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ANTI- INFLAMMATORY EFFECTS OF CANNABINOID 2 RECEPTOR AGONIST, GW405833, IN A MODEL OF CARRAGEENAN-INDUCED ACUTE INFLAMMATION OF THE RAT PAW

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Anti- inflammatory effects of cannabinoid 2 receptor agonist, GW405833, in a model of carrageenan-induced acute inflammation of the rat paw

INTRODUCTION

Cannabinoids

- Cannabis sativa
- Synthetic

Endocannabinoidergic system (ECS)

- The receptors
- The ligands
- The enzymes
- The effect mechanism

ECs and their effects

- Central
- Pheripferal

ECs and Inflammation

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM

CANNABIS SATIVA

- Cannabis sativa produces over 80 cannabinoids
- **Δ9-tetrahydrocannabinol (THC)** was identified as the main bioactive constituent of cannabis, the main psychotropic constituent of marijuana.
- The psychological addiction resulting from the abuse of cannabis is the main concern limiting its therapeutic use.
- Nonpsychoactive compound; cannabidiol (CBD) and cannabinol (CBN)

THE SYNTHETIC CANNABINOIDS

- Naphthoylindoles (e.g., JWH-018, JWH-073, JWH-398).
- Naphthylmethylindoles. (e.g., JWH-175, JWH-195, JWH-197).
- Naphthoylpyrroles.(e.g., JWH-030, JWH-156, JWH-243).
- Naphthylmethylindenes (e.g., JWH-176).
- Phenylacetylindoles (i.e., benzoylindoles, e.g., JWH-250, JWH-253, JWH-313).
- Cyclohexylphenols (e.g., CP 47,497 and homologs of CP 47,497).
- Classical cannabinoids (e.g., HU-210).

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM
I. THE RECEPTORS

- The cannabinoid receptors were found and cloned in the early 1990s
- The endogenous cannabinoid (EC) system consists of two **G-protein-coupled** cannabinoid i.e.

- **CB1** and
- **CB2** receptors

- However there are maybe additional receptors
- And, some EC effects result from the interaction with other receptors, such as the **vanilloid receptor**

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM
I. THE RECEPTORS

- The cannabinoid CB1 receptors are preferentially **located on brain** and also expressed in nerve terminals of peripheral tissues including heart and vessels.
- The cannabinoid CB2 receptors are mainly **located on peripheral non-neuronal cells** (mostly immune system cells) which exert a broad range of critical effects under physiological or pathological conditions

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM

II. ENDOCANNABINOID LIGANDS

- First endogenous ligands turned out to be fatty acid-derived molecules including
- **Anandamide** (arachidonoyl ethanolamide, **AEA**) and
- **2-arachidonoylglycerol (2-AG)**

- AEA has more affinity to CB1 than CB2,
- 2-AG shows similar affinity for CB1 and CB2

- CB2 receptors may also bind other endocannabinoid ligands; however, the signalling consequences of this binding is poorly known

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM

III. ENDOCANNABINOID-**BIOSYNTHETIC** AND **CATABOLIC** ENZYMES

AEA

- Ca2+-dependent N-acyltranferase
- N-acylphosphatidylethanolamine-hydrolyzing phospholipase D
- Fatty acid amide hydrolase (FAAH)

2-AG

- diacylglycerol lipase
- phospholipase Cβ
- Monoacylglycerol lipase (MAGL)

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM

III. ENDOCANNABINOID BIOSYNTHETIC AND CATABOLIC ENZYMES

- The biosynthesis of AEA is catalyzed by Ca2+-dependent N-acyl-phosphatidylethanolamine–hydrolyzing phospholipase D,
- AEA can also be produced by other routes,

- AEA is hydrolyzed mainly by fatty acid amide hydrolase (FAAH) into arachidonic acid and ethanolamine .

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM

III. ENDOCANNABINOID BIOSYNTHETIC AND CATABOLIC ENZYMES

- 2-AG is synthesized from its phospholipid precursor diacylglycerol by diacylglycerol lipases.

