_2 in the medium, and this may have little bearing on the effective PGE_2 concentration at its site of action.

TABLE I

COMPARISON OF THE EFFECTS OF VARIOUS INSULIN-LIKE AGENTS AND PGE_2 ON 2-DEOXY GLUCOSE UPTAKE[1]

Additions	2-Deoxy Glucose Uptake (% of control)
None	100
Insulin (5 ng/ml)	280 ± 31
Cysteine (0.5 nM)	221 ± 18
Diamide (0.2 mM)	202 ± 24
Spermine (20 μM)	227 ± 27
Cysteine + Insulin	285 ± 27
Diamide + Insulin	290 ± 32
Spermine + Insulin	272 ± 37
PGE_2 (10^{-7}M)	110 ± 6
Insulin + PGE_2	483 ± 42

[1]All studies were done at a 2-deoxy glucose concentration of 0.125mM. See legend to Fig. 1 and Reference 4 for details. Data represent mean (± SE) for 3 experiments.

like agents. As can be seen in Table I, cysteine, diamide, and spermine all stimulate basal rates of glucose transport in the absence of insulin, but in no case are their effects additive to those of insulin. In contrast PGE_2 does not appreciably increase basal uptake rates, and this was so even when PGE_2 levels as high as 10^{-5}M were used. Its effects are seen only in the presence of insulin, and result in rates of transport in excess of those seen with insulin alone. Furthermore, this effect is highly specific for the insulin stimulatory process, since PGE_2 did not enhance spermine, cysteine, or diamide stimulated rates of 2-deoxy glucose uptake (data not shown).

The ability of PGE_2 to augment insulin stimulated uptake is unlikely to be

TABLE II

COMPARISON OF THE EFFECTS OF OLEATE, $PGF_{2\alpha}$ AND PGE_2 ON BASAL AND INSULIN STIMULATED 2-DEOXY GLUCOSE UPTAKE[1]

Additions	2-Deoxy Glucose Uptake (% of control)
A.	
None	100
Insulin (5 ng/ml)	287 ± 30
Insulin + PGE_2 (10^{-12}M)	279 ± 37
+ PGE_2 (10^{-11}M)	351 ± 32
+ PGE_2 (10^{-10}M)	469 ± 42
+ PGE_2 (10^{-7}M)	486 ± 50
B.	
Insulin (5 ng/ml)	296 ± 36
Insulin + Oleate (1mM)	146 ± 21
Insulin + $PGF_{2\alpha}$ (10^{-7}M)	287 ± 33
+ $PGF_{2\alpha}$ (10^{-5}M)	294 ± 38

[1]These studies were done at a 2-deoxy glucose concentration of 0.125mM and represent the mean (± SE) of 3 experiments for parts A and B.

a non-specific effect of fatty acid-like compounds since oleic acid and $PGF_{2\alpha}$ either inhibited (oleate), or had no effect ($PGF_{2\alpha}$) on 2-deoxy glucose uptake (Table IIB).

The specific relationship between insulin receptors and insulin's cellular action is poorly understood (10). The results of these studies suggest a poten tial role of PGE_2 in the interaction between occupied insulin receptors and at least one of the cellular effector systems for insulin action -- the glucose transport system. However, the nature of this effect of PGE_2 is not clear

from these studies. The data in Fig. 1 suggest PGE_2 increases the Vmax of insulin stimulated glucose transport. Whether this involves the availability of additional glucose transport units under the influence of PGE_2, or whether there is an increase in the intrinsic mobility or activity of transport units which are normally activated by insulin remains to be defined.

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