Nutrition 27 (2011) 1161–1167

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Effects of flavonoids on α-glucosidase activity: Potential targets for glucose homeostasis

Danielle Fontana Pereira M.Sc. [a], Luisa Helena Cazarolli Dr. [b], Cristiane Lavado St. [a], Vanessa Mengatto St. [a], Maria Santos Reis Bonorino Figueiredo Dr. [a], Alessandro Guedes M.Sc. [c], Moacir Geraldo Pizzolatti Ph.D. [d], Fátima Regina Mena Barreto Silva Ph.D. [a,*]

[a] Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Campus Universitário, Bairro Trindade, Florianópolis, SC, Brazil
[b] Universidade Federal da Fronteira Sul, Campus Universitário Laranjeiras do Sul, Bairro Vila Alberti, Laranjeiras do Sul, PR, Brazil
[c] Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Regional de Blumenau, Campus III, Bairro Itoupava seca, Blumenau, SC, Brazil
[d] Departamento de Química–Centro de Ciências Físicas e Matemáticas, Universidade Federal de Santa Catarina, Campus Universitário, Bairro Trindade, Florianópolis, SC, Brazil

ARTICLE INFO

Article history:
Received 15 October 2010
Accepted 24 January 2011

Keywords:
Quercetin
Kaempferitrin
Rutin
Kaempferol
Disaccharidase
Acarbose

ABSTRACT

Objective: Flavonoids are naturally occurring compounds widely distributed in plants, which have hypoglycemic potential and have been described as glucosidase inhibitors. This study evaluated the effect of flavonoids on intestinal glucosidase activity after in vivo and in vitro treatment.
Methods: For the in vivo studies animals received quercetin by gavage and for the in vitro assays a segment of the small intestine was used. To obtain the oral glucose tolerance curve fasted normal rats were loaded with glucose plus flavonoids. The glycemia was measured by the glucose oxidase method.
Results: Quercetin reduced the effect of sucrase and maltase in the in vivo and in vitro treatments. It was observed in the in vitro studies that the maximum inhibitory effect of kaempferitrin was around 23% for maltase activity. Also, for the sucrose substrate the specific enzyme activity was significantly decreased. Aglycone, flavonoids, and kaempferol decreased significantly the maltase activity at all concentrations assayed. Finally, rutin reduced maltase-specific activity at all concentrations studied. According to the oral glucose tolerance curve, rutin reduced the serum glucose levels at 15, 30, and 60 min when administered by oral gavage 30 min before glucose overload in rats.
Conclusion: Based on these results, we can conclude that disaccharidases are targets of flavonoids in the regulation of glucose absorption and consequently glucose homeostasis.
© 2011 Elsevier Inc. Open access under the Elsevier OA license.

Introduction

Flavonoids are naturally occurring phenolic compounds that are widely distributed in plants and some of them have been described as glucosidase inhibitors [1,2]. Glucosidase inhibitors are potential agents for diabetes therapy since glucosidases are involved in several important and relevant biological processes [3]. Acarbose, the first α-glucosidase inhibitor to be identified, is currently used for the treatment of type 2 diabetes [4].

Diabetes mellitus is a complex metabolic disorder in the endocrine system characterized by abnormalities in insulin secretion and/or insulin action that leads to progressive deterioration of glucose tolerance and causes hyperglycemia. This disease is a major public health problem worldwide and is rapidly becoming more common [1,5]. One therapeutic approach to decreasing postprandial hyperglycemia is to retard the absorption of glucose via inhibition of carbohydrate-hydrolyzing enzymes, such as glucosidase, in the intestine [6]. The glucosidase enzymes are located in the brush border of the small intestine and are required for the breakdown of carbohydrates before monosaccharide absorption. The alpha-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glycemia and insulin peaks [7].

Flavonoids are potential antidiabetic agents because they exert multiple actions that are both hypoglycemic (insulinomimetic action) and antihyperglycemic (insulin secretagogue) [1,2]. Our group has investigated the effects of different classes of flavonoids on glucose control in hyperglycemic rats (glucose overloaded) and in chemically induced diabetic rats. In these studies it was

* Corresponding author. Tel.: +55-48.3721.69.12; fax: +55-48.3721.96.72.
E-mail address: mena@mbox1.ufsc.br (F. R. M. B. Silva).

0899-9007 © 2011 Elsevier Inc. Open access under the Elsevier OA license.
doi:10.1016/j.nut.2011.01.008

demonstrated that kaempferol-3,7-*O*-(α)-dirhamnoside (kaempferitrin) and kaempferol-3-neohesperidoside act through multiple sites, constituting strong evidence for their insulinomimetic role in assuring glucose homeostasis [8–12]. We also showed that api genin-6-C-(2"-O-α-L-rhamnopyranosyl)-β-L-fucopyranoside and apigenin-6-C-β-L-fucopyranoside can act either as insulin-secretagogues or as insulinomimetic agents [13,14]. Thus, it seems clear that flavonoids can act through a number of tissues to regulate serum glucose homeostasis. Based on these findings, the aim of this study was to investigate the effects of quercetin, kaempferitrin, rutin, and kaempferol on in vivo and/or in vitro intestinal glucosidase activity.

