Pharmacological Reports xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Involvement of endogenous opioid peptides in the peripheral antinociceptive effect induced by the coffee specific diterpene kahweol

Luciana S. Guzzo [a], Thiago R.L. Romero [a], Celso M. Queiroz-Junior [b], Marcelo V. Caliari [b], Adolfo O. Azevedo [c], Andréa C. Perez [a], Igor D.G. Duarte [a,*]

[a] Department of Pharmacology, Institute of Biological Sciences, ICB-UFMG, Belo Horizonte, MG, Brazil
[b] Department of Pathology, Institute of Biological Sciences, ICB-UFMG, Belo Horizonte, MG, Brazil
[c] Department of Pharmacology, UNILAVRAS, Lavras, MG, Brazil

ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 19 February 2015
Accepted 23 February 2015
Available online xxx

Keywords:
Coffee diterpene
Kahweol
Peripheral antinociception
Opioid system
β-Endorphin

ABSTRACT

Background: Kahweol is a diterpene present in the oil derived from coffee beans. Although several pharmacological activities of kahweol are already well described in the literature, no study was done in order to assess the analgesic activity of this substance. Thus, the aim of this study was to investigate the possible peripheral antinociceptive effect of kahweol. Considering that the opioid peptides have been implicated in peripheral antinociception induced by non-opioidergic compounds, the present study also evaluated the endogenous opioids involvement in this effect.
Methods: The rat paw pressure test was used, and hyperalgesia was induced by intraplantar injection of prostaglandin E_2 (2 μg/paw). All drugs were administered subcutaneously in the hindpaws of male Wistar rats. The expression of β-endorphin was examined by immunohistochemistry in the skin tissue samples of the plantar surface of rat right hindpaws.
Results: Intraplantar injection of kahweol (40 and 80 μg) induced significant peripheral antinociception. The antinociceptive effect of kahweol was due to a local peripheral action because the higher dose (80 μg/paw) did not produce any effect in the contralateral paw. The opioid receptor antagonist naloxone (50 and 100 μg/paw) prevented action of kahweol (80 μg/paw) and the aminopeptidases inhibitor bestatin (400 μg/paw) potentiated the antinociceptive effect of kahweol (40 μg/paw). Furthermore, kahweol treatment increased the intensity of β-endorphin immunoreactivity in the epithelium of rat paws.
Conclusions: The results discussed here provide evidence that kahweol treatment has peripheral antinociceptive effect and suggest that this effect is mediated by the release of endogenous opioids.

Introduction

Coffee is among the most valuable products of the global economy and is one of the most consumed beverages in the world. Coffee is a complex chemical mixture reported to contain a variety of potentially bioactive compounds, and the frequent consumption of this beverage has piqued interest in studies related to these compounds [1].

Kahweol and its dehydro derivative, cafestol, are diterpenes found only in the unsaponifiable lipid fraction from raw coffee. Brewing releases oil droplets containing the two diterpenes from ground coffee beans, but they are retained by paper filters. The highest concentrations of these constituents occurs in Turkish and Scandinavian style boiled coffee, while instant, drip-filtered and percolated coffee brews contain negligible amounts [2].

Kahweol and cafestol have been shown to exhibit both adverse and chemoiprotective properties. It is well documented that a mixture of these diterpenes increases blood cholesterol in both human and animal models, probable by increasing the serum activity of alanine aminotransferase and to a lesser extent aspartate aminotransferase, and reducing those of γ-glutamyl-transferase in the liver [3,4]. However, animal studies have shown that kahweol and cafestol afford protection against the action of well-known carcinogens [5,6]. The chemoprotective effects of kahweol and cafestol have thus far been related to the induction of apoptosis [7–9] and beneficial modifications of the xenobiotic

* Corresponding author.
E-mail address: dimitri@icb.ufmg.br (Igor D.G. Duarte).

http://dx.doi.org/10.1016/j.pharep.2015.02.009
1734-1140/

metabolism, such as the reduced activation of mutagens and carcinogens, mainly *via* the inhibition of cytochrome P450 enzymes [5,10,11], as well as the detoxification of carcinogens and mutagens *via* the induction of carcinogen-detoxifying enzyme systems such as glutathione S-transferase and UDP-glucuronosyl transferase [6,12–14]. Furthermore, the hepatoprotective [15], anti-oxidant [16,17] and anti-inflammatory [18] activities of kahweol have already been reported in the literature. The anti-inflammatory properties of kahweol may be due to the suppression of cell adhesion molecule expression [19] and inhibition of cyclooxygenase-2 and inducible nitric oxide synthase expression in macrophages *via* suppression of the pro-inflammatory transcription factor NF-κB *in vitro*, reducing the prostaglandin E_2 (PGE_2) and nitric oxide levels [20,21].

PGE_2 is a principal pro-inflammatory prostanoid that is the primary contributor to the hyperalgesia [22]. The inhibitory activity against PGE_2 production [20] suggested an anti-inflammatory and analgesic activity of kahweol. Although there have been studies that demonstrate the anti-inflammatory activity of kahweol [18] and the antinociceptive activity of cafestol [23], there has been no report designed to investigate the antinociceptive properties of this diterpene. Furthermore, the opioid peptides have been implicated in peripheral antinociception induced by non-opioidergic compounds, including nonsteroidal anti-inflammatories [24], the diterpene cafestol [23], α_2-adrenergic drugs [25–27] and selective endothelin-B receptor agonists [28,29]. Thus, the present study was undertaken to determine whether kahweol presents a peripheral antinociceptive effect and to evaluate the involvement of endogenous opioid peptides in this effect.

