0013-7227/04/$15.00/0
Printed in U.S.A.

Endocrinology 145(11):4985–4990

doi: 10.1210/en.2004-0764

Grape Seed-Derived Procyanidins Have an Antihyperglycemic Effect in Streptozotocin-Induced Diabetic Rats and Insulinomimetic Activity in Insulin-Sensitive Cell Lines

M. PINENT, M. BLAY, M. C. BLADÉ, M. J. SALVADÓ, L. AROLA, AND A. ARDÉVOL

Department of Biochemistry and Biotechnology, Rovira i Virgili University, 43005 Tarragona, Spain

Flavonoids are functional constituents of many fruits and vegetables. Some flavonoids have antidiabetic properties because they improve altered glucose and oxidative metabolisms of diabetic states. Procyanidins are flavonoids with an oligomeric structure, and it has been shown that they can improve the pathological oxidative state of a diabetic situation. To evaluate their effects on glucose metabolism, we administered an extract of grape seed procyanidins (PE) orally to streptozotocin-induced diabetic rats. This had an antihyperglycemic effect, which was significantly increased if PE administration was accompanied by a low insulin dose. The antihyperglycemic effect of PE may be partially due to the insulinomimetic activity of procyanidins on insulin-sensitive cell lines. PE stimulated glucose uptake in L6E9 myotubes and 3T3-L1 adipocytes in a dose-dependent manner. Like insulin action, the effect of PE on glucose uptake was sensitive to wortmannin, an inhibitor of phosphoinositol 3-kinase and to SB203580, an inhibitor of p38 MAPK. PE action also stimulated glucose transporter-4 translocation to the plasma membrane. In summary, procyanidins have insulin-like effects in insulin-sensitive cells that could help to explain their antihyperglycemic effect *in vivo*. These effects must be added to their antioxidant activity to explain why they can improve diabetic situations. (*Endocrinology* 145: 4985–4990, 2004)

FLAVONOIDS ARE PHENOLIC compounds that are widely found in fruits and vegetables (1). They have a broad range of biological activities (2–5). They function as powerful antioxidants, as phytoestrogens, and can alter the activities of important cell-signaling enzymes, such as tyrosine kinases, phosphodiesterases, and phosphoinositide kinases (6). Some may also have antidiabetic activity (7–10). Studies of the *in vivo* and *in vitro* effects of various flavonoids on glucose metabolism have shown opposite and often controversial results. This is probably because of the different structural characteristics of the molecules and the different experimental designs used (11–13). Procyanidins, a group of flavonoids, are oligomeric forms of catechins that are abundant in red wine, grapes, cocoa, and apples (1). It has been shown that they have a role in protecting against the altered oxidative state of diabetic situations (14–17). The only reported information about how they affect glucose metabolism derives from the recent work of Al-awwadi and colleagues (18) that describes an antidiabetic activity of a red wine polyphenolic extract in streptozotocin (STZ)-treated rats.

Hyperglycemia is characteristic of type I and type II diabetes mellitus, which is mainly caused by insulin deficiency and/or a failure of normal insulin levels to stimulate glucose uptake in tissues. Although defects in glucose homeostasis have been recognized for decades, the molecular mechanisms involved in impaired whole body glucose uptake are still not understood. It is now clear, however, that the aggressive control of hyperglycemia can attenuate the development of chronic complications. Significant progress has been made in identifying the molecular components and signaling pathways involved in the short-term regulation of glucose uptake (19, 20). Insulin binds to the cell surface insulin receptor and activates its intrinsic tyrosine kinase activity. The subsequent activation of phosphatidylinositol 3-kinase (PI3K) is necessary for the recruitment of glucose transporter-4 (GLUT-4) to the cell surface. In addition, emerging evidence suggests that a second signaling cascade, which functions independently of the PI3K pathway, is required for the insulin-dependent translocation of GLUT-4 (21, 22). However, GLUT-4 translocation to the plasma membrane does not account for all the increase in glucose uptake due to insulin stimulation. It has recently been shown that insulin also stimulates GLUT-4 activation through p38 MAPK signaling (23).

A suitable antidiabetic agent should have actions similar to those of insulin, or it should bypass the defects in insulin action characterized by insulin resistance. In this report we assess how effective a grape seed-derived procyanidin is at lowering hyperglycemia in an insulin-deficient rat model of diabetes: the STZ-induced diabetic rat. To analyze the biochemical mechanism of this postulated effect, we evaluate the insulinomimetic activity of this grape seed-derived procyanidin working with two cell culture lines of insulin-sensitive tissues: L6E9 myotubes and 3T3-L1 adipocytes.

Abbreviations: FBS, Fetal bovine serum; GLUT-4, glucose transporter-4; LDM, low density microsome; PE, grape seed procyanidin extract; PI3K, phosphatidylinositol 3-kinase; PM, plasma membrane; STZ, streptozotocin.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Materials and Methods

Cells, reagents, and materials

Grape seed procyanidin extract (PE) was provided by Les Dérives Résiniques et Terpéniques (Dax, France). According to the manufacturer, this PE extract contained essentially monomeric (21.3%), dimeric (17.4%), trimeric (16.3%), tetrameric (13.3%), and oligomeric (5–13 U; 31.7%) procyanidins.

