



Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

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

Full paper

Effects of chlorogenic acid on carbachol-induced contraction of mouse urinary bladder

Takeharu Kaneda ^{a,*}, Noriyasu Sasaki ^b, Norimoto Urakawa ^a, Kazumasa Shimizu ^a^a Laboratory of Veterinary Pharmacology, School of Veterinary Medicine, Nippon Veterinary and Life Science University, 7-1 Kyonan-cho 1-chome, Musashino, Tokyo 180-8602, Japan^b Laboratory of Veterinary Biochemistry, School of Veterinary Medicine, Nippon Veterinary and Life Science University, 7-1 Kyonan-cho 1-chome, Musashino, Tokyo 180-8602, Japan

ARTICLE INFO

Article history:

Received 31 July 2017

Received in revised form

19 November 2017

Accepted 6 December 2017

Available online xxx

Keywords:

Chlorogenic acid

Mouse urinary bladder

Phosphodiesterase

cAMP

ABSTRACT

Chlorogenic acid (CGA) is a polyphenol found in coffee and medicinal herbs such as *Lonicera japonica*. In this study, the effect of CGA-induced relaxation on carbachol (CCh)-induced contraction of mouse urinary bladder was investigated. CGA (30–300 µg/ml) inhibited CCh- or U46619-induced contraction in a concentration-dependent manner. SQ22536 (adenylyl cyclase inhibitor) recovered CGA-induced relaxation of CCh-induced contraction; however, ODQ (guanylyl cyclase inhibitor) did not have the same effect. In addition, 3-isobutyl-1-methylxanthine (IBMX) enhanced CGA-induced relaxation; however, forskolin or sodium nitroprusside did not have the same effect. Moreover, Ro 20–1724, a selective phosphodiesterase (PDE) 4 inhibitor, enhanced CGA-induced relaxation, but vardenafil, a selective PDE5 inhibitor, did not have the same effect. In the presence of CCh, CGA increased cyclic adenosine monophosphate (cAMP) level, whereas SQ22536 inhibited the increase of cAMP levels. Moreover, higher cAMP levels were obtained with CGA plus IBMX treatment than the total cAMP levels obtained with separate CGA and IBMX treatments. In conclusion, these results suggest that CGA inhibited CCh-induced contraction of mouse urinary bladder by partly increasing cAMP levels via adenylyl cyclase activation.

© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of Japanese Pharmacological Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Chlorogenic acid (CGA) is a polyphenol that is widely distributed in coffee, fruits, and medicinal herbs such as *Lonicera japonica*, *Caulis Lonicerae Japonicae*, and *Gardenia jasminoides*. The physiological activities of CGA have been recently widely studied. Many studies have revealed that CGA possesses antiinflammatory,¹ anti-oxidative,^{2,3} antitumor,⁴ and antiviral⁵ properties.

Recent studies have indicated that coffee intake increased the plasma and urine concentrations of CGA and its metabolites in humans.⁶ Moreover, in traditional Japanese medicine, *L. japonica* is used for treating suppurative disease and urinary tract inflammation. However, to our knowledge, there are no reports to show the effect of CGA on muscle contraction of the urinary bladder.

Furthermore, it has been reported that CGA increased cAMP and/or cGMP level in basophilic leukemia cell line⁷ and plate.^{8,9} Therefore, to elucidate the precise mechanism underlying CGA-induced relaxation of urinary bladder smooth muscles, we assessed the effect of CGA alone and in the presence of cyclic nucleotide-related agents on muscle contraction and investigated its effect of cAMP and cGMP levels in isolated mouse urinary bladder.

2. Materials and methods

2.1. Muscle preparation and tension measurement

Male mouse (ddy strain; weight, 20–25 g; Tokyo Laboratory Animals, Tokyo) were bled after stunning, and the urinary bladder was immediately excised. Trigonum vesicae, superficial tissues, fats, and mucous layer were removed. Longitudinal urinary bladder strips (approximately 10 mm in length and 3 mm in width) were incubated in physiological salt solution (PSS) containing 136.8 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl₂, 1.0 mM MgCl₂, 11.9 mM NaHCO₃,

* Corresponding author. Fax: +81 422 31 4457.

E-mail address: t-kaneda@nvl.u.ac.jp (T. Kaneda).

Peer review under responsibility of Japanese Pharmacological Society.

and 5.6 mM glucose. PSS was aerated with 95% O₂ and 5% CO₂ to adjust pH to 7.2 at 37 °C. This study was conducted according to the Guidelines for the Care and Use of Laboratory Animals of Nippon Veterinary and Life Science University (approval number 13–65).

Muscle tension was isometrically recorded using a strain gauge transducer (TB-611T; Nihon Kohden, Tokyo). One end of each strip was bound to a glass holder and the other end was connected with a silk thread to the transducer in an organ bath containing PSS, with a resting tension of 1 g. The muscle strips were equilibrated for 30 min to obtain stable contractility that was induced by hyperosmotically added 65 mM KCl.