Materials and methods

Materials

Dehydrated quercetin was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). NaOH, D(+)-monohydrated maltose and NaCl were acquired from Vetec (Duque de Caxias, Rio de Janeiro, Brazil); sucrose, lactose, and maleic acid were acquired from Nuclear (Diadema, São Paulo, Brazil), and the Glucose-PP kit was acquired from Analisa (Belo Horizonte, Minas Gerais, Brazil). The kaempferitrin, kaempferol, and rutin used in this study were available from our previous studies [15,16,17].

Experimental animals

Male Wistar rats, 50-55 day old and weighing around 180-200 g, were used. The animals were housed in cages (six animals per cage) and kept in an air-conditioned room (approximately 21°C) with controlled lighting (photoperiod of 12-h light and 12-h darkness). Pelleted food (Bio Base, Águas Frias, SC, Brazil) and water were available to the rats ad libitum until the start of the experiments. All the animals were carefully monitored and maintained in accordance with the ethical recommendations of the Brazilian Veterinary-Medicine Council and the Brazilian College of Animal Experimentation.

Rats were divided into five groups of six animals each: group I, control; group II, treated in vivo with quercetin 50 and 100 mg/kg (po; corresponding to 0.06 and 0.140 M, respectively) for 0, 30, and 60 min; group III, duodenal homogenates were incubated in vitro with quercetin, kaempferitrin, rutin, or kaempferol at different concentrations (3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 μM), for 5 min; group IV, treated in vitro with quercetin at 0.06 M for 5 min; group V, treated in vivo with rutin (50 mg/kg) 30 min before or at the same time as glucose (4 g/kg; 8.9 M), by oral gavage. Blood was collected at 0, 15, 30, 60, and 180 min for serum glucose measurements. Rats were fasted for 16 h before a single oral administration of quercetin by gavage for in vivo treatment or before euthanasia for in vitro treatment with quercetin, kaempferitrin, rutin, or kaempferol [9].

Disaccharidase extraction and assays

A segment of the small intestine was removed, washed in 0.9% NaCl solution, dried on filter paper, weighed, trimmed, and homogenized (300 rpm) with 0.9% NaCl (400 mg of duodenum per milliliter) for 1 min at 4°C. The resulting extract was centrifuged at 8000 rpm for 8 min. The supernatant was used for the measurement of in vitro and in vivo maltase, sucrase, and lactase activities and protein determination.

Maltase (EC 3.2.1.20), lactase (EC 3.2.1.23), and sucrase (EC 3.2.1.48) activities were determined using a glucose diagnosis kit based on the glucose oxidase reagent. For determination of disaccharidase activity in group I (control) and group II, the incubation system containing 0.112 μM of sucrose, lactose, or maltose dissolved in sodium maleate buffer (pH 6.0) and the appropriate enzyme extract volume was incubated for 10 min at 37°C [18]. At the end of this time, 1 mL of the color reagent solution containing glucose oxidase and peroxidase was added and incubated at 37°C for 10 min. The absorbance was read at 500 nm, and the activity calculation was based on a glucose standard. For groups III and IV, the homogenate was preincubated at 37°C for 5 min, in the absence (control) or in the presence of the flavonoids quercetin, rutin, kaempferitrin, or kaempferol (treated). Duodenum homogenates were then incubated at 37°C for 5 min with the substrate (corresponding to 0.056 μM of maltose, sucrose, or lactose).

One enzyme unit was defined as the amount of enzyme that catalyzed the release of 1 μmol of glucose per minute under the assay conditions. The specific activity was defined as enzyme activity per milligram of protein. Protein concentration was determined by the method described in Lowry et al. [19], using bovine serum albumin as the standard. The assays were performed in duplicate and conducted along with appropriate controls.

Determination of serum glucose levels

Blood samples from the tail vein were collected and centrifuged and the serum was used to determine the glycemia by the glucose oxidase method [20].

Data and statistical analysis

Data were expressed as mean ± SEM. One-way analysis of variance was employed followed by Bonferroni's post test, using GraphPad Software 3.01 for Windows 95 (San Diego, CA, USA). Differences were considered to be significant at $P \leq 0.05$.

