NPC Natural Product Communications

2015
Vol. 10
No. 6
1009 - 1012

Effect of Non-psychotropic Plant-derived Cannabinoids on Bladder Contractility: Focus on Cannabigerol

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Received: February 3rd, 2015; Accepted: March 5th, 2015

There are anecdotal reports that some *Cannabis* preparations may be useful for bladder dysfunctions. Here, we investigated the effect of a number of non-psychotropic phytocannabinoids, namely cannabidiol (CBD), cannabigerol (CBG), cannabidivarin (CBDV), Δ^9-tetrahydrocannabivarin (THCV) and cannabichromene (CBC) on mouse bladder contractility *in vitro*. CBG, THCV, CBD and CBDV, but not CBC, at concentration ranging from 10^{-8} M to 10^{-4} M, decreased (with similar potency), the contractions induced by acetylcholine without significantly modifying the contractions induced by electrical stimulation. The rank order of efficacy was CBG=THCV>CBD>CBDV. In depth studies on CBG showed that the effect of this phytocannabinoid on acetylcholine-induced contractions was not affected by CB_1 or CB_2 receptor antagonists. Additionally, CBG also reduced acetylcholine-induced contractions in the human bladder.

Keywords: Bladder, Cannabichromene, Cannabidiol, Cannabidivarin, Cannabigerol, Cannabinoid receptors, Δ^9-Tetrahydrocannabivarin, Phytocannabinoids, Sativex®.

Anecdotal reports have suggested that some preparations from the plant *Cannabis sativa* might have a beneficial effect on lower urinary tract symptoms [1]. Over 500 compounds have been isolated from *C. sativa*, with more than 100 being phytocannabinoids. Phytocannabinoids are lipid-soluble compounds that accumulate in the resin secreted from trichomes that are abundantly produced by female *C. sativa* plants [2,3]. This group of naturally-occurring molecules includes both psychotropic [(e.g. Δ^9-tetrahydrocannabinol (THC)] and non-psychotropic phytocannabinoids, such as cannabidiol (CBD), cannabigerol (CBG), cannabidivarin (CBDV), Δ^9-tetrahydrocannabivarin (THCV) and cannabichromene (CBC). In contrast to THC, non-psychotropic phytocannabinoids do not activate potently cannabinoid CB_1 and CB_2 receptors.

Research on phytocannabinoids has concentrated mostly on the psychoactive compound THC and on the primary and most abundant non-psychotropic phytocannabinoid, i.e. CBD. Relevant to this investigation, both THC and CBD have been shown to reduce bladder contractility *in vitro*. Specifically, THC has been shown to reduce electrically-evoked contractions in the mouse bladder via activation of CB_1 receptor [4-6]; conversely, a standardized *C. sativa* extract with high content of CBD, as well as pure CBD, has been reported to inhibit acetylcholine-induced contractions in the rat and human bladder through a cannabinoid receptor-independent mechanism [7].

In this study, we show that similar to THC and CBD the other cannabinoids, namely CBG, THCV, CBC and CBDV (Figure 1) have the ability to reduce bladder contractility. Experiments were performed in the isolated mouse and human bladder. CBD was previously evaluated on the rat and human bladder [7], and was also assessed here on the mouse bladder.

