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Analysis of the mechanisms underlying the antinociceptive effect of epicatechin in diabetic rats

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ABSTRACT

Aims: The purpose of this study was to investigate the antinociceptive effect of epicatechin as well as the possible mechanisms of action in diabetic rats.
Main methods: Rats were injected with streptozotocin to produce hyperglycemia. The formalin test was used to assess the nociceptive activity.
Key findings: Acute pre-treatment with epicatechin (0.03–30 mg/kg, i.p.) prevented formalin-induced nociception in diabetic rats. Furthermore, daily or every other day treatment for 2 weeks with epicatechin (0.03–30 mg/kg, i.p.) also prevented formalin-induced nociception in diabetic rats. Acute epicatechin-induced antinociception was prevented by L-NAME (N^{ω}-nitro-L-arginine methyl ester hydrochloride, 1–10 mg/kg, non-selective nitric oxide synthesis inhibitor), 7-nitroindazole (0.1–1 mg/kg, selective neuronal nitric oxide synthesis inhibitor), ODQ (1H-(1,2,4)-oxadiazolo(4,2-a)quinoxalin-1-one, 0.2–2 mg/kg, guanylyl cyclase inhibitor) or glibenclamide (1–10 mg/kg, ATP-sensitive K^+ channel blocker). Moreover, epicatechin (3 mg/kg)-induced antinociception was fully prevented by methiothepin (0.1–1 mg/kg, serotonergic receptor antagonist), WAY-100635 (0.03–0.3 mg/kg, selective 5-HT_{1A} receptor antagonist) or SB-224289 (0.03–0.3 mg/kg, selective 5-HT_{1B} receptor antagonist). In contrast, BRL-15572 (0.03–0.3 mg/kg, selective 5-HT_{1D} receptor antagonist) only slightly prevented the antinociceptive effect of epicatechin. Naloxone (0.1–1 mg/kg, opioid antagonist) did not modify epicatechin's effect.
Significance: Data suggest the involvement of the nitric oxide–cyclic GMP–K^+ channel pathway as well as activation of 5-HT_{1A} and 5HT_{1B}, and at a lesser extent, 5-HT_{1D}, but not opioid, receptors in the antinociceptive effect of epicatechin in diabetic rats. Our data suggest that acute or chronic treatment with epicatechin may prove to be effective to treat nociceptive hypersensitivity in diabetic patients.

Introduction

Diabetes mellitus is one of the most serious problems in developing as well as developed countries (Sharma et al., 2009), affecting about 347 million people around the world (WHO, 2012). Diabetic neuropathy is the most common of the complications associated to diabetes mellitus with nerve damage developing in over 50% of all diabetic patients (Calcutt, 2010; Tesfaye et al., 2011). Spontaneous pain, allodynia and hyperalgesia occur in diabetic patients and the etiology of these conditions is not well understood (Jolivalt et al., 2008; Obrosova, 2009). In fact, the management of painful diabetic neuropathy is complicated and relief of pain is frequently unsatisfactory (Tesfaye and Selvarajah, 2012). Therefore, more pharmacological treatments are needed to relieve this condition.

Epicatechin is a flavonoid present in cacao and its derivatives, green tea, grape seeds, strawberries and red wine (Engler et al., 2004; Schewe et al., 2008). Several studies in animals have demonstrated that epicatechin has beneficial effects in chronic degenerative diseases (Si et al., 2011; Al-Gayyar et al., 2011; Gómez-Guzmán et al., 2011; Mohamed et al., 2011). It has been recently shown that epicatechin exerts antinociceptive effects in several models of chemical nociception (Lopes et al., 2010, 2012). Since naloxone (opioid receptors antagonist), glibenclamide (ATP sensitive K^+ channel blocker), ketanserin (5-HT_{2A} receptor antagonist) and pindolol (5-HT_{1A} receptor antagonist), but not ondansetron (5-HT_3 receptor antagonist), prevented the antinociceptive effect of epicatechin, the authors suggested the participation of opioid receptors, ATP sensitive K^+ channels and 5-HT_{1A} and 5-HT_{2A} receptors in the antinociceptive effect of epicatechin (Lopes et al., 2012). In addition, epicatechin activates nitric oxide synthase through phosphorylation (Ramírez-Sánchez et al. 2012). Previous reports from our group have found that many drugs (morphine, some NSAIDs, gabapentin, etc.) that

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activate the nitric oxide release are able to produce antinociception (Ortiz et al., 2002, 2003, 2006, 2012). However, to date the participation of other 5-HT_1 receptor subtypes or the nitric oxide–cyclic GMP–K^+ channel pathway in epicatechin effects in diabetic rats has not been studied. Thus, the purpose of this study was to investigate the antinociceptive effect of acute and chronic treatment with epicatechin in streptozotocin-induced diabetic rats using the formalin test. Furthermore, we also assessed the possible mechanisms of action involved in the antinociceptive effect induced by this drug.

Materials and methods

Animals

Experiments were performed on adult female Wistar rats (body weight range, 220 to 240 g). Female rats were used based on the fact that previous experiments in our conditions (Wistar rats, formalin concentration 0.5–1% and weight of range 180–220 g) have not shown significant differences between males and females (unpublished data). Other authors have found differences only with other rat strains, greater weight or different formalin concentrations (Gaumond et al., 2002). The animals were obtained from our own breeding facilities and had free access to food and drinking water before experiments. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the Institutional Animal Care and Use Committee approved our study (Cinvestav, México City).

Induction of diabetes

Experimental diabetes type I was induced by a single intra-peritoneal injection of streptozotocin (50 mg/kg) (Courteix et al., 1993). Diabetes was confirmed 1 day or 2 weeks later by measuring the tail vein blood glucose levels with an Accu-Check glucometer (Roche, Mexico City). Only animals with blood glucose levels higher than 300 mg/dL were considered as diabetic and included in the study.