Results

Basal specific activity of maltase, sucrase, and lactase in the duodenum, jejunum, and ileum of rat small intestine

The basal in vitro activity of maltase, sucrase, and lactase was measured in the duodenum, jejunum, and ileum of rat small intestine. Figure 1A shows that although the activities of sucrase and lactase were detected in all portions of the intestine assayed, the activity of maltase was significantly higher than both of them in the duodenum, jejunum, and ileum. Figure 1B shows the time course of maltase, sucrase, and lactase activities measured in the duodenal homogenate. It can be seen that the activity decreased from the basal level as a function of time and the highest activity for all enzymes studied was detected after 5 min of in vitro

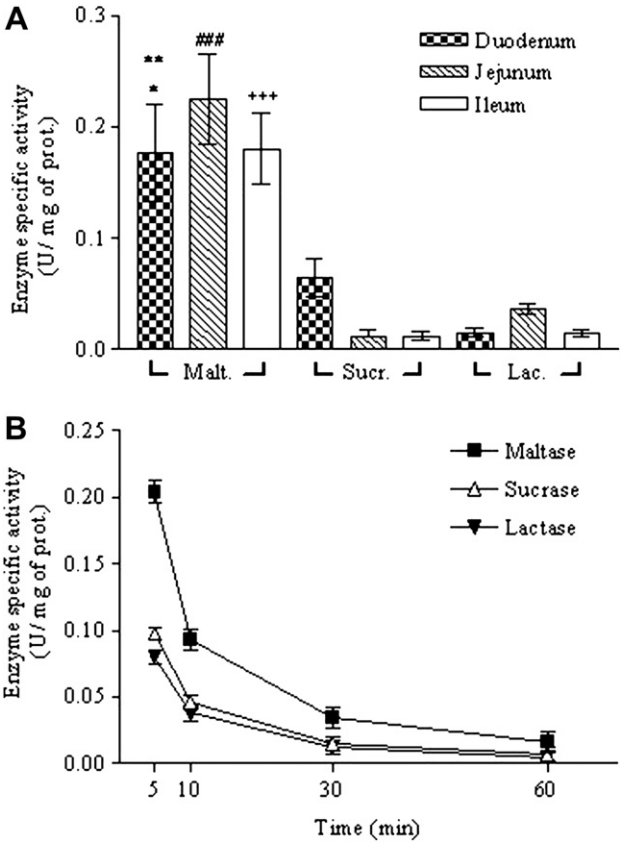

Fig. 1. (A) Basal specific activity of maltase, sucrase, and lactase in the duodenum, jejunum, and ileum from the small intestine of rats. Significant at $^{*}P \leq 0.05$ compared to duodenum sucrase group; $^{**}P \leq 0.01$ compared to duodenum lactase group; $^{###}P \leq 0.001$ compared to jejunum sucrase and lactase group; $^{+++}P \leq 0.001$ compared to ileum sucrase and lactase group. (B) In vitro time course of specific activity of maltase, sucrase, and lactase in the duodenal portion of rat intestine. Values are expressed as mean ± SEM; $n = 6$ for each group.