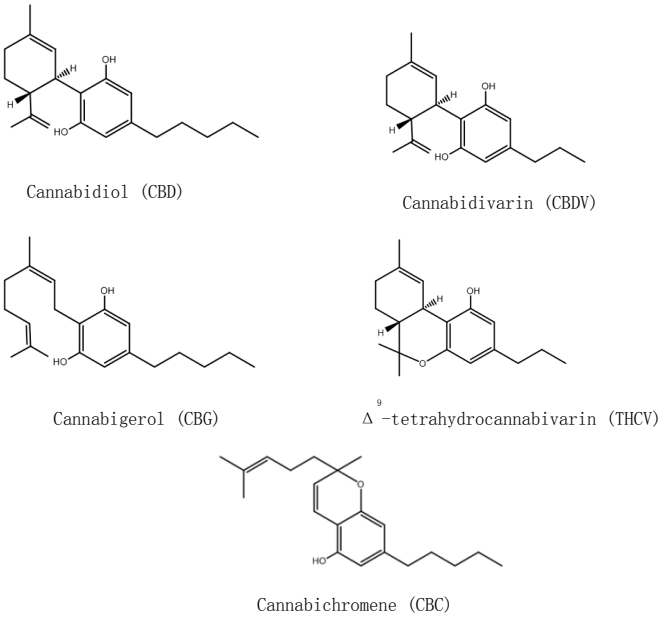

Figure 1: Chemical structures of non-psychotropic phytocannabinoids.

Mouse bladder: In preliminary experiments we found that the neuronal toxin tetrodotoxin ($3x10^{-7}$ M) abolished electrical field stimulation (EFS), but not acetylcholine-induced contractions. This suggests that the EFS-induced contractions were predominately due to the release of neurotransmitters from bladder neurons.

CBG, THCV, CBD and CBDV, at concentrations ranging from 10^{-8} M to 10^{-4} M, decreased the contractions induced by acetylcholine,

Table 1: Potency (indicated by the EC_{50} values) and efficacy (indicated by the E_{max} values) of cannabidivarin (CBDV), Δ^9-tetrahydrocannabivarin (THCV), cannabichromene (CBC), cannabigerol (CBG), and cannabidiol (CBD) (all tested in the 10^{-8}-10^{-4} M range of concentrations, n=6) on acetylcholine-induced contractions in the mouse isolated bladder

Phytocannabinoids	**EC_{50} [M]** (95% Confidence Intervals)	**E_{max} (%)** (95% Confidence Intervals)
CBDV	$9.54x10^{-6}$ ($4.79x10^{-6}$-$1.89x10^{-5}$)	66.53 (40.22-92.84)
THCV	$4.39x10^{-6}$ ($2.38x10^{-6}$-$8.25x10^{-6}$)	96.04 (80.17-111.9)
CBC	inactive	
CBG	$3.21x10^{-6}$ ($6.75x10^{-7}$-$1.54x10^{-5}$)	99.08 (63.49-148.0)
CBD	$2.52x10^{-6}$ ($7.19x10^{-7}$-$8.82x10^{-6}$)	75.35 (56.97-93.72)