Cell culture reagents were obtained from BioWhittaker (Verviers, Belgium), and SB203580 was purchased from Calbiochem (Merck, Darmstadt, Germany). Insulin (Actrapid) was obtained from Novo Nordisk (Bagsvaerd, Denmark). Bradford protein reagent was obtained from Bio-Rad Laboratories (Hercules, CA). L6E9 cells and anti-GLUT-4 antibody were provided by Dr. Marta Camps and Prof. Antonio Zorzano (University of Barcelona, Barcelona, Spain), and antirabbit peroxidase-conjugated antibody, wortmannin, and STZ were obtained from Sigma-Aldrich Corp. (St. Louis, MO). 2-Deoxy-D-[^{3}H]glucose and enhanced chemiluminescence detection reagent were purchased from Amersham Biosciences (Little Chalfont, UK).

Induction of experimental diabetes

Male Wistar rats, weighing 250 g, were purchased from Charles River Laboratories (Barcelona, Spain). The animals were housed in animal quarters at 22 C with a 12-h light, 12-h dark cycle and were fed *ad libitum*. All procedures were approved by the animal ethics committee of our university. Type 1 diabetes mellitus was induced by ip injection of a freshly prepared solution of STZ (70 mg/kg) in 50 mM citrate buffer, pH 4.5. The only diabetic animals used were those with polyuria, glycosuria, and hyperglycemia (~20 mM) 2–3 d postinduction. All studies were carried out 1 wk after STZ had been injected.

Animal experimental procedures

STZ-diabetic rats were divided into five groups of six or seven rats each. In the control group rats were fed an oral gavage with vehicle (tap water). In the PE group rats were given an oral gavage of PE in aqueous solution (250 mg/kg body weight). In the insulin group rats were given 9 nmol/animal insulin, ip, and fed an oral gavage with tap water. In the low insulin group rats were given 1.26 nmol/animal insulin, ip, and fed an oral gavage with tap water. In the PE plus low insulin group rats were given 1.26 nmol/animal insulin, ip, and fed an oral gavage with PE (250 mg/kg body weight).

At 1100 h, blood glucose levels were measured, and then (time zero) the respective treatments were administered. Blood samples were collected by tail bleeding, and glucose was measured at 30, 60, 90, 120, 150, 210, and 270 min.

An additional experiment was performed with the same experimental model, but only with fasted animals from the control and PE groups. Food was withdrawn at 0700 h, and at 1400 h (time zero), a blood sample was collected by tail bleeding. The respective treatments were then administered. Next, blood was again extracted after 60 min. Blood glucose was determined with a glucometer (Glucocard, Menarini, Barcelona, Spain).

Cell culture and differentiation

L6E9 myoblasts were cultured in supplemented DMEM [10% (vol/vol) fetal bovine serum (FBS), 1% penicillin/streptomycin, 2 mM glutamine, and 25 mM HEPES (pH 7.4)]. Preconfluent myoblasts (80–90%) were induced to differentiate by lowering FBS to a final concentration of 2% (vol/vol).

3T3-L1 preadipocytes were cultured and induced to differentiate as previously described (24). Briefly, confluent preadipocytes were treated with 0.25 μM dexamethasone, 0.5 mM 3-isobutylmethylxanthine, and 5 μg/ml insulin for 2 d in 10% FBS containing DMEM. Cells were switched to 10% FBS/DMEM containing only insulin for 2 d, then to 10% FBS/DMEM without insulin. Ten days after differentiation had been induced, cells were treated with PE and used for the experiments.

Cell treatment

Fully differentiated adipocytes and myotubes were washed twice with PBS and incubated at 37 C with serum-free, supplemented DMEM containing 0.2% BSA (depletion medium) for 2 and 5 h, respectively. Acute treatment with PE and/or insulin was carried out during the last 30 or 60 min of depletion in adipocytes and myotubes, respectively. All PE concentrations assayed were nontoxic for both 3T3-L1 adipocytes (24) and L6E9 myotubes (25).

Glucose uptake assay

Glucose transport was determined by measuring the uptake of 2-deoxy-D-[^{3}H]glucose. Cells were cultured on six- or 12-well plates. Transport assay was initiated by washing the cells twice in a transport solution (20 mM HEPES, 137 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, and 2 mM pyruvate, pH 7.4). Cells (myotubes and adipocytes) were then incubated for 7–10 min in the transport solution, which contained 0.1 mM 2-deoxy-D-glucose and 1 μCi 2-deoxy-D-[^{3}H]glucose (10 mCi/mmol). Glucose uptake was stopped by adding 2 vol ice-cold 50 mM glucose in PBS and washing twice in the same solution. Cells were disrupted by adding 0.1 M NaOH/0.1% PBS, and radioactivity was determined by scintillation counting (Packard 1500 Tri-Cab, Izasa SA, Madrid, Spain). Glucose transport values were corrected for protein content, which was determined by the Bradford method (26). Each condition was run in triplicate.

To determine whether PI3K and p38 MAPK were involved in the signaling pathways that were potentially used by PE, we performed assays using specific inhibitors of these kinases. Wortmannin (1 μM) and SB 203580 (100 μM) were added 15 and 20 min, respectively, before PE and insulin treatments. Glucose uptake was then assayed.

