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DUAL EFFECT OF ISOPRENALINE ON GLUCOSE TRANSPORT AND RESPONSE TO INSULIN IN ISOLATED ADIPOCYTES

HANS-GEORG JOOST,* RÜDIGER GÖKE† and HANS-JÜRGEN STEINFELDER
Institut für Pharmakologie und Toxikologie der Universität Göttingen, D-3400 Göttingen, Federal Republic of Germany

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Abstract—Glucose transport as assessed by the uptake rate of 3-O-methylglucose was stimulated in isolated rat fat cells by preincubation with isoprenaline or orciprenaline. The effect was apparently mediated by β_1-receptors, since (1) it was abolished by propranolol, (2) it closely paralleled the stimulation of lipolysis, and (3) isoprenaline was 10^2 times more potent that orciprenaline. Isoprenaline enhanced the effect of submaximal insulin concentrations as well as the basal transport rate but failed to increase the maximal effect of insulin. The stimulatory effect of isoprenaline was antagonized by adenosine deaminase which removes adenosine spontaneously released from the cells, and by bordetella toxin (IAP)‡ which blocks the inhibitory coupling component of adenylate cyclase. Moreover, bordetella toxin uncovered an inhibitory effect of isoprenaline on insulin stimulated glucose transport. There was no apparent correlation between the effects on glucose transport and the response of cellular cyclic AMP levels to the agents investigated. It is suggested that a step in the coupling of β-receptors and adenylate cyclase, but not total cellular cyclic AMP levels, may mediate stimulatory as well as inhibitory effects of catecholamines on glucose transport in the adipocyte.

Catecholamines reduce the glucose utilization of most tissues thereby antagonizing the action of insulin at crucial points of the intermediary metabolism [1]. In the adipocyte, however, the overall glucose utilization is increased reflecting enhanced lactate production and glyceride–glycerol synthesis [2]. Since hexose transport was considered to be rate limiting for glucose utilization [3], it was concluded that catecholamines increase glucose transport in the adipocyte [4]. More recently, the stimulatory effect of epinephrine was demonstrated with suitable methods to directly determine the hexose transport velocity [5, 6]. In contrast, two recent studies [7, 8] reported inhibitory effects of catecholamines on glucose transport.

Cyclic AMP plays an important role in the regulation of insulin action. Several agents which elevate cellular cyclic AMP levels reduce the transport rate, whereas agents which decrease them enhance the transport velocity or facilitate the action of insulin [9–11]. It has been suggested, therefore, that cyclic AMP mediates an inhibition of basal as well as insulin-stimulated glucose transport [9]. A stimulatory effect of catecholamines on glucose transport would obviously conflict with this concept. An alternate hypothesis has been developed, therefore, according to which cyclic AMP may mediate a stimulatory effect on glucose transport via elevated intracellular calcium levels [6].

The present study was performed to further investigate the effects of catecholamines on glucose transport. It was inspired by the previous finding that the glucose transport rate in isolated adipocytes may depend on adenosine [10] which is spontaneously released by incubated fat cells, and which is a potent inhibitor of adenylate cyclase [12]. The results show that isoprenaline stimulates basal as well as insulin-stimulated hexose transport if adenosine is present, and inhibits the effect of insulin if cells were pretreated with bordetella toxin. It is suggested that β-receptors may mediate a stimulation as well as an inhibition of hexose transport which might be related to the regulation of adenylate cyclase though not to total cellular cyclic AMP levels.