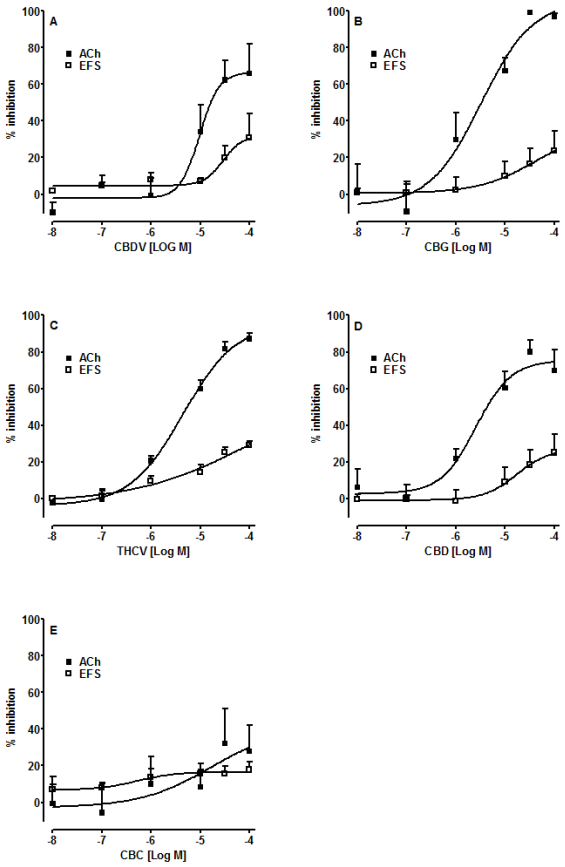

Figure 2: Effect of cannabidivarin (CBDV), cannabigerol (CBG), Δ^9-tetrahydrocannabivarin (THCV), cannabidiol (CBD) and cannabichromene (CBC) on the contractile response produced by either electrical field stimulation (EFS) or acetylcholine (10^{-6} M) in the isolated mouse bladder. Each point represents mean of 6 mice for each experimental group. Vertical lines show SEM. Note that the curve representing the inhibitory effect of the phytocannabinoids on EFS-induced contractions is significantly different (P<0.01) from the curve representing the inhibitory effect of phytocannabinoids on acetylcholine-induced contractions.

without significantly modifying the contractions induced by EFS (Figure 2A-2D). By contrast, CBC did not significantly affect either acetylcholine or EFS-induced contractions (Figure 2E). The IC_{50} (95% CL) and E_{max} (95% CL) values of phytocannabinoids-induced inhibition on acetylcholine-induced contractions are reported in Table 1.

Figure 3 shows the inhibitory effect of CBG (10^{-8}-10^{-4} M) on acetylcholine-induced contractions in the presence of drugs which antagonise cannabinoid receptors. Results show that neither rimonabant (10^{-6} M) nor SR144528 (10^{-7} M) modify the inhibitory effect of CBG. At the concentration tested, neither rimonabant nor SR144528 significantly modified acetylcholine-induced contractions (data not shown).

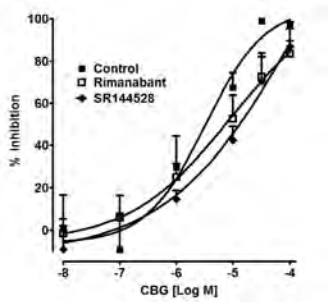

Figure 3: Acetylcholine-induced contractions in the isolated mouse bladder: effect of cannabigerol (CBG) either alone (vehicle) or in the presence of rimonabant (10^{-6} M, CB_1 receptor antagonist) or SR144528 (10^{-7} M, CB_2 receptor antagonist). Each point represents the mean of 6-7 mice for each experimental group. Vertical lines show SEM. No statistical significance among the three curves was observed.

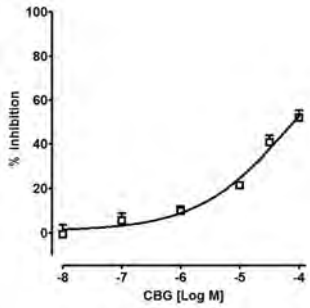

Figure 4: Effect of cannabigerol (CBG) on acetylcholine (10^{-6} M)-induced contractions in the human bladder. Each point represents mean of 4 experiments. Vertical lines show SEM.

Human bladder: Figure 4 reports the effect of CBG (10^{-8}-10^{-4} M) on acetylcholine-induced contractions in the human bladder. CBG inhibited acetylcholine-induced contraction, the effect being significant for the $3x10^{-5}$ M and 10^{-4} M concentrations. The IC_{50} (95% CL) value was $1.91x10^{-5}$ ($9.53x10^{-6}$-$3.86x10^{-5}$) M, and the E_{max} (95% CL) value 61.53 (49.29-73.78).

Subjects smoking *Cannabis* have reported an improvement in bladder dysfunctions [14]. Furthermore, nabiximols (Sativex®, GW Pharmaceuticals, Cambridge, UK), a patented cannabinoid oromucosal mouth spray containing THC, CBD and other minor phytocannabinoids is marketed for the relief of spasticity in multiple sclerosis patients, and has beeen shown to be effective for reducing bladder voids/day [15]. THC and CBD have been previously shown to depress *in vitro* bladder contractility [4,7]. Here, we have further extended the number of phytocannabinoids which are able to affect bladder contractility.

