Natural Diterpenes from Coffee, Cafestol, and Kahweol Induce Peripheral Antinociception by Adrenergic System Interaction

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- coffee
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Abstract
Cafestol and kahweol are diterpenes found only in the non-saponified lipid fraction of coffee. They are released during boiling and retained in the filtration process. Previous studies have shown peripheral antinociception induced by endogenous opioid peptides released by these diterpenes. Considering that the activation of the opioid system leads to a noradrenaline release, the aim of this study was to verify the participation of the noradrenergic system in the peripheral antinociception induced by cafestol and kahweol. Hyperalgesia was induced by an intraplantar injection of prostaglandin E2 (2 µg). Cafestol or kahweol (80 µg/paw) were administered locally into the right hindpaw alone, and after the agents α2-adrenoceptor antagonist yohimbine (5, 10 and 20 µg/paw), α2A-adrenoceptor antagonist yohimbine (5, 10 and 20 µg/paw), α2B-adrenoceptor antagonist RX 821002 (40 µg/paw), α1-adrenoceptor antagonist prazosin (0.5, 1 and 2 µg/paw), or β-adrenoceptor antagonist propranolol (150, 300 and 600 ng/paw), respectively. Noradrenaline reuptake inhibitor reboxetine (30 µg/paw) was administered prior to cafestol or kahweol low dose (40 µg/paw) and guanethidine 3 days prior to the experiment (30 mg/kg, once a day), depleting the noradrenaline storage. Intraplantar injection of cafestol or kahweol (80 µg/paw) induced a peripheral antinociception against hyperalgesia induced by PGE2. This effect was reversed by intraplantar injections of yohimbine, rauwolscine, prazosin and propranolol. Reboxetine injection intensified the antinociceptive effect of cafestol or kahweol low-dose, and guanethidine reversed almost 70% of the cafestol or kahweol-induced peripheral antinociception. This study gives evidence that the noradrenergic system participates in cafestol and kahweol-induced peripheral antinociception with the release of endogenous noradrenaline.

Introduction
Coffee is one of the most consumed beverages worldwide. Its high global consumption has stimulated several studies related to the grain and other constituents [1]. Cafestol and kahweol are diterpenes with similar chemical structures, differentiated only by the presence of a double bond in a ring of the cafestol molecule [2], as showed in Fig. 1. They are found only in the non-saponified lipid fraction of coffee, released during boiling, and retained in the filtration process [2]. Several studies have shown the antioxidant [3–5], antitumorigenic [6–12] and anti-inflammatory [13–15] effects of these diterpenes, and recently the involvement of cafestol and kahweol in peripheral antinociception was demonstrated [16].

Using the rat paw pressure test and hyperalgesia by intraplantar injection of prostaglandin E2 (2 µg/paw) our group showed that cafestol and kahweol were able to induce peripheral antinociception in Wistar rats. The mechanism suggested for this event was the release of endogenous opioid peptides and the activation of the opioid system [16]. Opioid system activation leads to a noradrenaline (NA) release in supra-spinal, spinal and peripheral sites [17–21]. Besides, other analgesic molecules, such as endocannabinoids, anandamide and N-palmitoyl-ethanolamine, were also able to induce a peripheral antinociception that interacts with adrenergic receptors leading to a NA release [22].

NA is an adrenergic agonist involved in the intrinsic control of pain inducing a pronociceptive effect in the primary afferent nociceptors [23] or a
The participation of their antinociceptive effect (data not shown). The difference between the chemical structures did not interfere in evaluated. The antinociceptive effects of these diterpenes were producing hyperalgesia or antinociception when administered alone effect of both molecules in a dose-dependent manner without interference in the peripheral antinociception induced by cafestol and kahweol.

Results

Cafestol and kahweol induced peripheral antinociception by interaction with \( \alpha_2 \)-adrenoceptors. In the present study the peripheral antinociceptive effect of cafestol and kahweol (80 µg/paw) against PGE2 hyperalgesia was evaluated. The antinociceptive effects of these diterpenes were similar in potency and time-response, indicating that the small difference between the chemical structures did not interfere in their antinociceptive effect (data not shown).

