Fitoterapia 113 (2016) 128–131

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Flavonoids stimulate cholecystokinin peptide secretion from the enteroendocrine STC-1 cells

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ARTICLE INFO

Article history:
Received 21 May 2016
Received in revised form 27 July 2016
Accepted 31 July 2016
Available online 2 August 2016

Keywords:
Cholecystokinin
STC-1 cells
Flavonoids
food intake

ABSTRACT

Animal experiments showed that flavonoids might have the potential for an anti-obesity effect by reducing weight and food intake. However, the exact mechanisms that could be involved in these proposed effects are still under investigation. The complex process of food intake is partially regulated by gastrointestinal hormones. Cholecystokinin (CCK) is the best known gastrointestinal hormone to induce satiety signal that plays a key role in food intake regulation. It is released from the endocrine cells (I cell) in response to the ingestion of nutrients into the small intestine. In this study, we investigated the possible effects of flavonoids (quercetin, kaempferol, apigenin, rutin and baicalein) on stimulation of CCK release in vitro using enteroendocrine STC-1 cells. In comparison with the control, quercetin, kaempferol and apigenin resulted in a significant increase in CCK secretion with quercetin showing the highest activity. On the other hand, no significant effect was seen by rutin and baicalein. To our knowledge, this is the first report to study the stimulation of CCK peptide hormone secretion from STC-1 cells by quercetin and kaempferol, rutin, apigenin and baicalein. Based on the cell-based results in this work, it can be suggested that the reported activity of flavonoids against food intake and weight could be mediated by stimulation of CCK signal which in turn is responsible for food intake reduction, but future animal and human studies are needed to confirm this conclusion at organism level.

1. Introduction

Obesity and overweight are a public health concern in both developing and developed countries. They are considered as risk factors for the development of diet-related diseases like type-2 diabetes, hypertension and cardiovascular diseases. Research studies focusing on prevention and treatment of obesity by the use of natural compounds have increased in the last decades. As such, flavonoids are one class of important natural compounds, which receive large attention in view of their potential health promoting effects. In vitro, in vivo and human studies provided indications of a potential role of flavonoids and foods rich in flavonoids in relation to treatment of obesity [2,11,16,22]. Quercetin is one of the most studied flavonoids in view of its potential role in management of diseases for which an anti-obesity effect has been proposed. This effect is based on a reduction in body weight gain observed in obese animals when treated with quercetin [6,13,20]. Also, rutin, the glycoside form of quercetin, resulted in a decrease in body weight gain when animals were given a high fat diet [5,10]. Other animal studies reported that kaempferol treatment caused a reduction in food intake and body weight [4,25]. In line with these results, other studies suggested that consumption of flavonoids-rich food may reduce food intake by stimulation of satiety-inducing hormones [19,26]. (See Figs. 1–4.)

Food intake is a complex process, partially regulated by gastrointestinal hormones, that mediates sensing and signaling related to food intake in the central nervous system [7,8]. Cholecystokinin (CCK) is one of the best known gastrointestinal hormones that induce satiety and as such play a key role in food intake regulation. It is released from the endocrine cells (I cell) in response to the ingestion of nutrients into the small intestine. The STC-1 cell line has already been proven to act as a suitable model to study CCK secretion [3].

In this study, we investigated the possible effect of different flavonoids (Fig. 1) on stimulation of CCK production and secretion in vitro using enteroendocrine STC-1 cells. The data should help in a better understanding of the mechanism(s) behind the reported activities of flavonoids and foods rich in flavonoids to lower food intake.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, Dulbecco's phosphate-buffered saline

Abbreviations: CCK, cholecystokinin; RIA, radioimmunoassay.

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http://dx.doi.org/10.1016/j.fitote.2016.07.016
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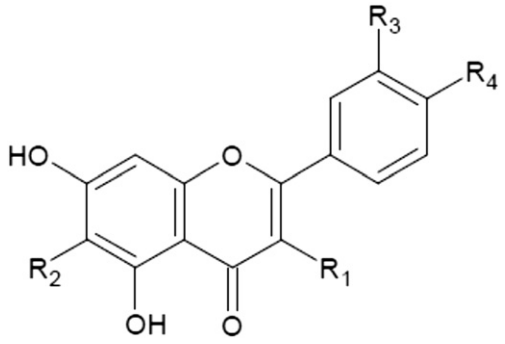

Fig. 1. Structure of flavonoids used in the study. Quercetin, R1 = R3 = R4 = OH, R2 = H; kaempferol, R1 = R4 = OH, R2 = R3 = H; rutin, R1 = glycoside, R2 = H, R3 = R4 = OH; apigenin, R1 = R2 = R3 = H, R4 = OH; baicalein, R1 = R3 = R4 = H, R2 = OH.

(DPBS), Hank's buffered salt solution (HBSS), Pierce™ IP Lysis Buffer, Halt™ Protease and Phosphatase Inhibitor Cocktail (100×), PrestoBlue™, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were purchased from Invitrogen (Paisley, UK). HEPES, forskolin, quercetin, kaempferol, rutin, apigenin, baicalein were purchased from Sigma-Aldrich (Bornem, Belgium; St. Louis, MO). RIA (radioimmunoassay) kit (EURIA-CCK) for CCK determination was purchased from EURO Diagnostica (Malmö, Sweden).