Assessment of nociception

Nociception in non-diabetic and diabetic rats (2 weeks) was assessed using the 0.5% formalin test (Juárez-Rojop et al., 2006; Sánchez-Ramírez et al., 2006). The rats were placed in open acrylic observation chambers for 30 min to allow them to acclimate to their surroundings; then they were removed for formalin administration. Fifty microliters of diluted formalin (0.5%) was injected subcutaneously into the dorsum of the right hind paw with a 30-gauge needle. The animals were returned to the chambers and nociceptive behavior was observed immediately after formalin injection. Mirrors were placed in each chamber to enable unhindered observation. Nociceptive behavior was quantified as the numbers of flinches of the injected paw during 1-min periods every 5 min up to 60 min after injection (Wheeler-Aceto and Cowan, 1991). Flinching was readily discriminated and was characterized as rapid and brief withdrawal or as flexing of the injected paw. Formalin induced flinching behavior was biphasic (Wheeler-Aceto and Cowan, 1991). The initial acute phase (0–10 min) was followed by a relatively short quiescent period, which was then followed by a prolonged tonic response (15–60 min). The animals were used only once and at the end of the experiment they were euthanized in a CO_2 chamber.

Drugs

(−)-Epicatechin [(−)-cis-3,3′,4′,5,7-pentahydroxyflavane, (2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol] was freshly prepared in deionized water. Methiothepin (1-[10,11-dihydro-8-(methylthio)dibenzo[b,f]thiepin-10-yl]-4-methylpiperazine mesylate salt), naloxone hydrochloride dihydrate (naloxone), BRL-15572 (3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol hydrochloride), WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt) and L-NAME (N^{ω}-nitro-L-arginine methyl ester hydrochloride) were dissolved in 0.9% saline solution. Glibenclamide (5-chloro-N-[4-(cyclohexylureidosulfonyl) phenethyl]-2-methoxybenzamide) and SB-224289 (1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl] carbonyl]-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3,4′-piperidine] hydrochloride) were dissolved in 5% DMSO. ODQ (1H-(1,2,4)oxadiazolo (4,3-A) quinoxalin-1-one) was dissolved in 25% DMSO. 7-nitroindazole (7-nitro-1H-indazole) was dissolved in 10% Tween 20. All drugs were purchased from Sigma-Aldrich (St. Louis, MO).

Study design

Independent groups of animals ($n = 6$) were used for each experimental condition. For the acute treatment, two week-diabetic rats received saline or epicatechin (3 mg/kg, i.p.) at 30, 60 or 90 min before formalin injection. It was observed that the best antinociceptive effect was reached with the 60 min pre-treatment. Thus, this pre-treatment time was used in subsequent experiments. In order to determine the dose–response relationship of epicatechin, two week-diabetic rats were administered with increasing doses of epicatechin (0.03, 0.3, 3 or 30 mg/kg, i.p.). As the best antinociceptive effect was reached with epicatechin 3 mg/kg, this dose was selected for the chronic treatment as well as for the mechanism studies.

For the chronic treatment, we choose 2 schemes of treatment. Diabetic rats (1 day after streptozotocin injection, blood glucose ≥ 300 mg/dL) were administered daily or every other day with epicatechin (0.03–30 mg/kg, i.p.) for 2 weeks. A group of diabetic rats received treatment with saline at the same times. One day after the last epicatechin administration, the animals were submitted to the 0.5% formalin test, as previously described. At the end of the experiment, the rats were euthanized in a CO_2 chamber.

Since repeated treatment with epicatechin may lead to side effects, the effects of the greatest tested doses of acute or chronic epicatechin on motor co-ordination were assessed in the diabetic rats as previously reported (Ambriz-Tututi and Granados-Soto, 2007).

In order to investigate the possible participation of the nitric oxide–cyclic GMP–K^+ channel pathway in the antinociceptive effect of epicatechin, we used the non-selective and selective neuronal nitric oxide synthase inhibitor L-NAME (1–10 mg/kg, i.p.) (Brignola et al., 1994) and 7-nitroindazole (0.1–1 mg/kg, i.p) (Bujalska and Gumułka, 2008), respectively, guanylyl cyclase inhibitor ODQ (0.2–2 mg/kg, i.p.) (Jiménez-Andrade et al., 2008) and the ATP-sensitive K^+ channel blocker glibenclamide (1–10 mg/kg, i.p.) (Ortiz et al., 2012). Furthermore, in order to investigate the possible involvement of 5-HT_1 receptor subtypes, the non-selective 5-HT receptor antagonist methiothepin (0.1–1 mg/kg, i.p.) (Berge, 1982), selective 5-HT_{1A} receptor antagonist WAY-100635 (0.03–0.3 mg/kg, i.p.) (Laporte et al., 1994), selective 5-HT_{1B} receptor antagonist SB-224289 (0.03–0.3 mg/kg, i.p.) (Gaster et al., 1998) and selective 5-HT_{1D} receptor antagonist BRL-15572 (0.03–0.3 mg/kg, i.p.) (Hagan et al., 1997) were used. Finally, to investigate the participation of opioid receptors, we used the non-selective opioid receptor antagonist naloxone (0.1–1 mg/kg, i.p.) (Halder et al., 2009). All antagonists were administered 10 min before epicatechin administration which was given 60 min before formalin injection.

Data and statistical analysis

Data of formalin-induced acute nociception are expressed as mean number of flinches or as the area under the curve (AUC), an expression of the duration and intensity of the effect. Area under the curve was calculated by the trapezoidal rule. Data are expressed as the mean ± S.E.M. of 6 animals.