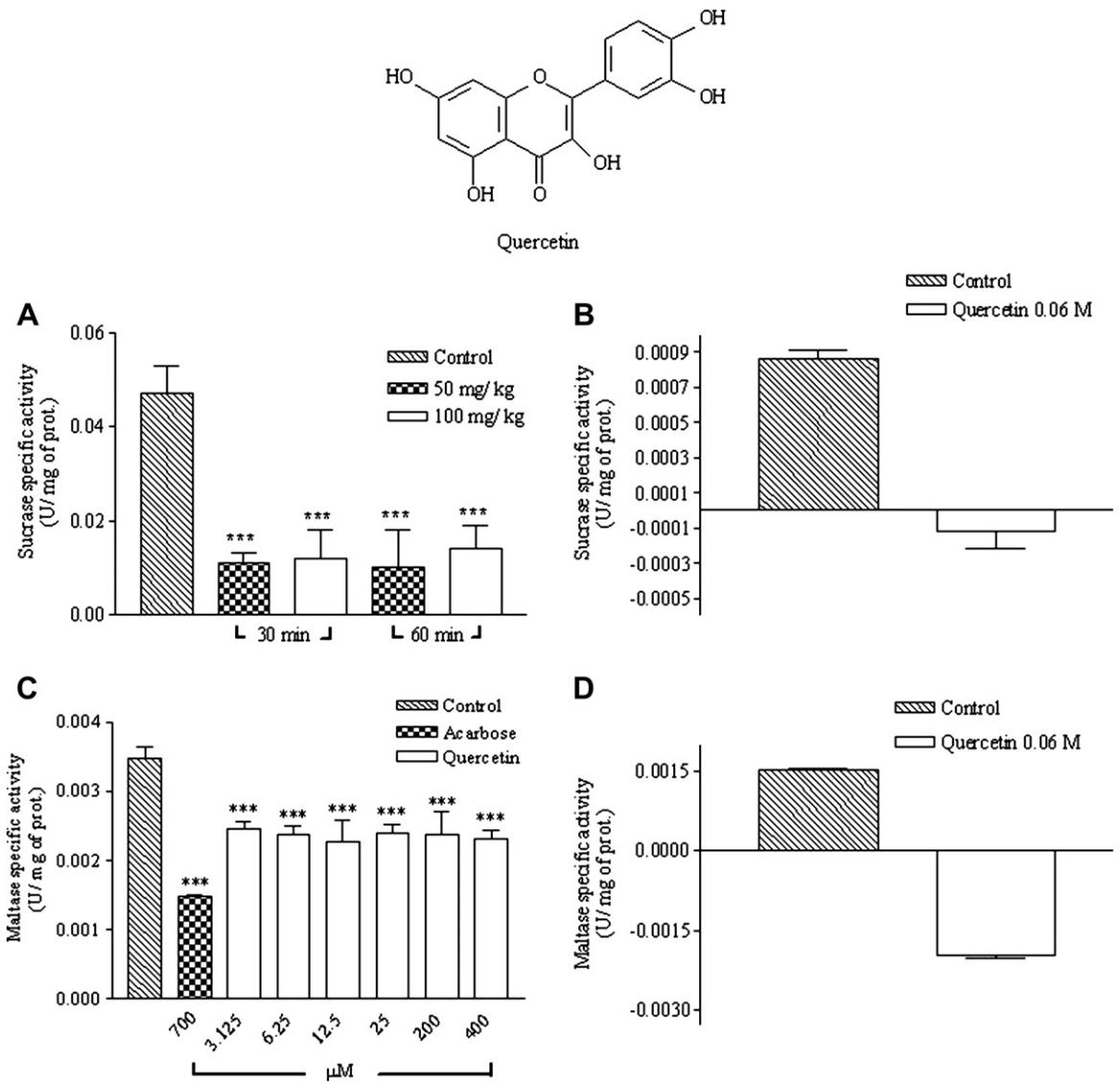

Fig. 2. In vivo effect of quercetin on specific activity of (A) sucrase and in vitro effect of quercetin on specific activity of (B) sucrase and (C, D) in vitro effect of quercetin on specific activity of maltase in the duodenal portion of rat intestine. Values are expressed as mean ± SEM; $n = 6$. Significant at $^{***}P \leq 0.01$ compared to control group.

incubation. Thus, 5 min of incubation was used in all the subsequent in vitro tests on enzyme activity in the duodenal portion of the small intestine.

In vivo and in vitro effect of quercetin on specific activity of duodenal sucrase and maltase

In the present study we investigated the effect of quercetin on intestinal disaccharidase enzymes at 30 and 60 min after oral treatment, the same time used to study the basal activity of disaccharidases on the substrate (Fig. 1A). Figure 2A illustrates the inhibitory effect of quercetin at 50 and 100 mg/kg (0.06 and 0.140 μM, respectively) on sucrase activity after short- and long-term in vivo treatment. However, this inhibitory effect was not replicated in the case of maltase and lactase activities, at least within the treatment periods studied (data not shown). Surprisingly, a complete inhibitory effect of quercetin on sucrase activity was detected in vitro when the concentration of quercetin used in vivo (50 mg/kg = 0.06 M) was tested for 5 min (Fig. 2B). Based on this finding, a wide range of concentrations was assayed to test the effect of the other flavonoids on sucrase, maltase, and lactase activities. Figure 2C shows a significant inhibitory effect of quercetin (3.125, 6.25, 12.5, 25, 100, 400 μM) on the specific activity of duodenal maltase after 5 min of in vitro incubation with this flavonoid, and in Figure 2D the inhibition of maltase activity in vitro was observed with quercetin incubated for 5 min using the concentrations tested in vivo (50 mg/kg = 0.06 M).

In vitro effect of kaempferitrin and kaempferol on specific activity of duodenal maltase and sucrase

Figure 3A shows a significant inhibitory effect of kaempferitrin (3.125, 6.25, 12.5, and 25 μM) on the specific activity of duodenal maltase after 5 min of in vitro incubation with this flavonoid. The maximum inhibitory effect obtained with kaempferitrin was around 23%. The doses of kaempferitrin chosen for in vitro studies were calculated from that which