Cell fractionation method

To obtain plasma membranes (PM) and low density microsomes (LDM), cells were grown on 75-cm^2 flasks and treated as described above. Then a subcellular fractionation was carried out with slight variations to the method used by Simpson and colleagues (27). Briefly, cells were homogenized, and the homogenate was centrifuged for 20 min at 15,800 $\times$ *g* to pellet the PM fraction. The supernatant was centrifuged for 75 min at 180,000 $\times$ *g* to pellet the LDM fraction. The fractions were then resuspended in HES (20 mM HEPES, 1 mM EDTA, and 255 mM sucrose, pH 7.4) and subjected to Western blotting analysis.

Electrophoresis and immunoblot analysis

Aliquots of fractions (PM and LDM) were resolved on 7.5% SDS-PAGE and transferred to nitrocellulose membranes, and the membranes were blocked with PBS 5% low-fat dry milk. Incubation with the primary antibody (anti-GLUT-4; 1:1000 dilution) was performed overnight. Membranes were washed in PBS/0.1% Tween and incubated with the peroxidase-conjugated secondary antibody (1:1000 dilution) for 2 h. Then they were washed again in PBS/0.1% Tween. Bands were visualized with enhanced chemiluminescence detection reagents.

Calculations and statistical analysis

Results are expressed as the mean $\pm$ SEM. Effects were assessed using one-way ANOVA or *t* test. We used Tukey's test of honestly significant differences to make pairwise comparisons. All calculations were performed using SPSS software (SPSS, Inc., Chicago, IL).

Results

Acute administration of procyanidins reduced hyperglycemia in STZ-induced diabetic rats

To assess the potential role of procyanidins in improving hyperglycemia, we used an animal model in which there is no, or very little, insulin secretion: STZ-induced diabetic rats. Figure 1A shows that an acute gavage of PE (250 mg PE/kg body weight) significantly reduced blood glucose levels. Although we started our experiment 2 h after the light period had begun, the control group showed that there was some intestinal glucose absorption. To ascertain whether the inhibition of glucose absorption in the intestine was not the

only thing to mediate the glycemia-lowering effect of PE, we administered the same dose of PE to fasted animals. In this case, Table 1 shows that 1 h after PE treatment, blood glucose levels also significantly decreased ($P = 0.004$) *vs.* those in the control group. Figure 1B shows that simultaneous administration of a low dose of insulin (1.26 nmol) and PE caused an additive effect, but it was not as high as that of an effective insulin dose (9 nmol). Table 2 summarizes the ANOVA results of all treatments on nonfasted animals and shows that the PE effect was greater than that caused by a low dose of insulin.

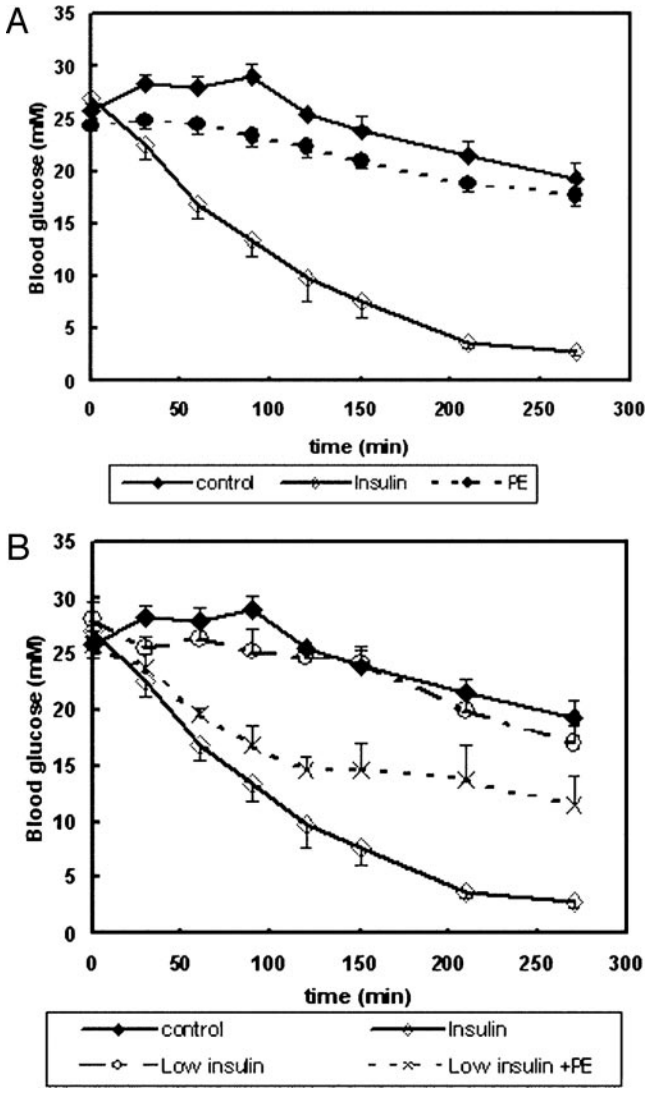

FIG. 1. Effect of PE treatments on glucose levels in STZ-induced diabetic rats. At 1100 h (time zero), a blood sample was collected by tail bleeding, and respective treatments were administered. Blood samples were extracted at the times indicated in the figure. Glucose levels were quantified by a glucometer. The data are the mean ± SEM of six animals. A, Effect of PE on glucose levels. B, Additive effect of a simultaneous treatment with PE plus a low dose of insulin. Table 2 summarizes the ANOVA results of the overall effect of the treatments shown in A and B. The *different letters* indicate statistically significant differences between treatments.

