Duality in the Mastoparan Action on Glucose Transport in Rat Adipocytes

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Abstract. Mastoparan, a tetradecapeptide purified from wasp venom, has been shown to stimulate glucose transport in rat adipocytes (Suzuki et al. Biochem Biophys Res Comm 189: 572–580, 1992) although the mechanism of its action has remained undefined. Here, we characterized the action of mastoparan on glucose transport in rat adipocytes. Mastoparan at a concentration of 20 μM or more caused a dose-dependent release of lactate dehydrogenase (LDH) from the cells, which closely correlated with its stimulatory effect on glucose uptake. The mastoparan-induced glucose uptake was inhibited neither by deprivation of ATP with KCN nor by addition of phloretin, a direct inhibitor of glucose transporter, suggesting that the ability of mastoparan to stimulate glucose uptake did not derive from activation of the glucose transport system (i.e., translocation or activation of GLUT4 and/or GLUT1). On the other hand, mastoparan at a lower concentration (15 μM or below), which showed an insignificant effect on LDH release, potentiated the insulin action on glucose transport and Akt phosphorylation in the presence of adenosine deaminase. The effect of mastoparan was not additive to that of phenylisopropyladenosine and was completely abolished by pretreatment of adipocytes with pertussis toxin (1 μg/ml for 2 hours). Thus, the present study disclosed duality in the action of mastoparan on glucose uptake in rat adipocytes. At a concentration of 15 μM or less, it enhances the insulin action on glucose transport by a pertussis toxin-sensitive Gi protein-dependent mechanism. At higher concentrations, however, mastoparan increases non-specific permeability of the plasma membrane, which causes LDH release as well as glucose uptake not mediated through glucose transporter.

Key words: Mastoparan, Adipocyte, Insulin, Glucose transport, GTP-binding protein

INSULIN stimulates glucose transport in adipocytes and skeletal/cardiac muscles mainly by promoting translocation of the glucose transporter isoform, GLUT4, from the intracellular compartments to the plasma membrane (for review see [1]). Although the molecular mechanism of insulin-induced GLUT4 translocation has yet to be fully defined, previous studies have indicated that heterotrimeric GTP-binding proteins are directly or indirectly involved in the insulin action on glucose transport. Firstly, while stimulation of Gs- or Gi-coupled receptors modulates insulin-stimulated glucose transport in a negative or positive fashion, respectively, without a significant change in the GLUT4 amount in the plasma membrane fraction in adipocytes [2], recent studies have demonstrated that these heterotrimeric GTP-binding proteins regulate the fusion of docked GLUT4-containing vesicles with the plasma membrane [3, 4]. Secondly, a heterodimeric phosphoinositide 3-kinase (PI 3-kinase1) isoform (p110β/p85) is synergistically activated by the βγ subunits of GTP-binding protein and a phosphotyrosyl peptide corresponding to the binding site of IRS-1 for the p85 regulatory subunit of PI 3-kinase [5]. Such a mechanism may be implicated in the potentiation by Gi-coupled receptor agonists such as adenosine of insulin-induced activation of Akt (protein kinase B) and glucose transport [6]. Thirdly, Goi2 was shown to be a negative regulator of protein-tyrosine phosphatase 1B...
Thus, activation of \(G_{\alpha_i2}\) causes suppression of PTP1B and mimics the insulin action on glucose transport and glycogen synthesis, whereas inhibition of \(G_{\alpha_i2}\) provokes insulin resistance by activation of PTP1B [8–10]. Finally, bradykinin was shown to trigger GLUT4 translocation via a Gq-dependent but PI 3-kinase-independent mechanism [11]. Subsequent studies demonstrated that the constitutively active mutant of Gq/G\(\alpha_11\) stimulates GLUT4 translocation and glucose transport whereas inactivation of Gq/G\(\alpha_11\) attenuates insulin-induced GLUT4 translocation [12, 13].