Specifically, we have demonstrated that CBG, CBD, THCV and CBDVwere significantly more active in inhibiting the contractions induced by acetylcholine (which contracts the bladder through direct activation of smooth muscles) than the contractions induced by EFS (which are mediated by neurotransmitters release from nerves). These results suggest a postsynaptic site of action. Under the same experimental conditions CBC, which has been previously shown to inhibit intestinal contractility [16], shows no effect. CBDV, CBG, THCV and CBD had comparable potency in the low micromolar range. The rank order of efficacy was CBG=THCV>CBD>CBDV.

Similar potency of the four active cannabinoids is consistent with their structural similarities (see Figure 1). In this aspect the structure of CBC significantly differs from the other compounds, which could explain its lack of activity. It is not understood yet why two

phytocannabinoids namely CBG and THCV are more eficacious than the other two.

From the available data on potency and efficacy (Table 1), CBG was found to be the most promising compound. Therefore, further studies were performed on this phytocannabinoid. In order to give some insights into the mode of CBG action, we hypothesized the possible involvement of cannabinoid receptors. This because: a) CBG is a partial agonist of CB_1 and CB_2 receptors, although exhibiting low affinity for these receptors [17] b) CBG is able to inhibit the reuptake of the endocannabinoid anandamide [18]; c) some of the pharmacological actions of CBG have been shown to be modulated by a CB_2 receptor antagonist [14,19]; d) components of the endocannabinoid system are involved in the regulation of bladder function [20]; e) CB_1 receptors have been shown to be involved in bladder contractility [4]. However, our experiments, performed with selective CB_1 and CB_2 receptor antagonists (i.e. rimonabant and SR144528), clearly showed that the inhibitory effect of CBG on acetylcholine-induced contractions does not involve cannabinoid receptors.

Finally, we tried to confirm the ability of CBG to reduce bladder contractility in human tissues. This is an important issue since the ability of cannabinoid agonists to inhibit contractility of the rodent bladder is not conserved across all mammalian species, including humans [5]. We found that CBG did inhibit acetylcholine- induced contractions in human bladder.

In conclusion, we have shown that four minor phytocannabinoids, CBG, CBD, THCV and CBDV, are able to reduce bladder contractility. Further studies on CBG showed that this phytocannabinoid acts via a cannabinoid receptors-independent mechanism and that it also inhibits human bladder contractility. Such results further provide a pharmacological basis able to explain, at least in part, the anecdotal use of *Cannabis* preparations for bladder dysfunctions.

EXPERIMENTAL

Bladder preparations and drug administration: Male Swiss mice (20 to 24 g) were purchased from Harlan Italy and maintained under controlled temperature (24±2°C) and humidity (60%) conditions until used. The mice had free access to water and food. All experiments complied with the Italian D.L. No. 116 of January, 27 1992 and the associated guidelines in the European Communities Council Directive of November 24, 1986 (86/609/ECC). The mice were euthanized by asphyxiation with carbon dioxide. The urinary bladder was removed and placed in Krebs solution (composition NaCl 119 mM, KCl 4.75 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, $MgSO_4$ 1.5 mM, $CaCl_2$ 2.5 mM, and glucose 11 mM). Strips were cut from the bladder body (2 strips from one mouse) and placed in 20 mL organ baths containing Krebs solution equilibrated with 95% oxygen and 5% carbon dioxide at 37°C. The tissues were connected to an isometric transducer (tension 9.81 mN). Contractions were recorded using a PowerLab system (Ugo Basile, Comerio, Italy). After a minimal 1 h equilibration period, the strips were subjected to EFS (8 Hz for 2 sec, 300 mA, 0.25-ms pulse duration, interval between stimulations: 2 min), delivered by electrodes placed around the tissue or stimulated with exogenous acetylcholine (10^{-6} M). Acetylcholine was left in contact with the tissue for 1 min and then washed out. Acetylcholine gave a contractile response that was similar in amplitude to that of EFS. Stable and reproducible contractions for 3 h were obtained with stimulation every 20 min. In preliminary experiments, the effect of tetrodotoxin ($3x10^{-7}$ M, contact time 10 min) on EFS- or acetylcholine-induced contractions was evaluated. After stable control contractions evoked by EFS or by acetylcholine were recorded, responses were observed in the presence of increasing phytocannabinoid concentrations (10^{-8}-10^{-4} M). The contact time for each concentration was 20 min. Preliminary experiments showed that this contact time was sufficient for the phytocannabinoids to achieve the maximal inhibitory effect.