- Phospholipase C-β releases diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate, which in turn is metabolized by diacylglycerol lipases (DAGLs) – with DAGLα and DAGLβ having prevalent roles in the brain and in several peripheral tissues, respectively – to produce 2-AG.

- The major degradative or inactivated pathway of 2-AG is its hydrolysis to arachidonic acid and glycerol by monoacylglycerol lipase (MAGL)

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM

III. ENDOCANNABINOID BIOSYNTHETIC AND CATABOLIC ENZYMES

- Although the hydrolysis pathway seems to be the primary fate of AEA and 2-AG, they can also be oxidized by cyclooxygenase-2 and lipoxygenase isozymes, thus producing oxidized endocannabinoids, which are involved in regulating brain synaptic transmission and other biological processes

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM

THE EFFECTS

- Accumulating evidence has indicated that EC and their major receptors CB1 and CB2 play a major role in the pathophysiology of diseases

- at a preclinical stage,
- the selective CB2 molecules are increased to interest as new targets in drug discovery
- Endocannabinoids can modulate levels of proinflammatory mediators and immune cell migration. Exogenously administered 2-AG and anandamide or several selective agonists to animal models of inflammation have also shown to be effective.

INTRODUCTION

THE CANNABINOIDERGIC SYSTEM
THE EFFECTS

- ECs are provided by a series of
- central and
- peripheral effects

- CB1 is more responsive to psychoactive cannabinoids (eg, THC) than to nonpsychoactive cannabinoids (eg, cannabidiol)

Introduction

The Cannabinoidergic system

The Effects

- ECs influence
- analgesia and motor function,
- energy balance and food intake,
- cardiovascular function,
- **immune and inflammatory responses**, and
- cell proliferation

Introduction

The Cannabinoidergic system And Inflammation

- the endocannabinoid system has been found to be involved in many inflammation-related conditions, such as

- Multiple sclerosis,
- Atherosclerosis,
- Inflammatory bowel disease,
- RA,
- Sepsis, and
- Allergic inflammation

Introduction

The Cannabinoidergic system And Inflammation

- The blockage of CB1 and activation of CB2 could inhibit inflammation in various animal models, mainly through restraining the activity of the immune system.
- The exogenous application of AEA and 2-AG exerts anti-inflammatory effects by decreasing the production of inflammatory mediators.
- **The exogenous application of selective CB2 agonists exerts anti-inflammatory effects by decreasing the production of inflammatory mediators.**
- Upregulating the level of endogenous cannabinoids by inhibiting their common metabolic enzyme, becomes an important strategy in the treatment process of inflammation-related diseases

INTRODUCTION

IN OUR PREVIOUS STUDIES

- We had evaluated some effects of the cannabinoid (CB)2 receptor activations' during the inflammatory processes of peripheral tissues after intestinal ischemia/reperfusion .

INTRODUCTION

AIM OF THIS STUDY

- This study was designed to investigate the anti-inflammatory effects of selective CB2 receptor agonist, **GW405833**,
  - in the carrageenan paw oedema test of rats.
  - in the capsaicin paw oedema test of rats.

Materials and Methods

Animals and Experimental Design

- The subjects weighed between 200 and 250g, and were housed in a temperature (20–22 °C) in their home cages and were maintained on a 12/12 h light/dark cycle.
- All rats were given standard rat chow and water ad libitum
- The sample size for each treatment group was 6 to 8 mice/group
- All animal protocols were approved by the Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health Guide for the care and use of Laboratory animals.
- After testing was completed, all mice were humanely euthanized via CO2 asphyxia, followed by rapid cervical dislocation.

Materials and Methods

Inflammation type

- **Carrageenan-induced paw edema -** Mix type of inflammation
- were induced by giving an intraplantar injection of carrageneen (50 µl, 1%) or

- **Capsaicin-induced paw edema -** Neurogenic type of inflammation
  - were induced by giving an intraplantar injection of capsaicin (50 µl, 0.1%) into the paw

Materials and Methods

Inflammation type

Paw edema

- Edema was expressed as the increase in paw thickness (mm) after carrageenan injection relative to the pre-injection value for each animal.