In an attempt to define the role of GTP-binding proteins in the insulin action, we previously reported that mastoparan, an amphiphilic tetradecapeptide from wasp venom, stimulates 3-O-methylglucose uptake in rat adipocytes [14] although the mode of action has yet to be characterized. Since mastoparan stimulates guanine nucleotide exchange by heterotrimeric GTP-binding proteins such as Gi and Go [15] and has been shown to promote exocytosis in a variety of cell types [16–21], one possibility is that mastoparan stimulates exocytic fusion of the GLUT4-containing vesicles with the plasma membrane by activation of a heterotrimeric GTP-binding protein(s). On the other hand, mastoparan shows diverse GTP-binding protein-independent actions, including activation of nucleoside diphosphate kinase [22], phospholipase C [23] and phospholipase D2 [24], and inactivation of ATP-sensitive potassium channels [25]. Furthermore, it has been shown that mastoparan possesses an activity to perturb membrane phospholipids resulting in an increase in membrane permeability independently of its action on GTP-binding proteins [26–29]. Thus it is also possible that mastoparan exerts its effect on glucose uptake through a GTP-binding protein-independent mechanism.

In the present study, we characterized the action of mastoparan on glucose transport in isolated rat adipocytes. Our data demonstrated duality in the mechanism of action of mastoparan on glucose transport. The action of mastoparan to stimulate glucose uptake, especially at 20 \(\mu\)M or more, derived mainly from its ability to increase non-specific permeability of the plasma membrane, but not from activation of the glucose transport system. On the other hand, mastoparan at 15 \(\mu\)M or below, which had an insignificant effect on membrane permeability, enhanced the insulin action on Akt phosphorylation and glucose transport by a pertussis-toxin sensitive mechanism in the absence of extracellular adenosine. In addition, our data also demonstrated that a pertussis-toxin-sensitive GTP-binding protein is not directly involved in the insulin action on glucose transport.

Materials and Methods

Materials

3-O-[\(^{3}\)H]Methyl-d-glucose was obtained from DuPont (Boston, MA). Mastoparan, Mas 7 and Mas 17 were purchased from Bachem (King of Prussia, PA) or Peptide Institute, Inc. (Osaka, Japan). Adenosine deaminase, wortmannin and pertussis toxin were from Sigma-Aldrich (St. Louis, MO). Wortmannin was dissolved to a concentration of 1 mM in dimethyl sulfoxide (DMSO). Phenylisopropyl adenosine (PIA) was from Wako Chemical (Kyoto, Japan). RHC 80267 was purchased from Biomol International (Plymouth Meeting, PA). Polyclonal antibodies against the C-terminal region of GLUT4 were raised in this laboratory as described previously [30]. Anti-phospho-Akt (Ser473) antibody was from Cell Signaling Technology, Inc. (Beverly, MA).

Preparation of rat adipose cells

Isolated adipocytes were prepared by the collagenase method from epididymal adipose tissues of Sprague-Dawley rats (from Charles-River, Japan, approximately 170–200 g) [31]. Unless otherwise specified, isolated cells were suspended in Buffer A (Krebs-Henseleit Hepes (25 mM) buffer, supplemented with 40 mg/ml bovine serum albumin (Fraction V) and 3 mM pyruvate, pH 7.4) or in high-K\(^+\)/low-Ca\(^{2+}\) buffer designated as Buffer X (118.0 mM KCl, 4.74 mM NaCl, 0.38 mM CaCl\(_2\), 1.0 mM EGTA, 1.18 mM MgSO\(_4\), 1.18 mM KH\(_2\)PO\(_4\), 23.4 mM Hepes/KOH, 40 mg/ml bovine serum albumin, 3 mM pyruvate, pH 7.4) [32].

Measurement of 3-O-methyl-d-glucose uptake

The cellular glucose transport activity was estimated by measuring the rate of 0.1 mM 3-O-methyl-d-glucose uptake by the oil-flotation method as described previously [32].
**Measurement of LDH release**

Adipocytes in Buffer A (cyclohex 0.213) were incubated for 15 minutes at 37°C. At the end of incubation, buffer was separated from the cells by centrifugation for 30 seconds at 1,000 × g. The LDH activity in the infranatant was assayed by using LDH-HA Test Wako assay kit (Wako Chemical, Kyoto).

**Phosphodiesterase assay**

Phosphodiesterase was assayed in the microsomal fraction of rat adipocytes as described previously [33].

**Lipolysis measurement**

Lipolysis, or free fatty acid production was followed by titrimetry as described previously [32, 34].