MATERIALS AND METHODS

Chemicals. Crystalline porcine insulin was a gift from the Hoechst AG, Frankfurt. Islet-activating protein (IAP) purified as described [13] was obtained from Dr. Jakobs, Institute of Pharmacology, Heidelberg. Bovine serum albumin (fraction V), adenosine, and phloretin were purchased from Serva, Heidelberg. Crude bacterial collagenase (type IV) was supplied by Worthington Biochemicals (Freehold, NJ), adenosine deaminase and all other enzymes were from Boehringer (Mannheim). It was assured that boiling destroyed the effects of the adenosine deaminase preparation on lipolysis as well as on glucose transport. Silicone oil (Cat. No. 6428 R-15) was purchased from A. H. Thomas (Philadelphia, PA), HEPES was from Sigma (St. Louis, MO). All radiochemicals were from Amersham-Buchler (Braunschweig).

Isolation of fat cells. Male albino Wistar rats bred in our institute were used throughout (body weight 130–180 g). The animals had free access to food and

* To whom all correspondence should be addressed.
† The results are in part from the MD Thesis of R. Göke, Göttingen 1984.
‡ Abbreviation: IAP, islet-activating protein.

water. Isolated fat cells were prepared by the method of Rodbell [14] with modifications described previously [10]. In brief, the epididymal adipose tissue of 3 rats was digested with collagenase (1 mg/ml) in Krebs–Ringer-HEPES buffer containing 4% of albumin. The cells were filtered through nylon mesh, washed, and distributed to the incubation vials to yield a concentration of approximately 10^5 cells per ml. An aliquot of the fat cell suspension was fixed with osmium tetroxide [15] and counted in a Fuchs-Rosenthal counting chamber. Total lipid volume was determined by centrifugation of an aliquot in a hematocrit tube [16]. The buffers used for washing and incubation of cells consisted of Krebs–Ringer-HEPES-buffer (pH 7.4) containing 1% of bovine serum albumin, 1 mM glucose, 10 mM HEPES, and 1 mM bicarbonate throughout.

Glucose transport assay. The uptake rate of 3-O-methylglucose was determined by the previously described method of Whitesell and Gliemann [17] with minor modifications. After preincubation with the agents under investigation the fat cell suspension was concentrated to yield samples of 200 μl containing approximately 4×10^5 cells. The samples were allowed to equilibrate at room temperature (22°), and were added to 5 μl of buffer containing 0.6 μCi of [^{3}H]methylglucose (final concentration 0.15 mM). The uptake of the glucose analogue was stopped after 3 sec by addition of 8 ml of ice-cold phloretin solution (1 mM, buffered with 1.3% $NaHCO_3$, pH 7.4). Silicone oil was added, and the samples were centrifuged (3 min, 3000 g) within 2 min after the termination of the transport. The cell layer was removed, and cell-associated radioactivity was determined. Timing of the transport was performed with a metronome set at half-second intervals. Blanks which had been added to the stopping solution before addition of radioactivity were included in each series, and all data were corrected accordingly. In control experiments with ^{14}C-labeled L-glucose it was assured that this procedure corrects for all of the nonspecific 'uptake' produced by trapped extracellular buffer. The blanks contained 30–50% of the total radioactivity taken up by the cells after 3 sec. In control experiments the time course of methylglucose transport was investigated: the uptake approached equilibrium after 30 seconds. After 3 sec, 10–20% of the equilibrium water space was filled in unstimulated cells, and 40–50% of equilibrium uptake was reached in insulin-stimulated cells.

Other assays. Glycerol was determined enzymatically as described [18]. Cyclic AMP was determined by radioimmunoassay [19] (testkit of Becton and Dickinson, Heidelberg). Samples were deproteinized with trichloroacetic acid, extracted with ether, freeze dried, and dissolved in acetate buffer (50 mM, pH 6.2).

RESULTS

When fat cells were incubated in the presence of isoprenaline or orciprenaline for 30 min prior to the transport assay, the basal 3-O-methylglucose uptake rate was significantly enhanced (Fig. 1). This stimulatory effect of the β-adrenergic agonists in our hands depended on the presence of glucose, and on a restricted cell concentration during the preincubation. In the absence of glucose and at cell concentrations exceeding 3×10^5/ml the stimulatory effect disappeared in parallel to a decrease of cellular ATP levels (data not shown).