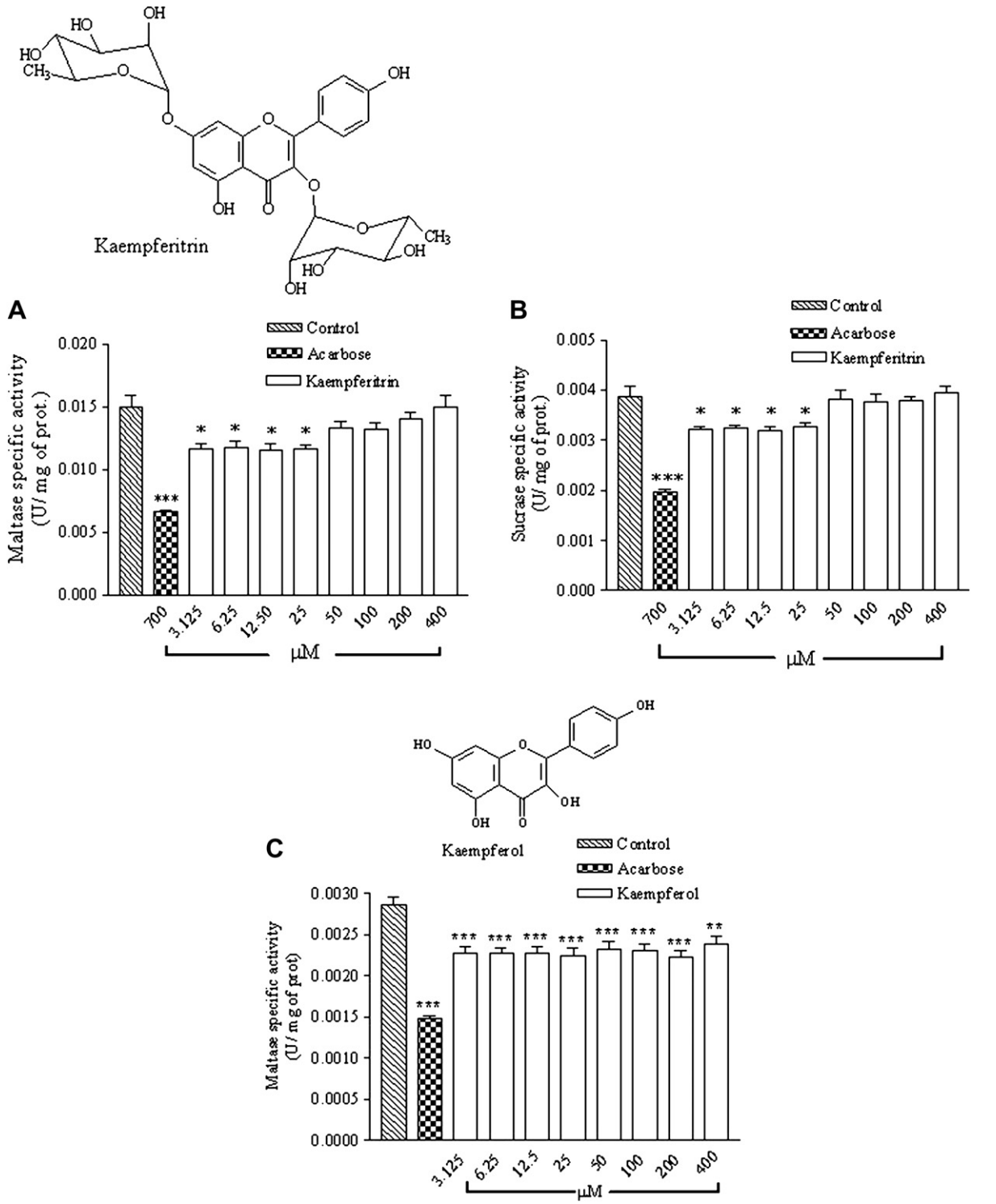

Fig. 3. In vitro effect of kaempferitrin on specific activity of (A) maltase, (B) sucrase, and in vitro effect of kaempferol on specific activity of (C) maltase in the duodenal portion of rat intestine. Incubation = 5 min. Values are expressed as mean ± SEM; $n = 6$ for each group. Significant at $^{*}P \leq 0.05$, $^{**}P < 0.01$ and $^{***}P \leq 0.001$ compared to their respective control group.

caused a hypoglycemic effect in vivo [9,10,21,22]. According to the data presented in Figure 3A, acarbose (700 μM), the well-known inhibitor of intestinal disaccharidases activity, diminished maltase activity by around 50%. Also, the specific activity of duodenal sucrase was significantly diminished in the presence of kaempferitrin (3.125, 6.25, 12.5, and 25 μM) by a similar extent to that of maltase (Fig. 3B). However, the specific activity of lactase was unaltered in the presence of this flavonoid (data not shown). The effect of kaempferol aglycone on the specific activity of maltase in the intestinal homogenate