**Immunoblotting**

Adipocytes in Buffer X were homogenized in homogenizing buffer (50 mM HEPES/Na, pH 7.5, 100 mM KCl, 10% glycerol, 0.2 mM EDTA, 2 mM EGTA, 1 mM DTT, 1 μM microcystin-LR, 1 μg/ml peptatin A, 20 KIU/ml aprotinin, 1 μg/ml leupeptin, 0.2 mM phenylmethylsulfonyl fluoride, 10 mM NaF, 50 mM β-glycerophosphate). After centrifugation for 2 minutes at 3, 000 × g, the infranatant fraction below the fat cake was subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting with anti-phospho-Akt (Ser473) antibody. The immunoblots were visualized and quantified by using ECL system (Amersham Biosciences) and LAS-3000 luminescent image analyzer (Fuji Photo Film, Tokyo).

All the results reported herein were confirmed by repeating the experiments with different batches of adipocytes on different occasions.

**Results**

*Mastoparan stimulated glucose uptake by an ATP-independent mechanism*

As shown in Fig. 1, mastoparan stimulated 3-O-methylglucose uptake in a concentration-dependent manner. The dose-response curve of the effect of mastoparan was bell-shaped; that is, the stimulation of glucose uptake was maximal at 50 μM but it declined at a higher concentration. Mas7, a more potent mastoparan analogue on GTP-binding proteins [35], also showed a biphasic pattern of stimulation of glucose uptake. The maximal effect of Mas7 was obtained at a lower concentration (30 μM) than mastoparan although it was not greater than that of mastoparan. On the other hand, Mas17, an inactive analogue of mastoparan [35], was without effect on glucose uptake. Thus, the stimulatory effect of mastoparan analogues on glucose uptake correlated with their ability to activate GTP-binding proteins. However, it seems unlikely that mastoparan stimulated glucose transport by exocytotic recruitment of GLUT4 and/or GLUT1 to the plasma membrane because the mastoparan-stimulated glucose transport activity was not affected by deprivation of metabolic energy with KCN to arrest the subcellular vesicle trafficking, whereas KCN treatment completely inhibited the effect of insulin (Fig. 2).

**Effect of mastoparan on LDH release from rat adipocytes**

In addition to the effect on GTP-binding proteins, mastoparan also have an activity to perturb membrane phospholipids leading to an increase in membrane permeability [26–29], which would increase glucose uptake not mediated through glucose transporter proteins.
To investigate the effect of mastoparan on membrane permeability, we measured the release of lactate dehydrogenase (LDH) from adipocytes. As shown in Fig. 3, a significant amount of LDH (23% of the total cellular content) was present in the incubation buffer even in the basal state, suggesting that isolated rat adipocytes are rather fragile under the experimental conditions, with a spontaneous release of LDH during preparation of the cells. Addition of mastoparan caused a further release of LDH in a concentration-dependent manner, indicating that the peptide increased permeability of the plasma membrane to macromolecules. The stimulation of LDH release was significant with mastoparan at $20 \mu M$ and more ($p<0.05$). While the effect of mastoparan on glucose uptake was maximal at $50 \mu M$ (see Fig. 1), mastoparan at this concentration elicited a release of 84.7% of total LDH. Mas7 was more potent in LDH release than mastoparan, whereas Mas17 was without effect (Fig. 3). Importantly, the effects of mastoparan and its analogues on LDH release closely correlated with their activity to stimulate glucose uptake. As almost whole cellular LDH was released with mastoparan at $100 \mu M$ or Mas7 at $50 \mu M$ and more, the decline in their effects on glucose uptake at such high concentrations (see Fig. 1) was probably brought about by cell damage associated with membrane perturbation. These findings raised the possibility that mastoparan enhances glucose uptake by increasing non-specific membrane permeability but not by activation of the glucose transport system, i.e., by promotion of translocation of GLUT4 and/or GLUT1 or by activation of the intrinsic transporter activity of these proteins.

Mastoparan-stimulated glucose uptake was not mediated through glucose transporter

To test this possibility, we examined the effect of phloretin, a direct inhibitor of glucose transporter, on the mastoparan-stimulated glucose transport activity. As shown in Fig. 4, phloretin completely blocked the glucose uptake stimulated with insulin, indicating that insulin-induced glucose uptake was mediated mainly through glucose transporter proteins (GLUT4 and GLUT1). By contrast, phloretin had little effect on the glucose uptake stimulated with $50 \mu M$ of mastoparan, suggesting that mastoparan stimulated glucose uptake by a mechanism unrelated to glucose transporter proteins. These findings were consistent with the notion that mastoparan especially at higher concentrations (20 $\mu M$ and more) stimulated glucose uptake by increasing permeability of the plasma membrane.
Effects of adenosine deaminase on the insulin action on glucose transport and Akt