The participation of \( \alpha_2 \)-adrenoceptor in the peripheral antinociception effect induced by cafestol or kahweol was verified using a non-selective \( \alpha_2 \)-adrenoceptor antagonist, yohimbine (5, 10 and 20 µg/paw), which antagonized the peripheral effect of cafestol or kahweol (80 µg/paw) in a dose-dependent manner, and without causing hyperalgesia or antinociception when administered alone (\( \circ \) Fig. 2A). We also investigated the selectivity of the \( \alpha_2 \)-subtypes involved in the peripheral antinociception. The \( \alpha_2 \)-C-adrenoceptor subtype was the only one that interacted with cafestol and kahweol producing peripheral antinociception. The \( \alpha_2 \)-C-adrenoceptor antagonist rauwolscine (10, 15 and 20 µg/paw) blocked the peripheral antinociceptive effect in a dose-dependent manner (\( \circ \) Fig. 2B). No effects were observed with blockers for \( \alpha_2 \)-A and \( \alpha_2 \)-D subtypes (\( \circ \) Fig. 3).

The participation of \( \alpha_1 \)-adrenoceptor in the peripheral antinociceptive effect induced by cafestol or kahweol (80 µg/paw) against PGE2 was verified using the selective \( \alpha_1 \)-adrenoceptor antagonist prazosin (0.5, 1 and 2 µg/paw) that antagonized the peripheral effect of both molecules in a dose-dependent manner without inducing hyperalgesia or antinociception when administered alone (\( \circ \) Fig. 4A, B). In the same way, the \( \beta \)-adrenoceptor antagonist propranolol (150, 300 and 600 ng/paw) blocked the peripheral antinociceptive effect of cafestol and kahweol (80 µg/paw) against PGE2 in a dose-dependent manner and no effect was verified when it was injected alone into normal or hyperalgesic paws (\( \circ \) Fig. 4C, D).

Reboxetine, 30 µg/paw, was administered into the right hind paw 30 min prior to cafestol or kahweol low dose injection (40 µg/paw). Cafestol or kahweol antinociception against PGE2, with a low dose, when administered with reboxetine (\( \circ \) Fig. 5) was similar to the antinociception observed with the injection of a high dose 80 µg/paw (\( \circ \) Fig. 5). Guanethidine (30 mg/kg) was administered into the peritoneal cavity 3 days prior to the experiment, to deplete peripheral sympathetic amines [27]. Cafestol or kahweol was injected 5 minutes prior to the PGE2 action peak. Depletion of peripheral sympathomimetic amines reversed the peripheral antinociceptive effect induced by both molecules against PGE2 (\( \circ \) Fig. 6).

Discussion

Cafestol and kahweol are diterpenes found only in the non-saponified lipid fraction of coffee, with similar chemical structures and functions. Our group demonstrated for the first time a peripheral antinociceptive effect of cafestol against PGE2-induced rat paw hyperalgesia [16]. Cafestol may induce a release of endogenous opioid peptides, leading to the activation of opioid receptors in nociceptive afferent neurons [16]. Moreover, opioids can produce peripheral antinociception by activation of adrenergic receptors after NA release [17]. NA has usually been associated with peripheral nociception [23], but in the last years NA has been shown to contribute to peripheral antinociception [13, 14]. In this way, the present study investigated the involvement of adrenergic system in the peripheral antinociception induced by cafestol and kahweol.

Yohimbine, the classical non-selective \( \alpha_2 \)-adrenoceptor antagonist, was used to verify the role of \( \alpha_2 \)-adrenoceptor in cafestol and kahweol antinociception, and it was able to antagonize this antinociceptive effect. \( \alpha_2 \)-adrenoceptor is classified in \( \alpha_2 \)-A, \( \alpha_2 \)-B, \( \alpha_2 \)-C and \( \alpha_2 \)-D subtypes, based on radioligand binding studies in the dorsal root ganglion (DRG) [28, 29], and on pharmacological studies [30, 31]. The antinociception induced by \( \alpha_2 \)-adrenoceptor agonists appears to be mediated by specific receptor subtypes [32, 33]. Thus, we investigated the selective role of \( \alpha_2 \)-A, \( \alpha_2 \)-B, \( \alpha_2 \)-C and \( \alpha_2 \)-D in cafestol and kahweol antinociception using specific adrenoceptor antagonists. Only the \( \alpha_2 \)-C-adrenoceptor subtype participated in the peripheral antinociceptive effect of cafestol or kahweol, because only rauwolscine was able to reverse the peripheral antinociceptive effect of both molecules, similar to the \( \alpha_2 \)-adrenoceptor agonists xylazine [34] and clonidine [30] that induce a peripheral antinociceptive effect mediated by the \( \alpha_2 \)-C-adrenoceptor subtype. Several studies have shown that others \( \alpha_2 \)-subtypes, such as \( \alpha_2 \)-A-adrenoceptors, induced antinociception at spinal [32, 33] and supra-spinal levels [32, 35], but this has not been associated with peripheral analogic effects [30, 34]. The \( \alpha_2 \)-D-adrenoceptor subtype is controversial, it is uncertain whether it exists as an actual separate subtype or as a more commonly accepted variant of subtype \( \alpha_2 \)-A. On the other hand, the \( \alpha_2 \)-D-adrenoceptor has also been associated with peripheral hyperalgesia [34].

