Effects of islet-activating protein on insulin- and isoprenaline-stimulated glucose transport in isolated rat adipocytes

H.G. Joost and R. Göke

Institut für Pharmakologie und Toxikologie der Universität Göttingen, Robert-Koch-Straße 40, D-3400 Göttingen, FRG

Received 2 December 1983

The effects of islet-activating protein (IAP), a *Bordetella pertussis* toxin, on insulin- and isoprenaline-stimulated glucose transport were studied in isolated rat adipocytes. Basal as well as insulin-stimulated glucose transport were not affected when cells were pretreated with IAP. In contrast, IAP pretreatment abolished the stimulatory effect of isoprenaline. When IAP-pretreated cells were exposed to a combination of insulin and isoprenaline, the catecholamine significantly reduced the stimulatory effect of insulin. Since IAP is supposed to specifically block the inhibitory component N_i of adenylate cyclase, the results suggest that: (a) the effect of insulin is unrelated to the regulation of adenylate cyclase; (b) isoprenaline may exert both stimulatory and inhibitory effects depending on activation of N_i. The inhibitory regulation of adenylate cyclase may thus be a pivotal link in the regulation of glucose transport.

Insulin *Isoprenaline* *Rat adipocyte* *Islet-activating protein* *Glucose transport*

1. INTRODUCTION

Glucose transport across the cell membrane is one of the rate limiting steps of glucose metabolism in the fat cell controlled by insulin [1]. The stimulatory action of insulin may be modulated, although probably not mediated as was initially proposed, by cellular cyclic AMP levels [2,3]. Recently, attention has been focused on the insulin antagonist action of catecholamines in the fat cell at the receptor and at the postreceptor level. Two groups reported that insulin receptor binding was decreased by catecholamines in parallel to a reduction of the response of glucose transport to insulin [4,5]. However, previous investigators have observed that catecholamines stimulate overall glucose incorporation [6,7] as well as the glucose transport rate which was assessed with non-metabolizable hexoses [8–10]. This discrepancy suggests that catecholamines may produce differential effects on hexose transport and raises the question for the conditions and mechanisms underlying the stimulatory and the inhibitory action.

The *Bordetella* toxin islet-activating protein (IAP) has been used as a tool to study the regulation of adenylate cyclase [11]. The toxin blocks the inhibition of the cyclase presumably by ADP-ribosylation of the hypothetical regulatory component N_i [12,13]. Here IAP has been used to investigate whether the action of insulin or of isoprenaline on glucose transport are dependent on the inhibitory regulation of adenylate cyclase. As anticipated, insulin action was not affected by the toxin. The stimulatory effect of isoprenaline, however, was abolished and reversed to an inhibitory action, suggesting a dual effect of isoprenaline on glucose transport.

2. MATERIALS AND METHODS

2.1. *Materials*

Cristalline porcine insulin was obtained as a gift from Hoechst AG (Frankfurt). IAP purified as in [14] was obtained from Dr Jakobs, Institute of Pharmacology (Heidelberg). Isoprenaline sulfate was supplied by Boehringer (Ingelheim). Bovine serum albumin (fraction V), adenosine and

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

phloretin were purchased from Serva (Heidelberg). Crude bacterial collagenase (type IV) was supplied by Worthington Biochemicals (Freehold, NJ). All other enzymes were from Boehringer (Mannheim). 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).

2.2. *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 water. Isolated fat cells were prepared as in [15] with modifications described in [16]. In brief, the epididymal adipose tissue of 2–3 rats was digested with collagenase (1 mg/ml) in Krebs-Ringer–Hepes buffer containing glucose (1 mM) and 4% 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. All subsequent incubations were carried out in Krebs-Ringer–Hepes buffer containing glucose (1 mM) and 1% albumin. An aliquot of the cell suspension was fixed with osmium tetroxide [17] and counted in a Fuchs-Rosenthal chamber. Total lipid volume was determined by centrifugation of an aliquot in a hematocrit tube [18].