The strips were contracted by carbachol (CCh, 3 μ M) or U46619 (10 μ M) and after CCh- or U46619-induced contraction reached steady level treated with CGA (30–300 μ g/ml) for 5–10 min at each concentration to obtain cumulative concentration–response curves. Moreover, the effect of CGA was evaluated in the presence of SQ22536 (100 μ M), an adenylyl cyclase inhibitor; 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 30 μ M), a soluble guanylyl cyclase inhibitor; forskolin (1 μ M), an adenylyl cyclase activator; sodium nitroprusside (SNP, 1 μ M), a guanylyl cyclase activator; 3-isobutyl-1-methylxanthine (IBMX, 10 μ M), a nonselective cyclic nucleotide phosphodiesterase (PDE) inhibitor; vardenafil (10 μ M), a selective cyclic guanosine monophosphate (cGMP) PDE isoenzyme (PDE5) inhibitor; and Ro 20–1724 (30 μ M), a selective cAMP PDE

isoenzyme (PDE4) inhibitor. The inhibitor concentrations used in this study were selected according to previous literature.^{10–13}

2.2. cAMP and cGMP assays

The cAMP and cGMP levels in the muscle strips were measured using enzyme immunoassays, as previously described.^{11,14} In brief, muscles strips were incubated with 1 μ M carbachol (CCh), CGA, IBMX, CGA + IBMX, or CGA + SQ22536 in an organ bath for 10 min. After incubation, the muscle strips were rapidly frozen in liquid nitrogen and stored at –80 °C until homogenization with 0.5 ml of 6% trichloroacetic acid. The homogenates were centrifuged at 3000 \times g for 15 min, and the supernatant was washed four times with 2.0 ml water-saturated diethyl ether. cAMP and cGMP levels were measured using an enzyme immunoassay kit (GE Healthcare/Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) and were expressed as p mol/g of wet weight.

2.3. Chemicals

Chemicals used were chlorogenic acid hemihydrate (Wako Pure Chemical, Osaka, Japan), carbamylcholine chloride (carbachol), forskolin, sodium nitroprusside, (Sigma–Aldrich, St. Louis, USA), Ro 20–1724 (LC Laboratories, Woburn, MA, USA), vardenafil (LKT

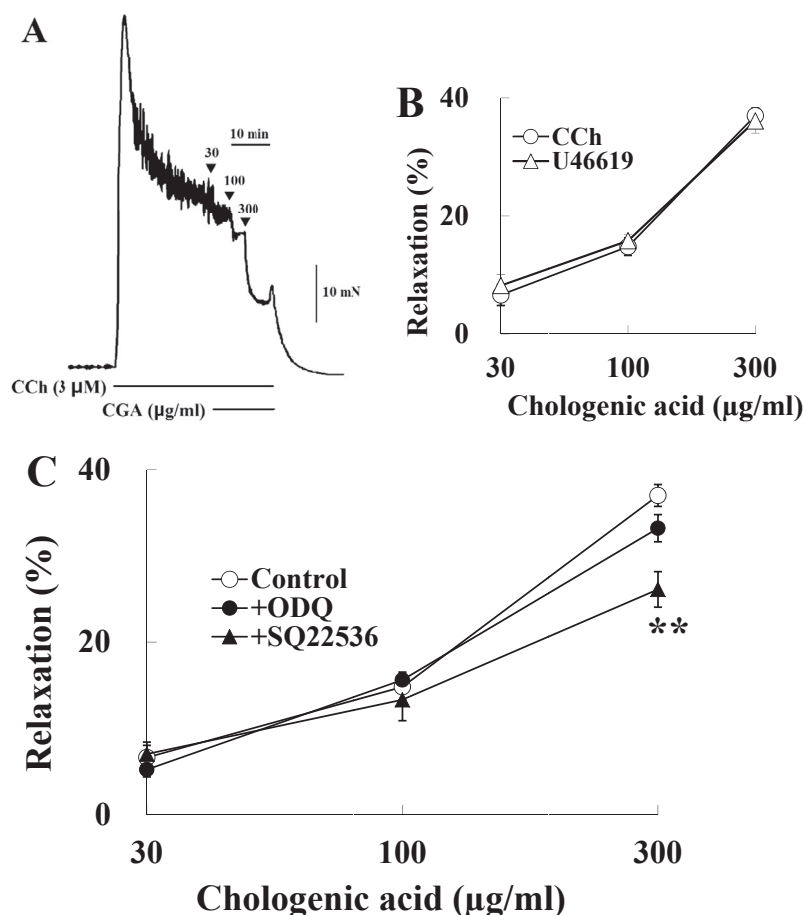


Fig. 1. Effect of chlorogenic acid (CGA) on carbachol (CCh)- or U46619-induced contraction. (A) A typical trace in which CGA inhibited CCh (3 μ M)-induced contraction in mouse urinary bladder, (B, C) CGA-induced relaxation in CCh (3 μ M)- or (B) U46619 (10 μ M)-precontracted urinary bladder, (B and C) Ordinate: relaxation. CCh- or U46619-induced contraction just before CGA treatment was considered to be 0%, and resting tension was considered to be 100%. Abscissa: CGA concentration. Each point represents the mean of 4–5 preparations. Vertical bars indicate SEM. +ODQ or +SQ22536: ODQ (30 μ M) or SQ22536 (100 μ M) were added to the organ bath 30 min before adding CCh. **: A significant difference between the control and +SQ22536 in CGA-induced inhibition with $P < 0.01$ using by ANOVA.

Download English Version:

<https://daneshyari.com/en/article/8532944>

Download Persian Version:

<https://daneshyari.com/article/8532944>

[Daneshyari.com](https://daneshyari.com)