In the DRG there are also other adrenoceptor subtypes, like \( \alpha_1 \) [36, 37] and \( \beta_2 \) [38] that were traditionally associated with peripheral hyperalgesia [27, 39–41] but are also involved in peripheral antinociceptive events [24, 43–45]. That is why we also investigated the role of \( \alpha_1 \)- and \( \beta \)-adrenoceptors in the antinociceptive effect of cafestol and kahweol. Administration of prazosin, an \( \alpha_1 \)-antagonist or propranolol, a \( \beta \)-antagonist, reversed
the cafestol or kahweol antinociception in a dose-dependent manner. The $\alpha_1$-adrenoceptor participated in the hyperalgesic effect induced by noradrenaline plantar injection [42], and $\beta$-adrenoceptors are involved in peripheral hyperalgesia induced by carrageenan and adrenaline [27,40]. Controversially, the adrenoceptors $\alpha_1$ and $\beta$ were involved in the antinociceptive events. Activation of $\beta$-adrenoceptor in patients with rheumatoid arthritis [43] and in rats with arthritis induced by adjuvants [44,45] provided analgesia. Binder et al. [24] showed that activation of receptors $\alpha_1$, $\alpha_2$, and $\beta_2$-adrenoceptor by NA on immune cells during inflammation may induce antinociception via release of endogenous opioids. In this sense, cafestol or kahweol may induce the release of NA, which induces peripheral antinociception indirectly by endogenous opioid release or directly by activation of the NO/cGMP analgesic pathway and consequent activation of K+ channels [25]. Alternatively, cafestol or kahweol injection may induce an opioid release [16] that produces antinociception directly by the activation of opioid receptors [21] or indirectly by the release of NA. Exogenous opioids, morphine, SNC80 and bremmazocine can also induce peripheral antinociception by NA release and activation of the adrenergic system [17]. This interaction could occur indirectly via cell activation, once that opioid receptors were demonstrated in cells of the immune system [46,47] and in keratinocytes [48–50] that can synthesize and release endogenous catecholamines [51,52]. Direct activation can also occur via receptor dimer complex. Aley and Levine [53] postulated a possible existence of a receptor complex composed of $\mu$ opioid, $\alpha_2C$-adrenoceptor and A1 adenosine receptors in the periphery, responsible for the peripheral antinociceptive effect induced by opioid, adrenergic and adenosine agonists. Jordan et al. [54] proposed a physical and functional association between $\mu$ opioid receptors and $\alpha_2A$-adrenoceptors on cells. In this way, endogenous cannabinoids, anandamide and N-palmitoyl-ethanolamine were able to elicit antinociceptive effect in rat paw prostaglandin $E_2$-induced hyperalgesia by the release of NA and interaction with $\alpha_1$, $\alpha_2$, and $\beta_2$-adrenoceptors [22]. The cannabinoid receptors were characterized in cells of the immune system [55] and in keratinocytes [55–57] that can synthesize and release endogenous catecholamines [58–60]. A previous study described a $CB_1/\beta_2$-receptor complex in human kidney embryonic cells, indicating a physical and functional interaction between the cannabinoid receptor and the adrenoceptor [61]. In this sense, several analgesic molecules may induce peripheral antinociception by NA release and adrenoceptors activation. To
Fig. 3 Subtypes of α-adrenoceptors α_2A, α_2B and α_2D did not interfere in the peripheral antinociception produced by cafestol (A) or kahweol (B) in hyperalgesic paws (PGE_2, 2 µg). RX (40 µg/paw), BRL (40 µg/paw) and IMI (40 µg/paw) were administered 30 min prior to cafestol or kahweol (80 µg/paw). Each column represents the mean ± S.E.M. (n = 4–5). * indicates significant differences compared to the (PGE_2 + veh 1) group (p < 0.05, ANOVA + Bonferroni test). Veh 1 = 10% DMSO in saline.