2.3. *Glucose transport assay*

The uptake rate of 3-*O*-methylglucose was determined as in [19], 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 to room temperature (22°C), and were added to 5 μl buffer containing 0.6 μCi [^{3}H]methylglucose (final concentration 0.15 mM). The uptake of the glucose analogue was stopped after 3 s by addition of 8 ml ice-cold phloretin solution (1 mM). Silicone oil was added, and the samples were centrifuged within 2 min after the termination of the transport. 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 L-[^{14}C]glucose it was assured that this procedure corrects for all of the unspecific 'transport' produced by trapped extracellular volume. The blanks ranged from 30–50% of the radioactivity found in the cells which was 0.05–0.1% of the total radioactivity. The basal uptake after 3 s was 10–20% of the equilibrium intracellular water space.

3. RESULTS AND DISCUSSION

Isolated fat cells were incubated for 90 min with IAP (85 ng/ml) and thereafter subjected to a second incubation (20 min) in the presence of insulin. As is shown in fig.1, IAP failed to significantly alter the response to insulin of the hexose transport rate. If fat cells were incubated in the presence of isoprenaline (1 μM) for 60 min prior to the transport assay, the basal hexose transport rate was increased by approximately

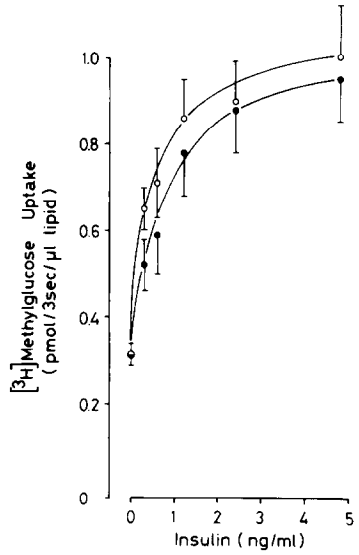

Fig.1. Effect of insulin on 3-*O*-methylglucose transport in IAP-treated adipocytes. Means ± SE of 6 experiments. Isolated fat cells (approx. 10^5/ml) were incubated for 90 min at 37°C in the presence (●) or absence (○) of IAP (85 ng/ml). The cell suspension was concentrated in order to obtain a sample size sufficient for the transport assay, and aliquots were distributed into incubation vials containing insulin as indicated. After 20 min of incubation at 37°C the samples were equilibrated to 22°C, and methylglucose transport was determined as described in section 2. Differences to control values were not significant throughout.

Table 1

Reversal of the effect of isoprenaline on basal 3-*O*-methylglucose transport in adipocytes

	3-*O*-Methylglucose uptake (pmol·3 s^{-1}·μl $lipid^{-1}$)		
	Control	Isoprenaline	*p*
No addition (11)	0.35 ± 0.03	0.52 ± 0.05	0.01
IAP (85 ng/ml) (6)	0.33 ± 0.06	0.30 ± 0.04	n.s.
Propranolol (10 μM) (5)	0.39 ± 0.07	0.42 ± 0.04	n.s.
Adenosine deaminase (2 μg/ml) (8)	0.33 ± 0.04	0.40 ± 0.04	n.s.

Isolated fat cells (approx. 10^5 cells) were incubated in the presence (or absence) of isoprenaline (1 μM) and the indicated agents for 30 min at 37°C. IAP was added 1 h prior to the addition of isoprenaline. The cell suspension was concentrated, allowed to equilibrate at 22°C, and methylglucose transport was determined as described in section 2. Data are means ± SE of a number of experiments indicated in parentheses. The 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.)

40% (table 1, fig.2). This effect was observed in the absence of insulin or in the presence of submaximally stimulating insulin concentrations, but disappeared at higher insulin concentrations, and seemed to be reversed to an inhibitory effect at maximal stimulation of hexose transport by insulin (fig.2).

When fat cells were subjected to a 90-min preincubation period with IAP (85 ng/ml), the stimulatory effect of isoprenaline on glucose transport was abolished (table 1). Moreover, in cells preincubated with IAP isoprenaline significantly inhibited the stimulatory effect of insulin on glucose transport (fig.3). Further, the stimulatory effect of isoprenaline on hexose transport was reduced by β-adrenergic blockade with propranolol, and by adenosine deaminase which removes adenosine spontaneously released by isolated fat cells [20].