The concentration response curves (Fig. 1) shows that isoprenaline was approximately 100 times more potent than orciprenaline in stimulating the transport rate. As can be seen from the upper part of the figure, the stimulation of glucose transport was well correlated with the effect of the catecholamines of lipolysis.

Figure 2 shows the effect of isoprenaline (1 μM) on the insulin-stimulated glucose transport. The catecholamine significantly stimulated the effect of submaximal insulin concentrations but failed to further enhance the maximal effect of insulin.

Table 1 shows the effects of isoprenaline on glucose transport in the presence of glucose and various agents affecting adenylate cyclase activity. Addition of adenosine failed to alter the stimulatory effect of isoprenaline. Since isolated fat cells spontaneously release adenosine, it was likely that the adipocytes of the control experiments were already exposed to an effective concentration of adenosine. Thus

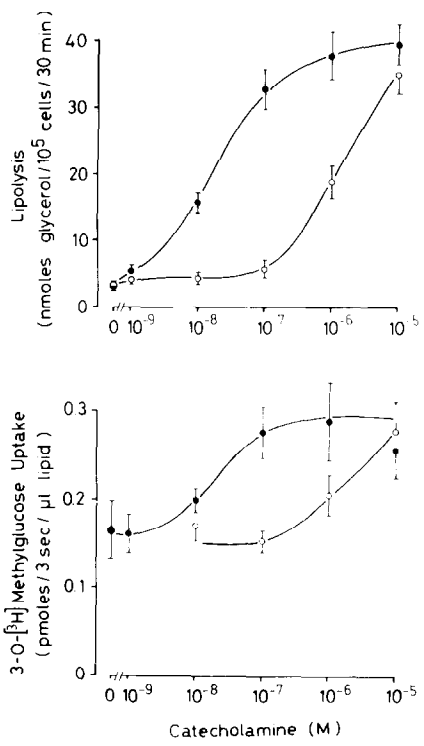

Fig. 1. Effects of isoprenaline (filled circles) and orciprenaline (open circles) on basal 3-O-methylglucose transport (lower part) and lipolysis (upper part) of isolated fat cells. Isolated rat adipocytes (approximately 10^5 cells/ml) were incubated with the catecholamines as indicated for 30 min. The medium was removed for glycerol assay, and transport was determined in the resulting concentrated cell suspension (approximately 4×10^5 cells/200 μl) after equilibration at room temperature (22°). Data represent means ± S.E. of six experiments.

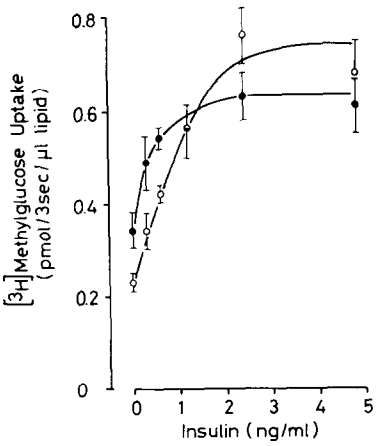

Fig. 2. Effect of isoprenaline (1 μM, filled circles) on insulin-stimulated 3-O-methylglucose transport. Isolated rat adipocytes (10^5 cells/ml) were preincubated with isoprenaline for 30 min. The cell suspension was concentrated to yield approximately 4×10^5 cells/200 μl, and insulin was added to the samples in the indicated concentrations. The samples were incubated for 20 min at 37°, and transport was determined after equilibration at room temperature. Data represent means ± S.E. of eight experiments. Differences to controls were significant ($P < 0.025$) at 0, 0.3 and 0.6 ng/ml insulin.

adenosine deaminase was added to remove the adenosine produced by the cells. In these experiments isoprenaline failed to significantly stimulate the hexose transport rate. Similarly, the effect of isoprenaline was abolished by propranolol added to block adrenergic β-receptors, and by the bordetella toxin (IAP) which is supposed to irreversibly block the inhibitory coupling unit of adenylate cyclase [13].