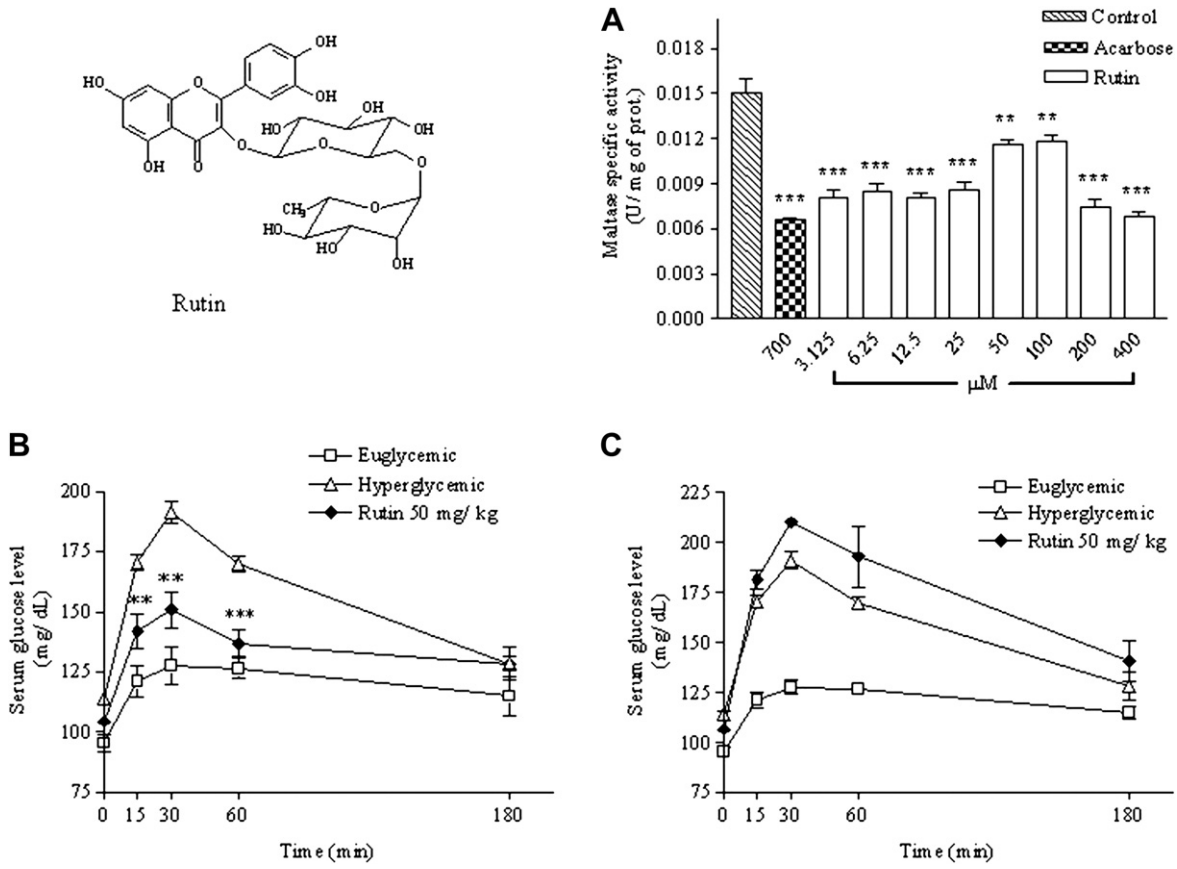

Fig. 4. In vitro effect of rutin on specific activity of (A) maltase in the duodenal portion of rat intestine. Incubation = 5 min. Effect of rutin on oral glucose tolerance curve. (B) Rutin by oral gavage 30 min before glucose loading in rats. (C) Rutin plus glucose by oral gavage at the same time. Values are expressed as mean ± SEM; $n = 6$. Significant at $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ compared to their respective control group.

was also studied. It can be seen in Figure 3C that kaempferol had a significant inhibitory effect on maltase activity at all concentrations tested. This effect was around 20% for kaempferol, compared to the control group. The inhibitory effects of kaempferol represent 48% of the effect of the powerful glucosidase inhibitor acarbose, studied under the same experimental conditions. However, kaempferol did not alter the sucrase and lactase activities at any concentration studied (data not shown).

In vitro effect of rutin on specific activity of duodenal maltase and on oral glucose tolerance curve

In this study, we observed that this *O*-glycoside reduced specific activity of maltase at all concentrations studied (3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 μM) after 5 min of in vitro incubation when compared with the basal group (Fig. 4A). The maximum effect of rutin was 55%, while that of acarbose was 56%. Furthermore, even with the lowest concentration of rutin, the inhibitory effect on maltase activity was similar to that observed for acarbose.

On the other hand, rutin did not affect the sucrase and lactase activity at any concentration tested (data not shown). The specificity of the action of rutin can be seen from the profile of the response in terms of the activities of the three enzymes analyzed in the intestinal homogenate. It is clear that rutin has a defined target in the intestine, and the powerful effect of rutin on maltase was corroborated by its significant serum glucose-lowering effect observed in the oral glucose tolerance curve (Fig. 4B, C). Rutin (50 mg/kg) reduced serum glucose levels at 15, 30, and 60 min when administered by oral gavage 30 min before glucose (4 g/kg; 8.9 M) overload in rats (Fig. 4B). However, when rats were overloaded with glucose and treated with rutin at the same time, no significant difference in glucose levels was detected (Fig. 4C).

Discussion

Quercetin is one of the most common flavonol aglycones and is widespread in nature [1,2]. This flavonoid activates hexokinase and glucokinase [23] and inhibits both glycogen phosphorylase a and b and hepatic glucose-6-phosphatase [24].

This significant biological action of quercetin on sucrase activity detected after in vivo treatment by oral gavage and in vitro after a short period of incubation is in line with the quercetin effect reported by Shimizu et al. [25], who demonstrated a marked reduction in glucose absorption, when compared with the control. In other reports it has been proposed that the inhibitory effect of quercetin on glucose absorption is due to competitive inhibition of the sodium-dependent glucose transporter-1 [1,2]. Considering the results of our previous

studies [10,12–14], these results verify multiple sites of action for different flavonoids in the modulation of glucose homeostasis.