IAP was used here as a tool to investigate whether the inhibitory regulation of adenylate cyclase might be involved in the stimulation of glucose transport by the agents under investigation. The results showed that treatment of cells with IAP failed to alter the effect of insulin on glucose transport. Similarly, recent studies have reported that pretreatment of rats with *Bordetella* toxin failed to affect the action of insulin on glucose oxidation [21] as well as the antilipolytic effect [22,23]. However, the stimulatory effect of insulin on phosphatidylinositol turnover was enhanced by the treatment [21], indicating that dif-

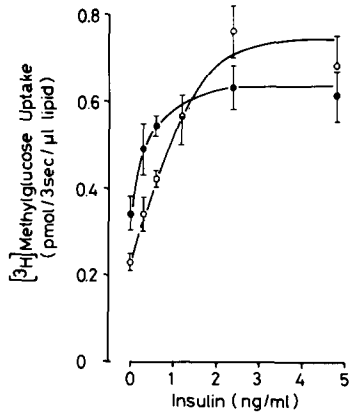

Fig.2. Effect of insulin on 3-*O*-methylglucose transport in the presence of isoprenaline. Means ± SE of 8 experiments. Isolated fat cells (approx. 10^5/ml) were incubated for 30 min at 37°C in the presence (●) and absence (○) of isoprenaline (1 μM). The cell suspension was concentrated, and insulin was added as indicated. After a second incubation period of 20 min the samples were equilibrated to 22°C and the transport rate was determined. Differences to controls were significant ($p < 0.05$) at 0, 0.6 and 2.4 ng/ml insulin.

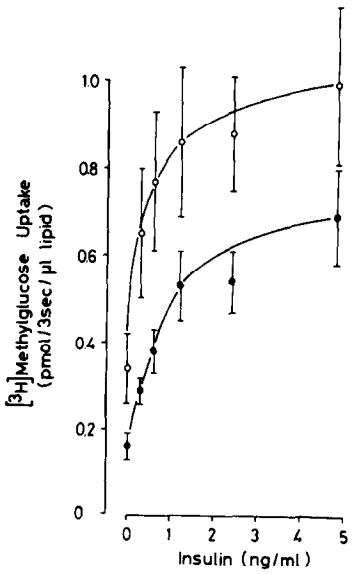

Fig.3. Effect of insulin and isoprenaline on 3-*O*-methylglucose transport in IAP-treated adipocytes. Means ± SE of 6 experiments. Isolated fat cells were incubated for 90 min at 37°C in the presence (●) and absence (○) of IAP (85 ng/ml). Isoprenaline (1 μM) was added 30 min prior to the end of the incubation. As in the experiments shown in fig.1 and 2, the fat cell suspension was concentrated and insulin was added as indicated. After further 20 min of incubation at 37°C the transport assay was performed as described in section 2. Differences to controls were significant ($p <$ 0.05) from 0 to 2.4 ng/ml insulin.

ferential mechanisms of insulin action might be revealed with the aid of IAP. The present results are consistent with the conclusion that the effect of insulin on glucose transport, like that on glucose metabolism and on lipolysis, is not mediated by the inhibitory regulation of adenylate cyclase.

In contrast to the effect of insulin, the stimulatory action of isoprenaline was abolished when cells were pretreated with IAP. Two explanations for this effect can be discussed. Firstly, the N_i component or an associated GTPase might mediate the stimulatory effect of isoprenaline on glucose transport. IAP has been reported to irreversibly block the membrane-bound GTPase [13], and evidence for a role of GTP in the regulation of glucose transport in muscle has previously been published [24]. However, this explanation of the stimulatory effect of isoprenaline is highly speculative, since it is based on the assumption that isoprenaline exerts a β-receptor-mediated action on the inhibitory component N_i, in addition to its stimulatory effect on adenylate cyclase via N_s.

Secondly, the stimulatory effect of isoprenaline might, if not mediated by the inhibitory regulation of adenylate cyclase, require a certain inhibition of the enzyme. Such an inhibitory effect is produced by adenosine which is spontaneously released by isolated fat cells during incubation [20]. Pretreatment of rats with IAP has been reported to block the effect of adenosine on adenylate cyclase and lipolysis in isolated fat cells [21]. This block of the hypothetical N_i component might produce a more pronounced response of adenylate cyclase to isoprenaline, thereby abolishing the stimulatory effect on glucose transport. In favour of the latter hypothesis is the finding that adenosine deaminase, like IAP, did inhibit the stimulatory effect of isoprenaline on glucose transport (table 1).