As anticipated, the addition of adenosine deaminase produced a large increase of cyclic AMP levels in response to isoprenaline. Added adenosine only moderately reduced the response to isoprenaline as compared to the control cells (no addition) which apparently had been exposed to endogenous adenosine spontaneously released by the cells. Bordetella toxin which abolished the stimulatory effect of isoprenaline on glucose transport increased the response of cyclic AMP to isoprenaline as compared to control conditions (no addition). However, the increase was only 2-fold as compared with a more than 10-fold stimulation produced by adenosine deaminase.

Figure 3 shows the effect of isoprenaline on insulin-stimulated glucose transport in cells pretreated with bordetella toxin for 90 min. It can be seen that bordetella toxin had reversed the stimulatory effect of isoprenaline (Fig. 1 and 2) to an inhibitory one, since basal as well as insulin-stimulated glucose transport were significantly reduced in the pretreated cells.

In order to investigate the action on glucose transport of cyclic nucleotide levels elevated independently from adenylate cyclase, cells were preincubated with dibutyryl cyclic AMP, and the 3-O-methylglucose uptake rate was determined. Figure 4 shows that the nucleotide significantly inhibited the uptake rate at concentrations which were lower than those stimulating the lipolysis.

DISCUSSION

Contradictory results have previously been reported on the effects of catecholamines on glucose transport in fat cells. Overall glucose utilization which mainly represents the conversion of glucose to triglycerides has been found enhanced by catecholamines. Thus on the basis of the assumption that glucose utilization is limited by the transport rate, catecholamines were supposed to stimulate the

Table 1. Effects of several agents affecting adenylate cyclase activity on glucose transport and cellular cyclic AMP levels in the presence and absence of isoprenaline (1 μM)

	Methylglucose uptake (pmole/3 sec/μl lipid)			Cyclic AMP (pmole/10^5 cells)		
	Control	Isoprenaline		Control	Isoprenaline	
No addition [1]	0.35 ± 0.03	0.52 ± 0.05	$P < 0.01$	23.0 ± 10.7 [8]	95.8 ± 14.1 [8]	$P < 0.001$
Adenosine (1 μM) [5]	0.33 ± 0.04	0.49 ± 0.04	$P < 0.025$	25.1 ± 8.7 [7]	57.3 ± 8.4 [8]	$P < 0.01$
Adenosine-deaminase (2 μg/ml) [8]	0.33 ± 0.04	0.40 ± 0.04	n.s.	48.6 ± 16.4 [8]	1106 ± 219 [8]	$P < 0.001$
Propranolol (10 μM) [5]	0.39 ± 0.07	0.42 ± 0.04	n.s.	—	—	
No addition [6]	0.34 ± 0.05	0.50 ± 0.08	$P < 0.025$	36.2 ± 14	44.0 ± 8	n.s.
IAP (85 ng/ml) [6]	0.33 ± 0.06	0.30 ± 0.04	n.s.	34.4 ± 11 [7]	93.0 ± 26 [7]	$P < 0.025$

Isolated fat cells were incubated in the presence of isoprenaline (1 μM) and the indicated agents for 30 min at 37°. IAP was added 1 hr prior to the addition of isoprenaline. Methylglucose uptake was determined at 22°, and data were calculated as pmole/3 sec/μl triglyceride and presented as means ± S.E. For determination of cyclic AMP levels samples were homogenized 5 min or, in IAP-treated cells, 10 min after addition of isoprenaline. Values in parenthesis represent the number of experiments. Differences were tested for statistical significance with the U-test of Wilcoxon, Mann and Whitney. If P was greater than 0.1, differences were considered to be not significant, n.s.