Material and methods

Animals

All experiments were performed on 180–220 g male Wistar rats (from CEBIO-UFMG). The rats were housed in a temperature-controlled room (23 ± 1 °C) on an automatic 12 h light/dark cycle (06:00–18:00 h). All tests were conducted during the light phase (08:00–15:00 h). Food and water were freely available until the onset of the experiments. The Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais (UFMG) approved all animal procedures and protocols.

Measurement of hyperalgesia

Hyperalgesia was induced by subcutaneous injection of prostaglandin E_2 (PGE_2; 2 μg/paw) into the plantar surface of the hindpaw. Hyperalgesia was measured according to the paw pressure test described by Green and Young [30] and Randall and Sellito [31]. An analgesiometer was used (Ugo-Basile, Monvalle VA, Italy) fitted with a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force to the hindpaw. The weight in grams (g) required to elicit the nociceptive response of paw flexion was determined as the nociceptive threshold. A cut-off value of 300 g was used to reduce the possibility of paw damage. The animals were habituated in the algesimeter one day prior to the experiment. The nociceptive threshold was measured in the right paw and determined as the average of the three consecutive trials recorded prior to and 3 h after PGE_2 injection. The threshold was calculated as the difference between these two averages (Δ of nociceptive threshold) and is expressed in grams.

Experimental protocol

Kahweol (20, 40 or 80 μg/paw) was administered subcutaneously in the right hindpaw 2:55 h after local injection of PGE_2. In the protocol used to determine whether kahweol was acting outside the injection paw, PGE_2 was injected into both hindpaws, while kahweol was administered 2:55 h later into the left paw, after which the nociceptive threshold was measured in both hindpaws. Naloxone (25, 50 or 100 μg/paw) and bestatin (400 μg/paw) were administered 30 min prior to kahweol injection.

The protocols concerning dose and time of administration of each drug used in this study were obtained through literature data and pilots experiments. Overall, two experimenters were necessary to conduct these experiments, the measurements made by one unaware of the treatments given.

Chemicals

The following chemicals were used: PGE_2 (Sigma, St. Louis, MO, USA), kahweol (Axxora, CA, USA), naloxone (Sigma) and bestatin (Tocris, Ellisville, MO, USA). The drugs were dissolved as follows: PGE_2 (2% ethanol in saline); kahweol acetate (10% DMSO in saline); naloxone and bestatin (saline). All drugs were administered subcutaneously using an injected volume of 50 μl/paw, with the exception of PGE_2, where an injected volume of 100 μl/paw was used.

Immunohistochemistry reaction for β-endorphin

Kahweol (80 μg/paw) was administered 2:55 h after local administration of PGE_2 (2 μg/paw). After 5 min, the animals were killed by cervical dislocation, and the skin tissue samples were collected from the rat right hindpaws. Samples were fixed in 10% buffered formalin solution (pH 7.2, for 48 h), processed in alcohol and xylene and embedded in paraffin. Paraffin-embedded tissues were sectioned (4 μm) and collected on glass slides coated with 2% 3-aminopropyltriethylsilane (Sigma Chemicals, St. Louis, MO, USA) for processing by a standard immunohistochemistry technique.

Slides were deparaffinized, hydrated with decreasing concentrations of ethanol solutions and washed in citrate buffer (pH 6.0; Sigma–Aldrich Co., St. Louis, MO, USA) for 20 min at 95 °C for antigen retrieval. Soon afterward, sections were incubated with 3% hydrogen peroxide diluted in phosphate buffered saline (PBS; pH 7.4) for 20 min to block endogenous peroxidase, and then they were placed in a 6% milk solution to block unspecific binding. Thereafter, samples were incubated at 4 °C overnight in a humidified chamber with the mouse monoclonal antibody anti-β-endorphin (B31.15, Santa Cruz Biotechnology, CA, USA) diluted at 1:100 in 1% phosphate buffered saline–bovine serum albumin (PBS–BSA). Following incubation with the primary antibody, sections were washed in PBS and treated with biotinylated goat IgG (Zymed Laboratories Inc., San Francisco, USA) and streptavidin (Zymed Laboratories Inc., San Francisco, USA). The staining was detected by a solution of 0.05% 3,3μ-diaminobenzidine (DAB) and 0.2% H_2O_2. Finally, after a wash with distilled water, the slides were counterstained with Mayer's hematoxylin and mounted. Negative controls consisted of sections in which primary antibodies were replaced by PBS–BSA.

Morphometric analyses

Following the immunohistochemistry procedures, 10 distinct images of the epithelium surface of each skin sample were visualized using a 40X objective for digitalization, through a JVC TK-1270/RGB camera (Yokohama, Japan) adapted to a microscope (magnification 400×). Images were processed, excluding the connective tissue and epithelium corneal layer of the fields, to allow the single evaluation of the epithelial surface. The area of β-endorphin immunostaining was calculated through the selection of the corresponding positive immunostained pixels and

Please cite this article in press as: Guzzo LS, et al. Involvement of endogenous opioid peptides in the peripheral antinociceptive effect induced by the coffee specific diterpene kahweol. Pharmacol Rep (2015), http://dx.doi.org/10.1016/j.pharep.2015.02.009