- Paw thickness was measured with electronic digital calipers, prior to and 1 or 4 h following capsaicin or carrageenan administration respectively, which corresponds to peak edema time.

Materials and Methods

Animals and Experimental Design

- **In the first group,**
- Plasma extravasations were measured via Evans blue dye method.
- The dye was injected in the tail vein 15 min before the end of the experiments.
- The anaesthetized animals were sacrificed by decapitation, and hind paws were incubated with formamide, and then the extracted dye was measured by spectrophotometry at 620 nm.

Materials and Methods

Animals and Experimental Design

- **In the second group**,
- paw thickness was measured with electronic digital callipers, prior to and 4 h following carrageenan or 1 h following capsaicin administration, which corresponds to peak oedema. This procedure has been used previously by studies.
- The anti-oedematous effects of GW405833 (3 mg/kg, i.v.) were compared to diclofenac (10 mg/kg, i.v.), a nonselective cyclooxygenase inhibitor, 15 min before these intraplantar injections of inflammatory agents.
- CB receptor involvement in the anti-inflammatory effects of GW405833 was evaluated by administration of the CB2 receptor antagonist, AM630 (1 mg/kg, i.v., 5 min before CB2 agonist injection).

Materials and Methods

Statistical Data Analysis

- All statistical analyses were carried out using GraphPad statistical software.
- All data were presented as mean ± standard error mean.
- Difference between groups was compared using student *t* test or one-way ANOVA followed by Tukey's Multiple Comparison.
- $P < 0.05$ was considered significant.