TABLE 1. Changes in blood glucose levels in fasted animals due to procyanidin treatment

	Blood glucose (mM)		
	0 h	1 h	Decrease
Control	19.31 ± 0.64	20.61 ± 1.40	1.31 ± 0.72
PE	19.65 ± 0.70	18.26 ± 1.12	−1.39 ± 0.52[a]

At 0700 h, food was removed from animal cages. At 1400 h (time zero), a blood sample was collected by tail bleeding, and respective treatments were administered. After 1 h, blood glucose was measured. The data are the mean ± SEM of six animals.

[a] Significant differences *vs.* the control group at $P < 0.05$.

TABLE 2. ANOVA results of all treatments on nonfasted animals

Treatment	Control	Low insulin	PE	Low insulin + PE	Insulin
ANOVA result	a	a, b	b	c	d

Different letters indicate statistically significant differences between treatments.

Procyanidins increase glucose uptake in insulin-sensitive cell lines (L6E9 myotubes and 3T3-L1 adipocytes)

To determine whether the effects of procyanidins *in vivo* were due to their action on insulin-sensitive tissues, we used the differentiated cell lines L6E9 myotubes and 3T3-L1 adipocytes. Figure 2 shows that PE treatment caused a dose-dependent increase in glucose uptake in both cell lines. These dose-response effects showed some differences between myotubes and adipocytes. L6E9 myotubes, at 0.14 mg PE/liter, increased their glucose uptake *vs.* untreated cells, and their maximal stimulation was 1.6-fold greater than basal rates. 3T3-L1 adipocytes, however, needed a higher concentration of PE to stimulate their glucose uptake (35 mg PE/liter), but at their maximal nontoxic PE concentration (140 mg PE/liter), its stimulation was stronger (7-fold above basal). Nevertheless, in both cell lines the effect of PE on glucose uptake at the maximal doses assayed was 50% that of insulin (55.3 ± 13.85% for L6E9; 45.32 ± 3.35% for 3T3-L1). To evaluate the effects of simultaneous administration of PE and insulin, we incubated adipocytes with a low dose of insulin (10 nM) and several doses of PE. Figure 3 shows that the presence of PE in the cell culture medium boosted the effect of a low insulin concentration. In 3T3-L1 adipocytes, 7 mg/liter of PE, a nonstimulating dose by itself, together with a low insulin dose (10 nM) almost doubled the capacity, induced by insulin in the cell, to capture glucose.

Procyanidin-induced increase in glucose uptake shares some mediators of insulin mechanism of action

Previous results show that procyanidins have an insulin-like effect. To elucidate the mechanism by which PE stimulates glucose uptake, we attempted to determine whether PE shared some of main cellular mediators of insulin signaling. Firstly, the combination of the highest stimulating concentration of PE and a supramaximal insulin concentration did not cause an additive stimulation of glucose transport in myotubes (Table 3). In adipocytes, there was some additive effect, although it did not reach the total sum of both the independent effects (Table 3). To determine whether the effects of PE on glucose transport required intact PI3K activity, wortmannin was added before PE to the cell culture

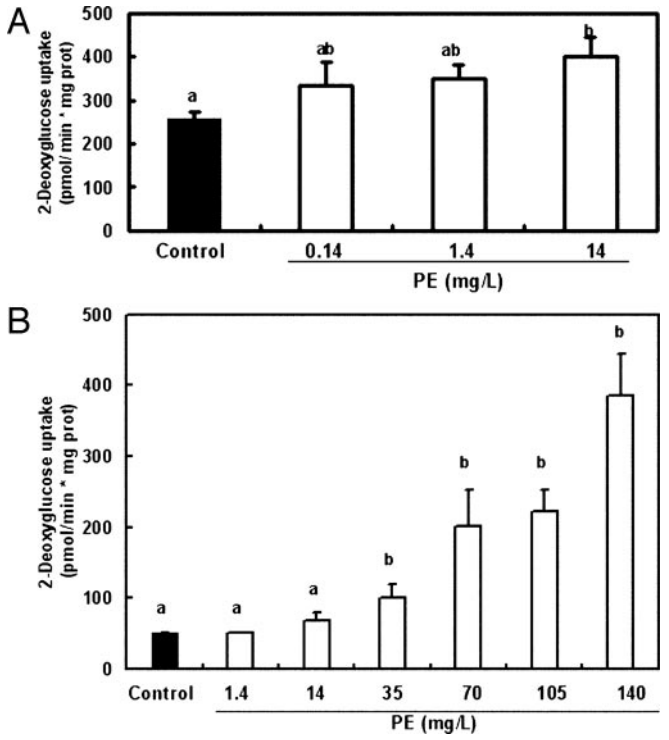

FIG. 2. Dose-response effects on glucose uptake due to PE treatment in L6E9 myotubes and 3T3-L1 adipocytes. Fully differentiated myotubes (A) and adipocytes (B) were incubated with serum-free medium supplemented DMEM containing 0.2% BSA for 5 and 2 h, respectively. Acute treatment with several doses of PE was carried out during the last 60 or 30 min of depletion in myotubes and adipocytes, respectively. At the end of the treatment, 2-deoxyglucose uptake was assayed. The data are the mean ± SEM of at least three different experiments. a and b, Statistically significant differences between PE concentrations.