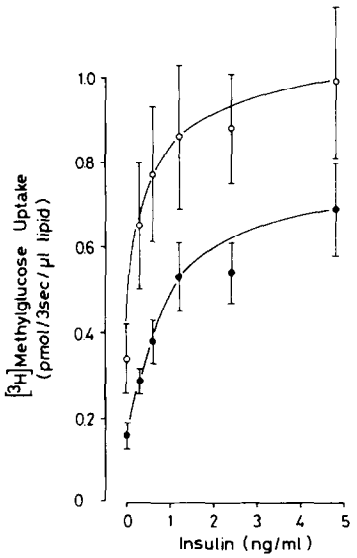

Fig. 3. Effect of insulin and isoprenaline on 3-O-methylglucose transport in bordetella toxin treated adipocytes. Isolated fat cells were incubated for 90 min at 37° in the presence (filled circles) and absence (open circles) of bordetella toxin (85 ng/ml). 1 µM isoprenaline (filled circles) was added 30 min prior to the end of the incubation. Like in the experiments shown in Figs. 1 and 2, the fat cell suspension was concentrated and insulin was added as indicated. After further 20 min of incubation at 37° the transport assay was performed as described at 22°. The data represent means ± S.E. of 6 experiments. Differences to controls were significant ($P < 0.05$) from 0 to 2.4 ng/ml insulin.

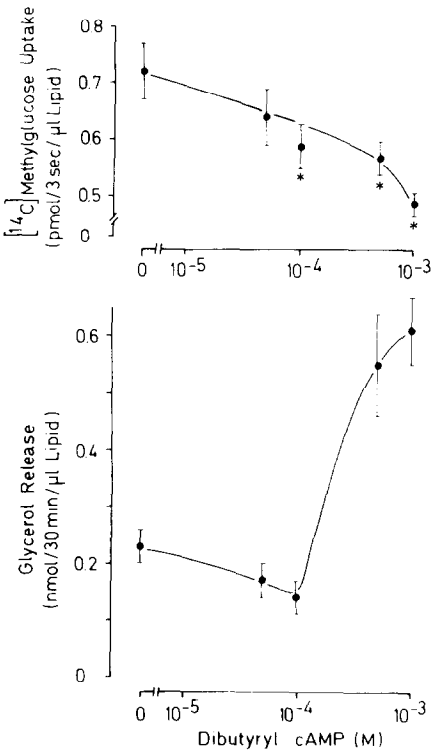

Fig. 4. Effects of dibutyryl-cyclic-AMP on 3-O-methylglucose transport (upper panel) and lipolysis (lower panel) in isolated rat fat cells. The incubation schedule was the same as described in Fig. 1. All incubation buffers contained glucose (1 mM). Data represent means ± S.E. of 7 experiments. Asterisks indicate significance ($P < 0.05$) of differences to the basal transport rate as tested with the D_i-test of Wilcoxon and Wilcox. Lipolysis was not tested for statistical significance.

glucose transport rate [4]. More recently, catecholamines were reported to stimulate the glucose transport rate [5, 6] as assessed with an improved method to directly determine the transport velocity with the non-metabolizable hexose 3-O-methylglucose [17]. Two recent studies, however, reported inhibitory effects of catecholamines on basal as well as on insulin-stimulated 3-O-methylglucose transport [7, 20].

The present results indicate that β-adrenergic agonists stimulate 3-O-methylglucose transport in isolated fat cells, and that certain conditions may reverse the stimulatory effect to an inhibitory one. The stimulatory effect closely paralleled the stimulation of lipolysis and was mediated by β-receptors, since it was abolished by propranolol. The β-receptors of the adipocyte are of the β_1-type [21], and were thus stimulated by isoprenaline in a 100-fold lower concentration than by orciprenaline (Fig. 1).