Based on these findings, we decided to study the short-term effects of the flavonoids quercetin, kaempferitrin, rutin, and kaempferol on duodenum maltase, sucrase, and lactase activities and to compare the results with the known effect of acarbose on α-glucosidases. It should be noted that to carry out these studies, a kaempferitrin dose of 100 mg/kg (0.0294 mmol/kg) was used, because this is the most efficient in terms of reducing serum glucose in diabetic rats, as previously reported by our laboratory [9–15].

We have previously demonstrated that oral administration of kaempferitrin led to a significant hypoglycemic effect in normal and alloxan-induced diabetic rats [9]. In addition, this compound stimulates in vitro glucose uptake in a target tissue of insulin and skeletal muscle [15]. More recently, we discussed that flavonoids regulate glucose homeostasis through a multitude of actions and by a complex intracellular signaling dictated by the particular structure of *O*- or *C*-glycosides or aglycone flavonoids [1,2]. Additionally, flavonoid action is dependent on the number, type, and position of glycosyl residues on the natural compounds. Specifically, for kaempferitrin, in previous studies we demonstrated that skeletal muscle is a significant target for the insulinomimetic effect of this compound; however, other important tissues in terms of glucose homeostasis, such as intestinal, were not studied. Thus, to our knowledge, this is the first time that intestinal disaccharidase activity has been investigated in the presence of kaempferitrin.

Among the reported biological properties of rutin are the antihyperglycemic and hypoglycemic effects in diabetic rats [21,26,27], an increase in insulin and C-peptide serum levels, a protective effect on pancreatic β-cells, restoration of glycogen content and hexokinase activity, and decreased glucose-6-phosphatase and fructose-1,6-bisphosphatase activities [28].

Our results for the in vitro effect of quercetin and kaempferol on the specific activity of duodenal maltase are in line with those reported by Matsui et al. [29], concerning the inhibitory effects of kaempferol, chrysin, galangin, and luteolin on α-glucosidase activity, observed in both in vitro and in vivo studies on the absorption and metabolism of carbohydrates. The putative inhibitory effects of some flavonoids on α-glucosidase activity were confirmed by Kim et al. [30], who showed that luteolin, amentoflavone, luteolin 7-O-glucoside, and daidzein are strong inhibitors of glucosidases. Also, Andrade-Cetto et al. [5] reported that flavonoid-enriched extracts from some Mexican plants efficiently inhibited α-glucosidase activity and significantly reduced serum-glucose levels in diabetic rats.

Conclusions

Based on the results reported herein, it can be concluded that disaccharidases are targets of flavonoids in the regulation of glucose absorption and consequently glucose homeostasis. Rutin, a glycosylated flavonoid, showed an acarbose-like inhibitory effect on maltase activity and this was highly specific to maltase. In addition, the effect of rutin in the intestine was reinforced because this flavonoid was able to reduce glycemia when administered before glucose overload. Because it is evident that multiple sites of action for flavonoids can contribute significantly to the serum glucose balance, studies are underway to investigate the molecular action of some selected flavonoids on enterocytes.

Acknowledgments

This work was supported by grants from Conselho Nacional de Desenvolvimento e Tecnológico (CNPq), Coordenação de Pessoal de Nível Superior (CAPES)/PPG-Bioquímica,and Fundação de Amparo à Pesquisa do Estado de Santa Catarina (FAPESC). The authors express their appreciation to Dr. Siobhan Wiese for assistance with the English correction of the manuscript.