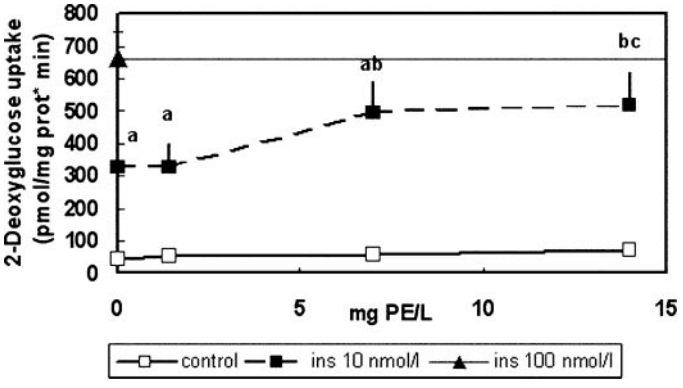

FIG. 3. Dose-response effect on glucose uptake due to simultaneous treatment with a low dose of insulin plus PE in 3T3-L1 adipocytes. Fully differentiated adipocytes were serum-depleted as indicated in Fig. 2. Simultaneous treatment with several doses of PE with or without 10 nM insulin was applied during the last 30 min of depletion. Cells treated with 100 nM insulin were used as a positive control. At the end of the treatment, the 2-deoxyglucose uptake was assayed. The data are the mean ± SEM of three different experiments. a, b, and c, Statistically significant differences between PE concentrations.

medium. Figure 4 shows that PE-induced glucose transport was inhibited by wortmannin in both cell lines. As positive control, we simultaneously treated 3T3-L1 adipocytes and

TABLE 3. Effects due to simultaneous treatment with high doses of PE and insulin in L6E9 myotubes and 3T3-L1 adipocytes

	L6E9	3T3-L1
Insulin	100	100
PE	43.18 ± 29.97	45.32 ± 3.35
Insulin + PE	113.7 ± 10.41	121.9 ± 6.23[a]

Fully differentiated myotubes and adipocytes were serum-depleted as described in Fig. 2. They were treated with their respectives high doses of PE (L6E9, 14 mg/liter; 3T3-L1, 140 mg/liter), without or with insulin 1 μmol/liter, during the last 60 or 30 min of depletion in myotubes and adipocytes, respectively. At the end of treatment, 2-deoxyglucose uptake was assayed. Results are expressed as the percentage of the insulin effect (100%). The data are the mean ± SEM of three different experiments.

[a] Significant differences *vs.* insulin treatment at $P < 0.05$.

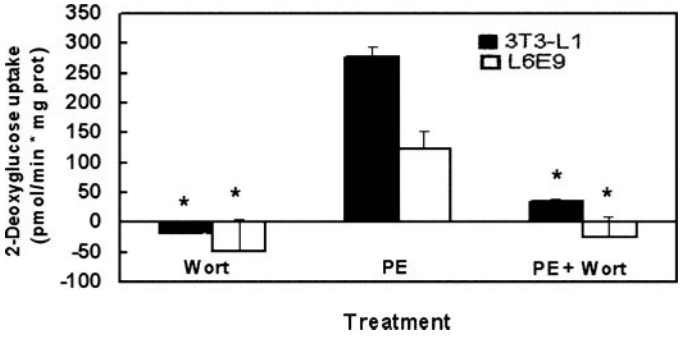

FIG. 4. Effects of wortmannin on PE-stimulated glucose uptake in L6E9 myotubes and 3T3-L1 adipocytes. Differentiated cells were serum-depleted as indicated in Fig. 2. L6E9 myotubes and 3T3-L1 adipocytes were treated with wortmannin (1 μM) before PE (14 mg/liter for 1 h for L6E9; 140 mg/liter for 30 min for 3T3-L1) was added to the cell culture medium. At the end of the treatment, 2-deoxyglucose uptake was assayed. The data are the mean ± SEM of three different experiments. *, Significant differences *vs.* PE-treated group at $P < 0.05$.

L6E9 myotubes with insulin or insulin plus wortmannin. In both cell lines, wortmannin inhibited insulin-induced glucose uptake (data not shown). These results prove that PE requires PI3-kinase activity to be intact if it is to have a stimulatory effect on cellular glucose uptake.

Insulin stimulation of glucose uptake activates two responses on GLUT-4: a very well established effect recruiting GLUT-4 transporters from intracellular reserves to the plasma membrane, and an increase in the intrinsic activity of the transporter, a p38 MAPK-mediated effect. We studied whether PE also activated both responses on GLUT-4. 3T3-L1 adipocytes treated with 140 mg/liter PE increased the amount of GLUT-4 in the plasma membrane (PE, 9.26 ± 4.55; control, 3.61 ± 1.33; Fig. 5). To determine whether the effects of PE on glucose transport required p38MAPK activity, SB203580 (100 μM) was added 20 min before PE treatment. Figure 6 shows that SB203580 inhibited PE-induced glucose transport to a similar extent as it inhibited insulin-induced glucose transport.