Fig. 4 Prazosin (A, B) and propranolol (C, D)-induced antagonism of the peripheral antinociception produced by cafestol (A, C) or kahweol (B, D) in hyperalgesic paws (PGE_2, 2 µg). Prazosin (PRA 0.5, 1 and 2 µg/paw) and propranolol (PROP 150, 300 and 600 ng/paw) were administered 30 min prior to cafestol or kahweol (80 µg/paw). Each column represents the mean ± S.E.M. (n = 4–5). * and # indicate significant differences compared to (PGE_2 + veh 1 + veh 2) or (PGE_2 + veh 1 + cafestol or kahweol)-injected groups, respectively (p < 0.05, ANOVA + Bonferroni test). Veh 1 = 10% DMSO in saline, Veh 2 = 2% ethanol in saline.
evaluate this hypothesis we verified the effect of cafestol or kahweol in rats treated with reboxetine, a NA reuptake inhibitor [62], and guanethidine that induce a gradual depletion of NA stores in nerve endings [27]. Acute reboxetine injection provided an intensification of the peripheral antinociceptive effect induced by low-doses of cafestol or kahweol (40 µg/paw). In addition, guanethidine injection resulted in an almost 70% reversal of the peripheral effect of cafestol or kahweol (80 µg/paw), indicating that the antinociception induced by these molecules was partially dependent of NA release.

These results suggest that NA release seems to be an important mechanism in which cafestol and kahweol exert its antinociceptive effect against hyperalgesia induced by PGE2 peripherally. Therefore, these diterpenes present in coffee have a potential applicability in painful conditions, expanding prospects for future research.

Materials and Methods

Animals

The experiments were performed on 180–220 g male Wistar rats from CEBIO-UFMG (Animal House of the Federal University of Minas Gerais). The animals were housed in a temperature-controlled room (23°C) on an automatic 12 h light/dark cycle (6:00am–6:00 pm light phase). All tests were conducted during the light phase (8:00am–3:00 pm). Food and water were freely available until the beginning of the experiments. The animals were sacrificed after experiments. All animal procedures and protocols were approved in 06/04/2013 by the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais (UFMG) protocol 50/2013.
Measurement of hyperalgesia

Hyperalgesia was induced by a subcutaneous injection of prostaglandin E2 (PGE2) (2 µg) into the plantar surface of rat’s hindpaw and measured according to the paw pressure test [26]. An analgesimeter (Ugo-Basile) was used with a cone-shaped rounded tip paw-pressor which applies a linearly increasing force to the hindpaw. The weight in grams (g) required to elicit a nociceptive response, paw flexion, was determined as the nociceptive threshold. A cut-off value of 300 g was used to prevent damage to the paws. The nociceptive threshold was measured in the right paw and determined as the average of the three consecutive trials recorded before and 3 h after the PGE2 injection. The difference between these two averages (Δ of nociceptive threshold) was calculated and also was expressed in grams.

Chemicals

The drug used as hyperalgesic agent was PGE2 (Cayman). The stock solution of PGE2 was prepared in ethanol, and further dilutions were made in saline; the final concentration of ethanol was 98% or more. The drug used in this study were obtained through literature data and Sigma-Aldrich) were dissolved in saline. The α2-adrenoceptor antagonist yohimbine (YOH; Sigma-Aldrich), the α2A-adrenoceptor antagonist BRL 44480 [(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isodoindole maleato (BRL; Tocris), the α2B-adrenoceptor antagonist imiloxan [RS 21361/2-(1-Ethyl-2-imidazoyl)methyl-1,4-benzodioxan hydrochloride] (IMI; Tocris), the α2C-adrenoceptor antagonist rauwolscine (corynanthidine α-yohimbine/17α-hydroxy-20α-yohimbana-16β-carboxylic acid, methyl ester hydrochloride) (RAU; Tocris), the α2D-adrenoceptor antagonist RX 821002[2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazole hydrochloride] (RX; Tocris), the α1-adrenoceptor antagonist prazosin (PRA; Sigma-Aldrich), the β-adrenoceptor antagonist propranolol (PROP; Sigma-Aldrich), the noradrenaline reuptake inhibitor reboxetine (REB; Pfizer), and a dopaminergic neurons from 6-hydroxydopamine-derived oxidative stress. FEBS Lett 2002; 76: 209–217

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Conflict of Interest

The authors indicate no conflicts of interest.
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