Evidence gathered over the past two decades suggests that adenosine, a nucleoside released by adipocytes, influences the insulin sensitivity by acting as an extracellular humoral signal. Thus, adenosine deaminase has been shown to impair the insulin sensitivity for glucose transport by inactivating extracellular adenosine [36, 37]. The sensitivity can be restored by treatment with an adenosine analogue phenylisopropyl-adenosine (PIA) [2]. Adenosine exerts its effect through the adenosine A1 receptor coupled to a pertussis toxin-sensitive Gi protein. Because mastoparan also stimulates GDP/GTP exchange by Gi, we next studied whether mastoparan, like adenosine, affects the insulin sensitivity for glucose transport. To this end, we used mastoparan at a concentration that does not induce LDH release. In addition, to minimize the effect of membrane permeabilization, we examined the effect of mastoparan in cells suspended in a high-K+/low-Ca\(^{2+}\) buffer (designated as Buffer X, see Materials and Methods) because, as we previously reported, the insulin effect is profoundly reduced by membrane permeabilization in cells suspended in Buffer A (Krebs-Henseleit Hepes buffer) but not in Buffer X [32].

As shown in Fig. 5A, the addition of adenosine deaminase (2 units/ml) caused a rightward shift of the dose-response curve of the insulin effect on glucose transport without a significant change in the maximal effect, suggesting that endogenous adenosine enhances
the apparent insulin sensitivity for glucose transport under the experimental conditions. We next investigated whether this attenuation of the insulin action derived from inhibition of insulin signaling. Treatment with adenosine deaminase inhibited insulin-induced Akt phosphorylation by more than 50% (Fig. 5, B and C), in agreement with the observation by other investigators that adenosine deaminase treatment caused a significant inhibition of the insulin action of PI 3-kinase [6]. Interestingly, while adenosine deaminase mainly affected the insulin sensitivity for glucose transport without affecting the maximal effect, it impaired the maximal effect of insulin on Akt phosphorylation. The reason for the differential effects of adenosine deaminase on the insulin action on glucose transport and Akt is unclear from the present study (see Discussion below).

**Mastoparan restored the insulin action by activation of a pertussis-toxin-sensitive GTP-binding protein**

In the next series of experiments, we investigated whether mastoparan would fully restore the insulin action attenuated with adenosine deaminase, and whether mastoparan, like adenosine, exerts the effect by activation of a pertussis toxin-sensitive GTP-binding protein(s). Effectiveness of pertussis toxin treatment was monitored by lipolytic activity in adipocytes. Previous studies have shown that pertussis toxin treatment markedly promotes lipolysis in adipocytes by blocking the tonic inhibition of adenylate cyclase with Gi-coupled receptor agonists (e.g. adenosine) [38]. As shown in Fig. 6A, treatment of isolated adipocytes with 1 μg/ml of pertussis toxin caused lipolysis after about 1 hour of delay, indicating that the toxin effectively inactivated the ability of Gi protein to interact with the adenosine A1 receptor. Under the experimental conditions, however, pertussis toxin-induced lipolysis reached a plateau at 3 hours of incubation, probably due to oversaturation of extracellular albumin with released free fatty acid, which would lead to reduction of ATP levels [39]. To eliminate this adverse effect, we treated adipocytes with pertussis toxin in the presence of RHC 80267, an inhibitor of diacylglycerol lipase [40]. Addition of 20 μM RHC 80267 markedly inhibited the pertussis toxin-evoked lipolysis and prevented oversaturation of extracellular albumin with free fatty acid (Fig. 6A). The importance of such circumvention of oversaturation of albumin with free fatty acid was shown by the observation that the insulin stimulation of cyclic AMP phosphodiesterase was not affected in cells treated with pertussis toxin for 3 hours in the presence of RHC 80267, whereas it was profoundly inhibited without the inhibitor (Fig. 6B). According to these findings, we treated cells with 1 μg/ml of pertussis toxin for 2 hours in the presence of RHC 80267 (20 μM) in the following experiments.