Discussion

In this study we show that PE mimics the role of insulin *in vivo* and *in vitro*. We show that an acute dose of PE has an antihyperglycemic effect on an insulin-deficient animal

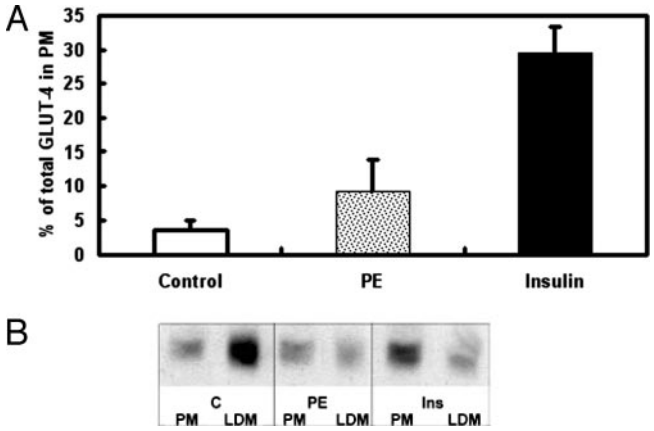

FIG. 5. Effect of PE on distribution of GLUT-4 between plasma membrane and intracellular membranes in 3T3-L1 adipocytes. Fully differentiated adipocytes were serum-depleted as indicated in Fig. 2. They were treated with 140 mg/liter PE or 100 nM insulin or were untreated during the last 30 min of depletion. PM and LDM fractions were obtained and assayed by Western blot to determine the abundance of the GLUT-4 glucose transporter. A, Percentage of total GLUT-4 that was located in the PM. B, Representative autoradiogram [30 μg (PM) and 5 μg (LDM) protein/lane]. The data are the mean ± SEM of at least three different experiments.

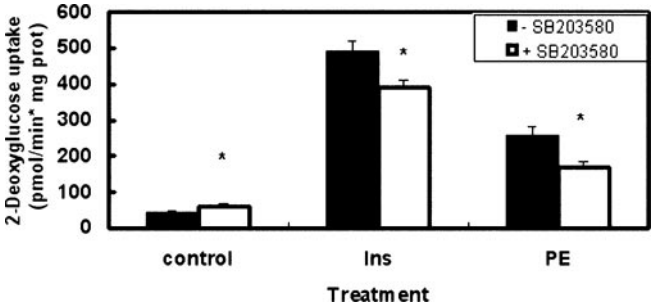

FIG. 6. Effects of SB203580 on PE-stimulated glucose uptake in 3T3-L1 adipocytes. Adipocytes were serum-depleted as indicated in Fig. 2. Cells were pretreated with SB203580 (100 μM) for 20 min before 140 mg/liter PE were added to the cell culture medium. After 30 min of simultaneous treatment, 2-deoxyglucose uptake was assayed. The data are the mean ± SEM of at least three different experiments. *, Significant differences *vs.* non-SB203580 group at $P < 0.05$.

model, the STZ-induced diabetic rat. The antihyperglycemic role of PE by itself was moderate, but simultaneous administration with a low dose (one seventh the effective dose) of insulin boosted the effect to such an extent that it was similar to that of other typical antihyperglycemic agents, such as metformin and troglitazone (28). To our knowledge, this is the first report of an antihyperglycemic effect mediated by PE *in vivo*. Previous studies have shown that wine flavonoids improve the altered oxidative status of the diabetic situation, but they have not found any effect on glycemia. Landrault and colleagues (14) reported that the daily administration of an ethanol-free white wine enriched in phenolics to STZ-induced diabetic rats improved the oxidative stress associated with diabetes. Also, Ceriello and colleagues (16) found that acute moderate red wine consumption during a meal (300 ml red wine, ~300 mg of flavonoids) improved oxidative status in the absorptive phase in human type 2 diabetic patients.

The differences between our results and those obtained by others may be due to the different composition and dose of procyanidins used and the diabetic situation tested, although little work has been done with procyanidins. The blood glucose-lowering activity of other flavonoids, however, has been defined for epicatechin (29), catechin (30), and epicatechin gallate (7, 31). These monomeric forms act through different mechanisms: epicatechin induces pancreatic β-cell regeneration (29), catechin inhibits intestinal glucose absorption (30), and epicatechin gallate increases hepatic glycogen synthesis (7, 31). Only Al-awwadi and colleagues (18) observed that a polyphenol extract from a red wine administered for 6 wk to STZ-induced diabetic rats has an antidiabetic activity, but they do not describe any possible mechanisms to explain this effect. Our *in vivo* experiments suggest that PE can lower glucose levels by at least two mechanisms: a delay in intestinal glucose absorption and an insulin-like effect on insulin-sensitive tissues. Both effects were present in nonfasted animals: 1 h after PE treatment, the glucose level of PE-treated animals was 24.51 ± 1.00 mM, approximately 4 mM lower than that of the control group (28.01 ± 1.08 mM). And when the animals had fasted for 7 h, thus eliminating the effect on glucose absorption, we found a smaller difference (~2 mM) between the glycemia in the PE-treated group (18.26 ± 1.12 mM) and that in the control group (20.61 ± 1.4 mM). To explain the biochemical basis of this last effect, we hypothesized that procyanidins act on insulin-sensitive tissues. It has been reported that some flavonoids stimulate glucose uptake in cultured cells (8, 12, 13). Others, however, are *in vitro* inhibitors of glucose transport in rat adipocytes (11, 32–34).