2.2. Cell culture

The intestinal neuroendocrine tumor STC-1 cells were purchased from ATCC, USA, and cultured according to instructions from ATCC. Cells were grown in Dulbecco's modified Eagle's Medium (DMEM, 4.5 g/L glucose) supplemented with 10% FBS, 1% penicillin and streptomycin. Then, incubation was done at 37 °C in the presence of 10% CO_2.

2.3. Stimulation of CCK secretion

Forskolin was used as a positive control as it is already reported to stimulate CCK secretion from STC-1 cells [24]. Cells were seeded in T25 flasks for 2 days. On the third day, when confluency reached almost 70%, the medium was removed and cells were washed twice with Hanks' Balanced Salt Solution buffer (HBSS) supplemented with 20 mM HEPES, pH 7.4. Samples (with forskolin, quercetin, kaempferol, apigenin, rutin and baicalein) were prepared in the same buffer used for washing in the presence of 0.1% DMSO. Cells were incubated with flavonoid samples for 2 h at 37 °C, under 10% CO_2 atmosphere. As a final concentration, 100 μM of forskolin and 20 μM of flavonoids were used. The concentration of forskolin was selected based on reported literature, while the flavonoid concentration was based on their solubility and the limitation to avoid possible complexation for CCK as already reported in previous work [1,18]. Control flasks contained only buffer. Culture medium were collected and kept at −80 °C until CCK determination.

After collecting the culture medium, cells were washed once with ice cold phosphate-buffered saline (PBS). Afterwards, 1 mL of cell lysis buffer supplemented with 1% protease and phosphatase inhibitor was added and flasks were incubated on ice for 5 min with periodic mixing. Next, cell lysate was collected with a scraper and transferred to Eppendorf tubes and centrifuged at 13,000*g* for 10 min. Supernatants were transferred to other tubes and kept in −80 °C for determination of CCK concentration.

CCK measurement was done by RIA using a rabbit antiserum raised against CCK-8 sulphate conjugated to bovine serum albumin. The cross-reactivities of this antiserum were really low for the non-sulfated member of the gastrin/CCK family that share the same C-terminal penta-peptide (Cholecystokinin 26–33 non-sulfated < 0.01%, Cholecystokinin 30–33 < 0.01%, Gastrin-17 sulphate 0.5%, and Gastrin-17 non-sulfated < 0.01%) but it should bind all biological active forms with equimolar potency (Cholecystokinin 26–33 sulphate 100.0%, Cholecystokinin-33 sulphate 134.0%). The sensitivity of the assay has been optimized at 0.3 pmol/L.

2.4. Analysis of cell viability upon exposure to phenolic compounds

Viability of STC-1 cells treated with forskolin and flavonoids was evaluated by the PrestoBlue™ and MTT assay. PrestoBlue™ reagent is a resazurin-based solution in which resazurin, blue in color and non-fluorescent, is converted to resorufin under the reducing power of viable cells. Resorufin has a red color and is highly fluorescent, thus can be used to determine cell viability and cytotoxicity. The MTT assay is a colorimetric assay for assessing mitochondrial activity of viable cells. Briefly for both assays, cells were seeded for 2 days at 40,000 cells per well in 96 well plates. Afterwards, medium was removed and cells were treated with 100 μM forskolin, 20 μM quercetin, kaempferol, apigenin, rutin and baicalein. After incubation for 2 h, cells were washed with buffer and a fresh culture medium was added. Both assays were carried out as reported before [1,18].

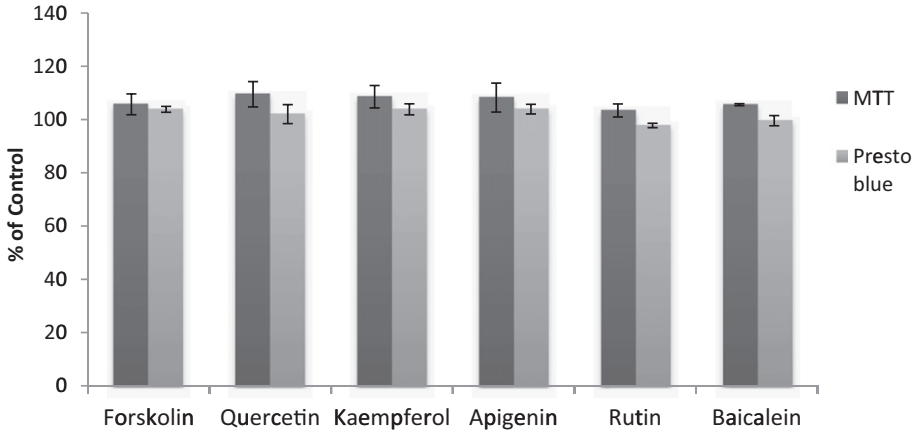

Fig. 2. Viability of STC-1 cells upon exposure to 100 μM of forskolin and 20 μM of flavonoids. Two assays were used, Presto blue and MTT. Each value is three biological replicates and expressed as the mean ± SEM. Values are presented as a percentage of the control (non-treated cells).