References

[1] Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MSRB, Folador P, Damazio RG, et al. Flavonoids: prospective drug candidates. Mini Rev Med Chem 2008;8:1429–40.
[2] Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MSRB, Folador P, Damazio RG, et al. Flavonoids: cellular and molecular mechanism of action in glucose homeostasis. Mini Rev Med Chem 2008;8:1032–8.
[3] De Melo EB, Carvalho I. α e β-Glucosidases como alvos moleculares para desenvolvimento de fármacos. Quim Nova 2006;29:840–3.
[4] Yee HS, Fong NT. A review of the safety and efficacy of acarbose in diabetes mellitus. Pharmacotherapy 1996;16:792–805.
[5] Andrade-Cetto A, Jiménez JB, Vázquez RC. Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes. J. Ethnopharmacol 2008;116:27–32.
[6] Holman RR, Cull CA, Turner RC. A randomized double-blind trial of acarbose in type 2 diabetes shows improved glycemic control over 3 years. Diabetes Care 1999;22:960–4.
[7] Stuart AR, Gulve EA, Wang M. Chemistry and biochemistry of type 2 diabetes. Chem Rev 2004;104:1255–82.
[8] Silva FRMB, Szpoganicz B, Pizzolatti MG, Willrich MAV, De Sousa E. Acute effect of Bauhinia forficata on serum glucose levels in normal and alloxan-induced diabetic rats. J Ethnopharmacol 2002;83:33–7.
[9] De Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Szpoganicz B, et al. Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-(alpha)-dirhamnoside from Bauhinia forficata leaves. J Nat Prod 2004;67:829–32.
[10] Cazarolli LH, Zanatta L, Jorge AP, De Sousa E, Horst H, Voehl VM, et al. Follow-up studies on glycosylated flavonoids and their complexes with vanadium: their anti-hyperglycemic potential role in diabetes. Chem-Biol Interact 2006;163:177–91.
[11] Zanatta L, Rosso A, Folador P, Figueiredo MSRB, Pizzolatti MG, Leite LD, et al. Insulinomimetic effect of kaempferol 3-neohesperidoside on the rat soleus muscle. J Nat Prod 2008;71:532–5.
[12] Cazarolli LH, Folador P, Pizzolatti MG, Silva FRMB. Signaling pathways of kaempferol-3-neohesperidoside in glycogen synthesis in rat soleus muscle. Biochimie 2009;91:843–9.
[13] Cazarolli LH, Folador P, Moresco HH, Brighente IMC, Pizzolatti MG, Silva FRMB. Mechanism of action of the stimulatory effect of apigenin-6-C-(2"-O-alpha-l-rhamnopyranosyl)-beta-l-fucopyranoside on (14)C-glucose uptake. Chem-Biol Interact 2009;179:407–12.
[14] Cazarolli LH, Folador P, Moresco HH, Brighente IMC, Pizzolatti MG, Silva FRMB. Stimulatory effect of apigenin-6-β-L-fucopyranoside on insulin secretion and glycogen synthesis. Eur J Med Chem 2009;44:4668–73.
[15] Jorge AP, Horst H, De Sousa E, Pizzolatti MG, Silva FRMB. Insulinomimetic effects of kaempferitrin on glycaemia and on 14C-glucose uptake in rat soleus muscle. Chem-Biol Interact 2004;149:89–96.
[16] Pizzolatti MG, Cunha AJ, Szpoganicz B, De Sousa E, Braz-Filho R, Schripsema J. Flavonóides glicosilados das folhas e flores de Bauhinia forficata (Leguminosae). Química Nova 2003;26:466–9.
[17] Machado DG, Bettio LEB, Cunha MP, Santos ARS, Pizzolatti MG, Brighente IMC, et al. Antidepressant-like effect of rutin isolated from the ethanolic extract from Schinus molle L. in mice: evidence for the involvement of the serotonergic and noradrenergic systems. Eur J Pharmacol 2008;587:163–8.
[18] Dahlqvist A. Assay of intestinal disaccharidases. Scan J Clin Lab Invest 1984;44:169–72.
[19] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[20] Varley H, Gowenlock AH, Bell M. In: Heinemann IW, editor. Practical biochemistry. London; 1976. vol. 1, p. 389–91.
[21] Shin JS, Kim KS, Kim MB, Jeong JH, Kim BK. Synthesis and hypoglycemic effect of chrysin derivatives. Bioorg Med Chem Lett 1999;9:869–74.
[22] Folador P, Cazarolli LH, Gazola AC, Reginatto FH, Schenkel EP, Silva FRMB. Potential insulin secretagogue effects of isovitexin and swertisin isolated from Wilbrandia ebracteata roots in non-diabetic rats. Fitoterapia 2010;81:1180–7.
[23] Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozotocin induced diabetic rats. Comp Biochem Physiol C Toxicol Pharmacol 2003;135:357–64.

[24] Jakobs S, Fridrich D, Hofem S, Pahlke G, Eisenbrand G. Natural flavonoids are potent inhibitors of glycogen phosphorylase. Mol Nutr Food Res 2005;50:52–7.
[25] Shimizu M, Kobayashi Y, Suzuki M, Satsu H, Miyamoto Y. Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. J Agric Food Chem 2000;48:5618–23.
[26] Velussi M, Cernigoi AM, De Monte A, Dapas F, Caffau C, Zilli M. Long-term (23 months) treatment with an anti-oxidant drug (silymarin) is effective on hyperinsulinemia, exogenous insulin need and malondialdehyde levels in cirrhotic diabetic patients. J Hepatol 1997;26:871–9.
[27] Hnatyszyn O, Mino J, Ferraro G, Acevedo C. The hypoglycemic effect of Phyllanthus sellowianus fractions in streptozotocin-induced diabetic mice. Phytomedicine 2002;9:556–9.
[28] Prince SM, Kamalakkannan N. Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes. J Biochem Mol Toxicol 2006;20:96–102.
[29] Matsui T, Kobayashi MK, Hayashida S, Matsumoto K. Luteolin, a flavone, does not suppress postprandial glucose absorption through an inhibition of α-glucosidase action. Biosc Biotechnol Biochem 2002;66:689–92.
[30] Kim JS, Kwon CS, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Biosc Biotechnol Biochem 2000;64:2458–61.