As shown in Fig. 7, the glucose transport activity

![Fig. 6. Effect of pertussis toxin treatment on lipolysis and the insulin action on phosphodiesterase. A, Aliquots of pooled adipocytes were suspended in Buffer X, and were incubated with pertussis toxin (1 μg/ml) in the absence (open circle) or presence (closed triangle) of 20 μM RHC 80267 at 37°C. At the indicated time points, the free fatty acid accumulated was assayed by titrime-try. Each point indicates the mean of duplicate determinations. B, Adipocytes in Buffer X were incubated without or with pertussis toxin (1 μg/ml) for 3 hours at 37°C in the absence or presence of 20 μM RHC 80267. Then cells were divided into a number of aliquots and were stimulated without or with 10 nM insulin for 15 minutes. At the end of incubation, the phosphodiesterase activity was assayed as described under Materials and Methods. Results are expressed as mean ± S.E.M. (n = 3–6).](image-url)
stimulated with 10 nM of insulin was attenuated by 42% in the presence of adenosine deaminase (2 units/ml), which was fully restored by the addition of 15 μM of mastoparan to the level equivalent to that with PIA. There was no additivity between the effects of mastoparan and PIA. Pertussis toxin treatment (1 μg/ml, 2 hours) itself reduced the insulin-stimulated glucose transport to the level comparable to that with insulin plus adenosine deaminase in the control cells, probably due to inactivation of the Gi-coupled adenosine A1 receptor-mediated signaling. The addition of adenosine deaminase did not further inhibit the insulin effect, and the stimulatory effects of mastoparan or PIA were abolished in pertussis toxin-treated cells. Likewise, the impaired Akt phosphorylation in the presence of adenosine deaminase was reversed with mastoparan or PIA to the level comparable to that with insulin alone (Fig. 8). Pertussis toxin treatment reduced the insulin-stimulated Akt phosphorylation to the level with insulin plus adenosine deaminase in the control cells. The effect of mastoparan or PIA to enhance the insulin-stimulated Akt phosphorylation was completely abolished in pertussis toxin-treated cells. These results suggest that, in the absence of extracellular adenosine, mastoparan potentiated the insulin action on Akt and glucose transport by activation of a pertussis toxin-sensitive GTP-binding protein. In addition, these data are consistent with the notion that the signals originated from the insulin receptor and the Gi-coupled adenosine A1 receptor synergistically activate PI 3-kinase and its downstream effector Akt, leading to stimulation of glucose transport. Furthermore, our data indicated that pertussis toxin-sensitive GTP-binding proteins are not directly involved in the insulin signal to stimulate glucose transport and cyclic AMP phosphodiesterase (see Discussion below).

**Discussion**

The present data disclosed duality in the action of mastoparan on glucose transport in isolated rat adipocytes. Although we previously reported that mastoparan stimulates glucose uptake in isolated adipocytes, this action of mastoparan, as shown in the first part of this study, did not derive from activation of the glucose transport system (i.e. translocation or activation of glucose transporter protein(s)). First, mastoparan at a concentration of 20 μM or higher caused a significant increase in permeability of the plasma membrane, leading to LDH release in a dose-dependent manner. This mastoparan-evoked LDH release was closely corre-
lated with its ability to stimulate 3-O-methylglucose uptake. Additionally, the Mas7-induced glucose uptake also was correlated with LDH release whereas Mas17, which was inactive in LDH release, did not stimulate glucose uptake. Second, the glucose transport activity stimulated with 50 μM of mastoparan was not affected by deprivation of metabolic energy with KCN to arrest subcellular vesicle trafficking, indicating that the mastoparan action is unrelated to GLUT4 or GLUT1 translocation. Finally, the mastoparan-stimulated glucose uptake was not inhibited with phloretin, a direct inhibitor of glucose transporter, which completely inhibited the insulin-stimulated glucose transport, suggesting that mastoparan-induced glucose uptake was not mediated through phloretin-sensitive glucose transporter proteins including GLUT1 and GLUT4. Thus, these results are consistent with the notion that mastoparan enhanced glucose uptake by an increase in permeability of the plasma membrane derived from its ability to perturb membrane phospholipids.

On the other hand, mastoparan at a lower concentration (15 μM or less), which showed a minor effect on glucose transport or LDH release, significantly enhanced the insulin action on Akt phosphorylation and glucose transport in the absence of extracellular adenosine. Since these effects of mastoparan were completely abolished by treatment of the cells with pertussis toxin, mastoparan enhanced the insulin action by activation of a pertussis toxin-sensitive GTP-binding protein(s). Although mastoparan activates guanine nucleotide exchange by Gi and Go, both of which are the substrates of pertussis toxin, the major target of mastoparan action seems to be Gi, because (a) rat adipocytes does not express a pertussis toxin substrate with a molecular size of 39 kDa corresponding to that of brain Goz [41, 42], (b) pertussis toxin-treatment itself attenuated the insulin action on glucose transport to the level comparable to that with insulin plus adenosine deaminase in control cells, and (c) there was no additivity between the effects of mastoparan and PIA, an agonist for the Gi-coupled adenosine A1 receptor. Thus, mastoparan seems to mimic the action of endogenous adenosine by activation of a pertussis toxin-sensitive GTP-binding protein, probably Gi.