Stimulatory [8–10] as well as inhibitory effects [4,5] of isoprenaline on glucose transport have been reported by different groups. The present results suggest that these seemingly conflicting data reflect a dual action of catecholamines which might depend on the conditions of incubation applied. In our experiments the stimulatory effect of isoprenaline prevailed, provided adenosine was present in the incubation medium. When cells were pretreated with IAP, the inhibitory effect of isoprenaline on insulin-stimulated glucose transport was revealed. Thus the N_i component may be a pivotal link in the regulation of adipocyte glucose transport.

ACKNOWLEDGEMENTS

The authors are indebted to Dr K.H. Jakobs, Heidelberg, for a generous gift of purified IAP. The experiments are part of the MD-thesis of R.G.

REFERENCES

[1] Crofford, O.B. and Renold, A.E. (1965) J. Biol. Chem. 240, 14–21.
[2] Hepp, K.D. and Renner, R. (1972) FEBS Lett. 20, 191–194.
[3] Taylor, W.M. and Halperin, M.L. (1979) Biochem. J. 178, 381–389.
[4] Häring, H.U., Baumgarten, M., Deufel, T. and Kemmler, W. (1982) Diabetes 31, 479 (abstr.).

[5] Pessin, J.E., Gitomer, W., Oka, Y., Oppenheimer, C.L. and Czech, M.P. (1983) J. Biol. Chem. 258, 7386–7394.
[6] Jeanrenaud, B. (1961) Metabolism 10, 535–581.
[7] Luzio, J.P., Jones, R.C., Siddle, K. and Hales, C.N. (1974) Biochim. Biophys. Acta 362, 29–36.
[8] Ludvigsen, C., Jarett, L. and McDonald, J.M. (1980) Endocrinology 106, 786–790.
[9] Rasmussen, M.-J.K. and Clausen, T. (1982) Biochim. Biophys. Acta 693, 389–397.
[10] Göke, R. and Joost, H.G. (1983) Akt. Endokrinol. 4, 80 (abstr.).
[11] Hazeki, O. and Ui, M. (1981) J. Biol. Chem. 256, 2856–2862.
[12] Katada, T. and Ui, M. (1982) J. Biol. Chem. 257, 7210–7216.
[13] Aktories, K., Schultz, G. and Jakobs, K.H. (1983) FEBS Lett. 156, 88–92.
[14] Cowell, J.L., Sato, Y., Sato, H., An der Lan, B. and Manclark, C.R. (1982) in: Seminars in Infectious Disease (Weinstein, L. and Fields, B.N. eds) vol.4, pp.371–379, Georg Thieme, New York, Stuttgart.
[15] Rodbell, M. (1964) J. Biol. Chem. 239, 375–380.
[16] Joost, H.G. and Steinfelder, H.J. (1982) Mol. Pharmacol. 22, 614–618.
[17] Hirsch, J. and Gallian, E. (1968) J. Lipid Res. 9, 110–119.
[18] Gliemann, J., Østerlind, K., Vinten, J. and Gammeltoft, S. (1967) Biochim. Biophys. Acta 286, 1–6.
[19] Whitesell, R.R. and Gliemann, J. (1979) J. Biol. Chem. 254, 5276–5283.
[20] Schwabe, U., Ebert, R. and Erbler, H.C. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 276, 133–148.
[21] Moreno, F.J., Mills, J., García-Sáinz, J.A. and Fain, J.N. (1983) J. Biol. Chem. 258, 10938–10943.
[22] García-Sáinz, J.A. (1981) FEBS Lett. 126, 306–308.
[23] Kather, H., Aktories, K., Schulz, G. and Jakobs, K.H. (1983) FEBS Lett. 161, 149–152.
[24] Walaas, O., Walaas, E., Lystad, E., Alertsen, A.R. and Horn, R.S. (1979) Mol. Cell. Endocrinol. 16, 45–55.