To date, however, the effects of procyanidins on glucose uptake in insulin-sensitive cells have not been studied. In this respect we show that PE has an insulin-like effect on glucose uptake in two insulin-sensitive cell lines: 3T3-L1 adipocytes and L6E9 myotubes. In both cell lines, PE alone did not cause maximal insulin stimulation, but there was a synergic effect between PE and insulin in 3T3-L1. With this simultaneous treatment, a low dose of insulin and PE, we managed to stimulate glucose uptake to a similar extent as that caused by a maximal insulin concentration. There are various molecular mechanisms by which this stimulation of glucose uptake could take place. Waltner-Law and colleagues (31) showed that epigallocatechin gallate has an insulin-like effect on hepatocytes mediated by changes in the redox state that affect the functional states of some intracellular mediators of insulin signaling. We do not discard this possibility, because procyanidins are also very well described antioxidants (35). However, the high affinity of procyanidins for binding to proteins is also very well described (36–38). Our present results show that the PE stimulation of glucose uptake does not use a different or complementary mechanism to that of insulin in myotubes, because simultaneous treatment with saturating doses of insulin and PE did not cause an additive effect. We do not discard a complementary mechanism in adipocytes, because we observed that simultaneous treatment leads to some addition, but it did not reach the sum of both independent effects. Therefore, procyanidins in adipocytes must also use insulin mechanisms.

Our results also support the idea that the PE stimulation of glucose uptake in adipocytes and myotubes acts through

some of the main intracellular mediators described for the insulin signaling pathway (PI3K and p38 MAPK). In both cell lines, the inhibition of PE-stimulated glucose uptake caused by wortmannin was similar to that caused by insulin stimulation. The p38 MAPK inhibitor, SB203580, led to a similar situation. PE also increased the amount of insulin-sensitive glucose transporter, GLUT-4, in the plasma membrane. Like the effect of PE on the stimulation of glucose uptake, the effect of PE on GLUT-4 translocation was less than that of insulin.

In conclusion, our results indicate that PE is an antihyperglycemic agent with insulinomimetic properties. We have shown that procyanidins mimic and/or influence insulin effects by directly acting on specific components of the insulin-signaling transduction pathway. This insulin-like role of procyanidins must be added to their very well described effect of improving altered oxidative states.

Acknowledgments

The manuscript was corrected by John Bates of our university's language service.

Received June 17, 2004. Accepted July 15, 2004.

Address all correspondence and requests for reprints to: Dr. Anna Ardévol, Department of Biochemistry and Biotechnology, Plaza Imperial Tarraco 1, 43005 Tarragona, Spain. E-mail: aag@astor.urv.es.

This work was supported by Grant CO3/O8 from the Fondo de Investigación Sanitaria and Grant AGL2002–00078 from the Comisión Interministerial de Ciencia y Tecnología of the Spanish government. M.P. is the recipient of a fellowship from the autonomous government of Catalonia.