In a very recent publication [20] a biphasic concentration response curve of the effect of isoprenaline was observed: Low concentrations of the catecholamine stimulated the glucose transport rate, whereas higher concentrations were inhibitory in parallel to an increase of cyclic AMP levels. In contrast, we failed to observe an inhibitory effect of higher catecholamine concentrations. The difference might be due to the experimental design of the former study in which the transport was assayed after incubation of a 40% cell suspension (approximately 60×10^5 cells/ml). In our hands, at a cell concentration exceeding 3×10^5 cells/ml the stimulatory effect of the catecholamines disappeared, and a decrease of cellular ATP-levels reflected accumulation of fatty acids [22]. The present experiments were therefore carried out at lower cell concentrations (10^5 cells/ml), and the cell suspensions were concentrated prior to the transport assay.

In adipose tissue catecholamines activate adenylate cyclase via β-receptors giving rise to an elevation of cellular cyclic AMP levels. At present the role which cyclic AMP plays in the regulation of glucose transport is a controversial issue. Taylor and Halperin [9] concluded from experiments with agents modulating cellular cyclic AMP levels that the nucleotide inhibits glucose transport and antagonizes insulin action.

The inhibitory effect of dibutyryl cyclic-AMP (Fig. 4) is in accordance with this concept, whereas the stimulatory effect of isoprenaline, which was paralleled by an elevation of cyclic AMP, obviously disagrees. An alternative model was advanced by Rasmussen and Clausen [6] who suggested that an increase in cyclic AMP mobilizes calcium ions from intracellular pools which in turn activates the glucose transport system [23]. This concept would be com-

patible with the stimulatory effect of isoprenaline. It conflicts, however, with the effect of adenosine deaminase as observed in the present study. When the response of cyclic AMP to isoprenaline was enhanced by adenosine deaminase more than 10-fold, the stimulation of glucose transport was reversed (Table 1). It appears, therefore, that the stimulatory effect of isoprenaline depends on the presence of adenosine, which inhibits adenylate cyclase [12, 24], rather than on an elevation of cyclic AMP levels.

According to the concept of Taylor and Halperin [9] one might conclude that the large response of cyclic AMP produced by adenosine deaminase had reversed the stimulation of glucose transport. However, bordetella toxin as well abolished the effect of isoprenaline but failed to trigger an increase of cyclic AMP levels comparable to that produced by adenosine deaminase. The present experiments failed, therefore, to correlate the effects on glucose transport with cellular cyclic AMP levels. They do not fully rule out a regulatory role of cyclic AMP, however, since it cannot be excluded that a major proportion of the nucleotide is located in a compartment without influence on the glucose transport system.

The role of adenosine as a modulator of adipocyte metabolism is well recognized since the work of Schwabe *et al.* [12, 24, 25]. Adenosine inhibits lipolysis [12] by inhibition of adenylate cyclase [24, 25], and enhances insulin sensitivity of fat cells at a step between insulin receptors and the glucose transport system [10]. In fat cell membranes the inhibitory effect of adenosine on adenylate cyclase depends on the presence of GTP [26]. It has been reported that the nucleoside stimulates a GTPase at the stimulatory coupling unit N_s of adenylate cyclase [27] which upon hydrolysis of GTP to GDP triggers a deactivation of the cyclase. Bordetella toxin has recently been reported to irreversibly block a GTPase at the presumed inhibitory coupling unit N_i of adenylate cyclase in fat cells [13]. In the present experiments bordetella toxin exerted effects on glucose transport similar to those of removal of adenosine by adenosine deaminase. The stimulatory effect of isoprenaline was abolished, and was reversed to an inhibition of the insulin-stimulated glucose transport (Fig. 3) [28]. Similarly adenosine deaminase has recently been reported to uncover an inhibitory effect of isoprenaline on insulin-stimulated 2-deoxyglucose transport [8].

It is tempting to speculate, therefore, that the regulatory component(s) of adenylate cyclase and/or the associated GTPase(s) represent a pivotal link in the regulation of glucose transport of the adipocyte.

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