In different sets of experiments, the effect of CBG on acetylcholine-induced contractions was also evaluated in the presence of either rimonabant (10^{-6} M, CB_1 receptor antagonist) or SR1442558 (10^{-7} M, CB_2 receptor antagonist) [8]. These concentrations were selected on the basis of previous published work [7]. Finally, the effect of CBG was evaluated on the contractions evoked by acetylcholine in human tissues. Bladder was taken from patients who underwent radical cystectomy for carcinoma of the bladder or transvescicaladenomectomy due to adenomatous hyperplasia of the prostate. Bladder strips (approx 10 mm) were set up under a resting tension of 9.81 mN. The experimental protocol, including drug administration and contact time, was identical to that described for mouse bladder.

Drugs: CBD, CBG, CBC, CBDV and THCV were extracted and isolated from strains of *Cannabis sativa* plants as described below. Extraction and isolation were performed in the GW Pharmacueticals laboratory (Cambridge, UK). Acetylcholine hydrochloride, and tetrodotoxin were purchased from Sigma (Milan, Italy), and rimonabant and SR144528 from SANOFI-Aventis (Montpellier, France). Rimonabant, SR144528 (*N*-[-1S-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide, CBG, CBDV, CBC and THCV were dissolved in dimethyl sulfoxide (DMSO), and CBC in ethanol. The other drugs were dissolved in distilled water. Neither DMSO nor ethanol (less than 0.01%) significantly affected bladder contractility.

Phytocannabinoids extraction and isolation from strains of* Cannabis sativa *plants: CBD, CBG, CBC, CBDV or THCV were extracted from the corresponding phytocannabinoid-predominant *Cannabis sativa* plants. *C. sativa* chemotypes, cloned from the same plant to have a controlled high amount of a specific phytocannabinoid (i.e. CBD, CBG, CBC, CBDV or THCV) were used [9-10]. The mechanism that is responsible for the accumulation of a specific phytocannabinoid in certain phenotypes of *C. sativa* is described in detail elsewhere [11]. *C. sativa* was grown in highly secure computer-controlled glasshouses. All aspects of the growing climate, including temperature, air change and photoperiod, were computer controlled and the plants were grown without the use of pesticides. *Cannabis* dry flowers and leaves were extracted at room temperature with CO_2 to give an extract which, evaporated to dryness, was a brownish solid. A portion of the extract was dissolved in methanol for high-performance liquid chromatography (HPLC) analysis (Agilent 1100) using a C18 column (150 × 4.6 mm and 1 mL/min flow rate) [12,13]. CBD, CBG, CBDV, CBC and THCV were crystallized from the corresponding extracts using alkanes as solvents. The identity and purity of the phytocannabinoids (CBG, 95%, CBD 99.3%, CBDV, 95.0%, CBC, 95.0%, THCV, >95%) were assessed by various chromatographic techniques (i.e. HPLC, gas chromatography, melting point, infrared spectroscopy).

Statistical analysis: Results are expressed as the mean±SEM. Comparisons between two sets of data were made by Student's t test for paired data. When multiple comparisons against a single control were made, ANOVA was used, followed by the Tukey-Kramer multiple comparisons test. The concentrations of phytocannabinoids

that produced 50% inhibition of acetylcholine-induced contractions (IC_{50}) or maximal inhibitory effect (E_{max}) were used to characterize phytocannabinoid potency and efficacy, respectively. The IC_{50} and E_{max} values were calculated with the aid of a computer program (Graphpad Prism 5).

Conflict of interest: Angelo A. Izzo receives research support from GW Pharmaceuticals (Cambridge, UK), which markets Sativex®, a phytocannabinoid-based medicine. The other authors declare that they have no conflict of interest.

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