References

1. **Scalbert A, Williamson G** 2000 Dietary intake and bioavailability of polyphenols. J Nutr 130(8S Suppl): 2073S–2085S
2. **Ross JA, Kasum CM** 2002 Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr 22:19–34
3. **Knekt P, Kumpulaineu J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, Hakulinen T, Aromaa A** 2002 Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 76:560–568
4. **Middleton EJR, Kandaswami C, Theoharides TC** 2000 The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673–751
5. **Havsteen BH** 2002 The biochemistry and medical significance of the flavonoids. Pharmacol Ther 96:67–202
6. **Gamet-Payrastre L, Manenti S, Gratacap M-P, Tulliez J, Chap H, Payrastre B** 1999 Flavonoids and the inhibition of PKC and PI 3-kinase. Gen Pharmacol 32:279–286
7. **Kao Y-H, Hiipakka RA, Liao S** 2000 Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology 141:980–987
8. **Ong KC, Khoo H** 1996 Insulinomimetic effects of myricetin on lipogenesis and glucose transport in rat adipocytes but not glucose transporter translocation. Biochem Pharmacol 51:423–429
9. **Ahmad FKP, Khan MM, Rastogi AK, Kidwai JR** 1989 Insulin like activity in (−)epicatechin. Acta Diabetol Lat 26:291–300
10. **Vessal M, Hemmati M, Vasei M** 2003 Antidiabetic effects of quercetin in streptozotocin-induced diabetic rats. Comp Biochem Physiol C 135:357–364
11. **Harmon A, Patel YM** 2003 Naningerin inhibits phosphoinositide 3-kinase activity and glucose uptake in 3T3–L1 adipocytes. Biochem Biophys Res Commun 305:229–234
12. **Kamei RKM, Kitagawa Y, Hazeki O, Oikawa S** 2003 2′-Benzyloxychalcone derivatives stimulate glucose uptake in 3T3–L1 adipocytes. Life Sci 73:2091–2099
13. **Jarvill-Taylor JK, Anderson RA, Graves DJJ** 2001 A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3–L1 adipocytes. Am Coll Nutr 4:327–336
14. **Landrault N, Poucheret P, Azay J, Krosniak M, Gasc F, Jenin C, Cros G, Teissedre PL** 2003 Effect of a polyphenols-enriched chardonnay white wine in diabetic rat. J Agric Food Chem 51:311–318
15. **Maritim ADB, Sanders RA, Watkins III JB** 2003 Effects of pycnogenol treatment on oxidative stress in streptozotocin-induced diabetic rats. J Biochem Mol Toxicol 17:193–199
16. **Ceriello A, Bortolotti N, Motz E, Lizzio S, Catone B, Assaloni R, Tonutti L, Taboga C** 2001 Red wine protects diabetic patients from meal-induced oxidative stress and thrombosis activation: a pleasant approach to the prevention of cardiovascular disease in diabetes. Eur J Clin Invest 31:322–328
17. **Caimi GCC, Lo Presti R** 2003 Diabetes mellitus: oxidative stress and wine. Curr Med Res Opin 19:581–6
18. **Al-Awwadi NAJ, Poucheret P, Cassanas G, Krosniak M, Auger C, Gasc F, Rouanet JM, Cros G, Teissedre PL** 2004 Antidiabetic activity of red wine polyphenolic extract, ethanol, or both in streptozotocin-treated rats. J Agric Food Chem 52:1008–1016
19. **Lange K** 2001 Regulation of glucose uptake in differentiated cells. Front Biosci 6:D630–D359
20. **Zierath JR, Wallberg-Henriksson H** 2002 From receptor to effector: insulin signal transduction in skeletal muscle from type II diabetic patients. Ann NY Acad Sci 967:120–134
21. **Khan AHPJ** 2002 Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. Diabetologia 45:1475–1483
22. **Simpson F, Whitehead J, James D** 2001 GLUT4: at the cross roads between membrane trafficking and signal transduction. Traffic 2:2–11
23. **Michelle Furtado L, Poon V, Klip A** 2003 GLUT4 activation: thoughts on possible mechanisms. Acta Physiol Scand 178:287–296
24. **Ardévol A, Bladé C, Salvadó MJ, Arola L** 2000 Changes in lipolysis and hormone-sensitive lipase expression caused by procyanidins in 3T3–L1 adipocytes. Int J Obesity 24:319–324
25. **Blay M, Pinent M, Baiges I, Ardévol I, Salvadó M, Bladé C, Arola L** 2003 Grape seed proanthocyanidins increase glucose uptake in muscular cell line L6E9. In: Lonvaud-Funel A, deRevel G, Darriet P, eds. Oenologie '03. 7th International Symposium of Oenology. Bordeaux: Editions Tec&Doc; 669–673
26. **Bradford MM** 1976 A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254
27. **Simpson IA, Yver DR, Hissin PJ, Wardzala LJ, Karnieli E, Salans LB, Cushman SW** 1983 Insulin-stimulated translocation of glucose transporters in the isolated rat adipose cells: characterization of subcellular fractions. Biochim Biophys Acta 763:393–407
28. **Reed M, Meszaros K, Entes L, Claypool M, Pinkett J, Gadbois T, Reaven G** 2000 A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism 49:1390–1394
29. **Kim M-J RG, Chung J-S, Sim SS, Min DS, Rhie D-J, Yoon SH, Hahn SJ, Kim M-S, Jo Y-H** 2003 Protective effects of epicatechin against the toxic effects of streptozotocin on rat pancreatic islets: in vivo and in vitro. Pancreas 26:292–299
30. **Shimizu M, Kobayashi Y, Suzuki M, Satsu H, Miyamoto Y** 2000 Regulation of intestinal glucose transport by tea catechins. Biofactors 13:61–65
31. **Waltner-Law ME, Wang XL, Law BK, Hall RK, Nawano M, Granner DK** 2002 Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem 277:34933–34940
32. **Smith RM, Tiesinga JJ, Shah N, Smith JA, Jarett L** 1993 Genistein inhibits insulin-stimulated glucose transport and decreases immunocytochemical labeling of GLUT4 carboxyl-terminus without affecting translocation of GLUT4 in isolated rat adipocytes: additional evidence of GLUT4 activation by insulin. Arch Biochem Biophys 300:238–246
33. **Shisheva A, Shechter Y** 1992 Quercetin selectively inhibits insulin receptor function in vitro and the bioresponses of insulin and insulinomimetic agents in rat adipocytes. Biochemistry 31:8059–8063
34. **Kotyk A, Kolinska J, Veres K, Szammer J** 1965 Inhibition by phloretin and phlorizin derivatives of sugar transport in different cells. Biochem Z 342:129–138
35. **Counet CCS** 2003 Effect of the number of flavanol units on the antioxidant activity of procyanidin fractions isolated from chocolate. J Agric Food Chem 51:6816–6822
36. **Hagiwara M, Inoue S, Tanaka T, Nunoki K, Ito M, Hidaka H** 1988 Differential effects of flavonoids as inhibitors of tyrosine protein kinases and serine/threonine protein kinases. Biochem Pharmacol 37:2987–2992
37. **Brunet MJ, Bladé C, Salvado MJ, Arola L** 2002 Human apo A-I and rat transferrin are the principal plasma proteins that bind wine catechins. J Agric Food Chem 50:2708–2712
38. **Moini HGQ, Packer L** 2000 Enzyme inhibition and protein-binding action of the procyanidin-rich French maritime pine bark extract, pycnogenol: effect on xanthine oxidase. J Agric Food Chem 48:5630–5639