While several lines of evidence have indicated that heterotrimeric GTP-binding proteins are directly or indirectly involved in the insulin action (see Introduction), our findings suggest that a pertussis toxin-sensitive GTP-binding protein(s) is not directly involved in the acute insulin action on glucose transport because insulin was able to stimulate glucose transport in pertussis toxin-treated cells to the level not less than that with insulin plus adenosine deaminase in control cells (Fig. 7). Thus, pertussis toxin modifies the insulin action on glucose transport by eliminating the Gi-mediated effect of extracellular adenosine, but does not inhibit the effect of insulin itself.

Although the precise mechanism of Gi-mediated potentiation of the insulin action on glucose transport has yet to be fully elucidated, recent studies have led us to a better understanding of the mechanism of modulation of the insulin action by heterotrimeric GTP-binding proteins. Previous studies indicated that the effect of adenosine is independent of changes in cyclic AMP level or cyclic AMP-dependent protein kinase activity [2, 43], and that it is not associated with a significant change in the amount of glucose transporter in the plasma membrane fraction [2]. While the latter finding was initially interpreted to suggest that the Gi-mediated signal modulates the intrinsic activity of the glucose transporter present in the plasma membrane, more recent works have shown that agonists for Gs- or Gi-coupled receptors regulate insulin-stimulated glucose transport by influencing the rate of fusion of docked GLUT4-containing vesicles with the plasma membrane [3, 4]. On the other hand, another series of studies has demonstrated synergistic activation of heterodimeric PI 3-kinase consisting of p85 and p110β by the βγ subunits of G proteins and phosphotyrosyl peptide [5, 6, 44], which would be implicated in the in vivo enhancement by adenosine of insulin-stimulated glucose transport [6]. These studies also suggested that the Gβγ-sensitive p85/p110β isoform of PI 3-kinase, rather than the p85/p110α one, would play a major role in the insulin action on glucose transport. Unfortunately, the former series of studies did not measure PI 3-kinase activity while the latter did not look at GLUT4 and/or GLUT1 translocation. Thus, it is unclear whether these two mechanisms are interrelated or not.

Although our data that adenosine deaminase considerably attenuated the insulin-induced Akt phosphorylation (Fig. 5, B and C) was in agreement with the second mechanism, PI 3-kinase (p85/p110β) may not be the only target of Gi protein, because adenosine deaminase inhibited the insulin-stimulated Akt phosphorylation by more than 50%, whereas it only caused a rightward
shift of the dose-response curve of the insulin effect on glucose transport with a minor change in the maximal effect (Fig. 5A). The reason for such differential effects of adenosine deaminase on the insulin-stimulated Akt phosphorylation and glucose transport is unclear. One possibility is that, as shown by Takasuga et al. [6], the relationship between PI 3-kinase activity and glucose transport is not linear, and glucose transport is maximally stimulated with 30–50% activation of PI 3-kinase. Thus, the attenuation of glucose transport by PI 3-kinase inhibition would be more prominent with submaximal stimulation of glucose transport. Alternatively, it is also possible that insulin stimulation of glucose transport requires activation of two distinct signaling pathways, and that the attenuated PI 3-kinase activity would be compensated by a PI 3-kinase-independent signal(s) with a supermaximal concentration (i.e. 100 nM under our experimental conditions) of insulin. In this regard, recent observations have indicated that insulin-induced translocation and activation of GLUT4 is regulated by separate signals [45, 46].

Further studies will be needed to clarify the mechanism of modulation by GTP-binding proteins of the insulin action on glucose transport.

In summary, the present study showed that mastoparan has GTP-binding protein-dependent and independent effects on glucose uptake in rat adipocytes. At a concentration of 15 μM or less, it enhances the insulin action on glucose transport by activation of a pertussis toxin-sensitive GTP-binding protein. At higher concentrations, however, mastoparan increases non-specific permeability of the plasma membrane, which causes LDH release as well as glucose uptake not mediated through glucose transporter.

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