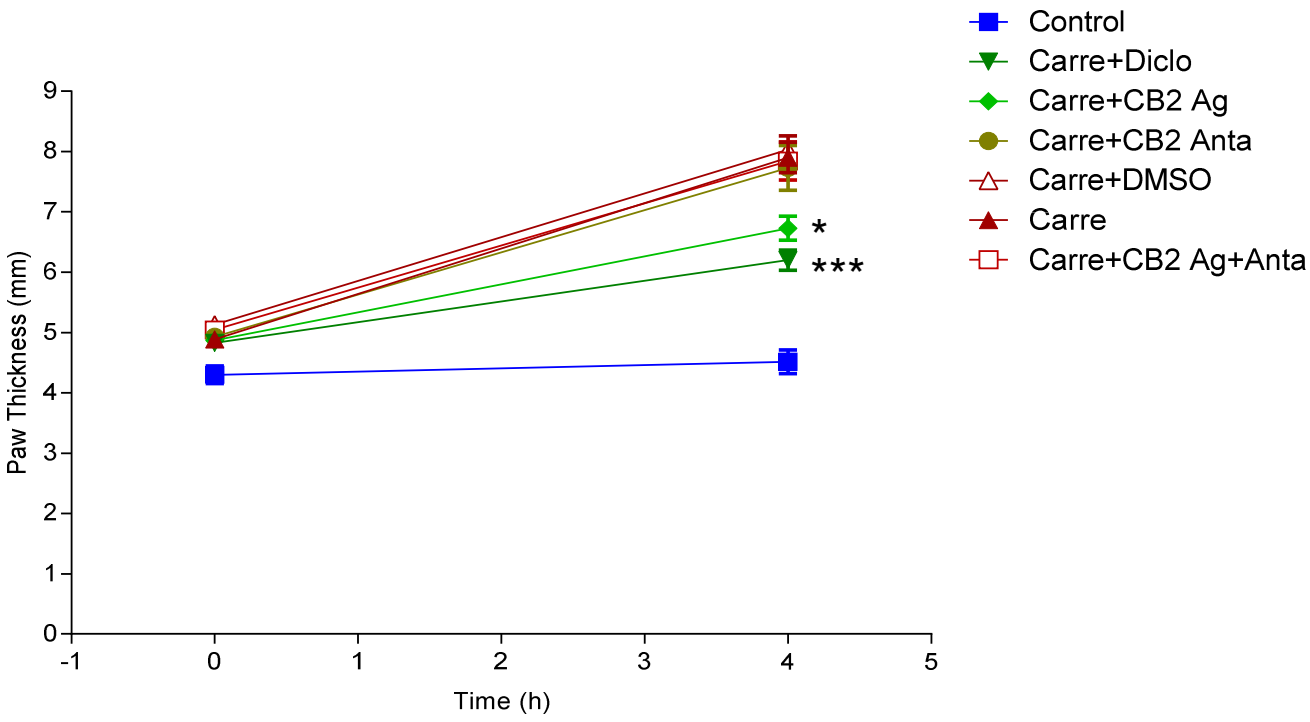
RESULTS

- I. **The carrageenan-induced paw oedema**

- Administrate of carrageenan caused clear edema in paw tissue ($P < 0.001$)

- Pretreatment of rats with both GW405833 ($P < 0.05$) and diclofenac ($P < 0.001$) significantly attenuated carrageenan-induced paw oedema compared to vehicle-treated group.
- CB2 receptor antagonist, AM630, significantly reversed the effect of CB2 agonist ($P < 0.01$)

Figure 1. The carrageenan-induced paw oedema and the effect of CB2 agonist

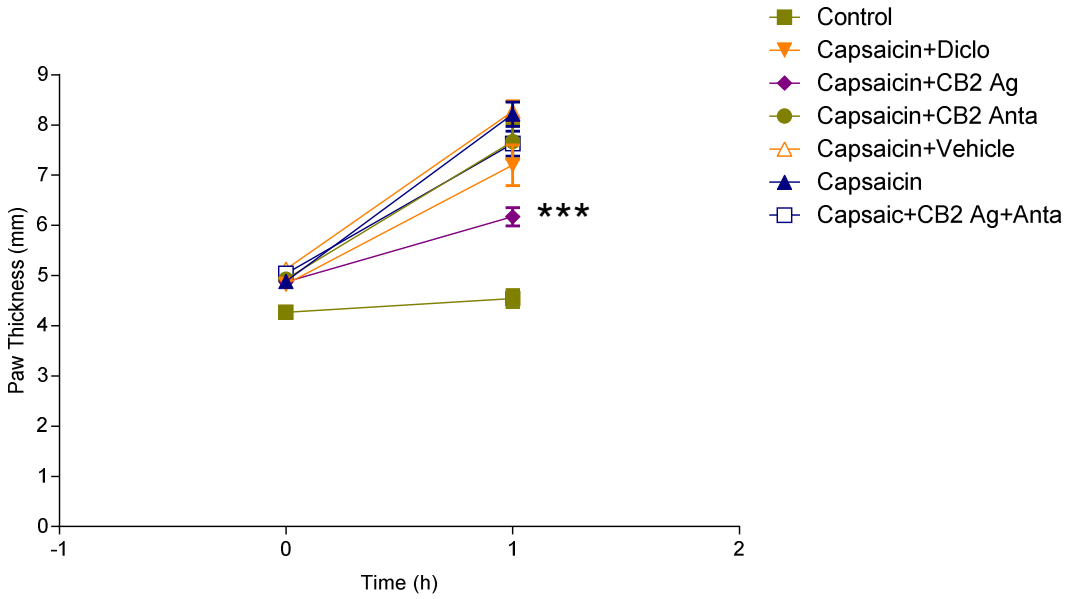


RESULTS

- II. **The capsaicin-induced paw oedema**

- Administrate of capsaicin caused clear edema in paw tissue ($P < 0.001$)
- Pretreatment of rats with GW405833 ($P < 0.001$) significantly attenuated capsaicin-induced paw oedema compared to vehicle-treated group.
- CB2 receptor antagonist, AM630, significantly reversed that effect ($P < 0.001$)

Figure 2. The capsaicin-induced paw oedema and the effect of CB2 agonist

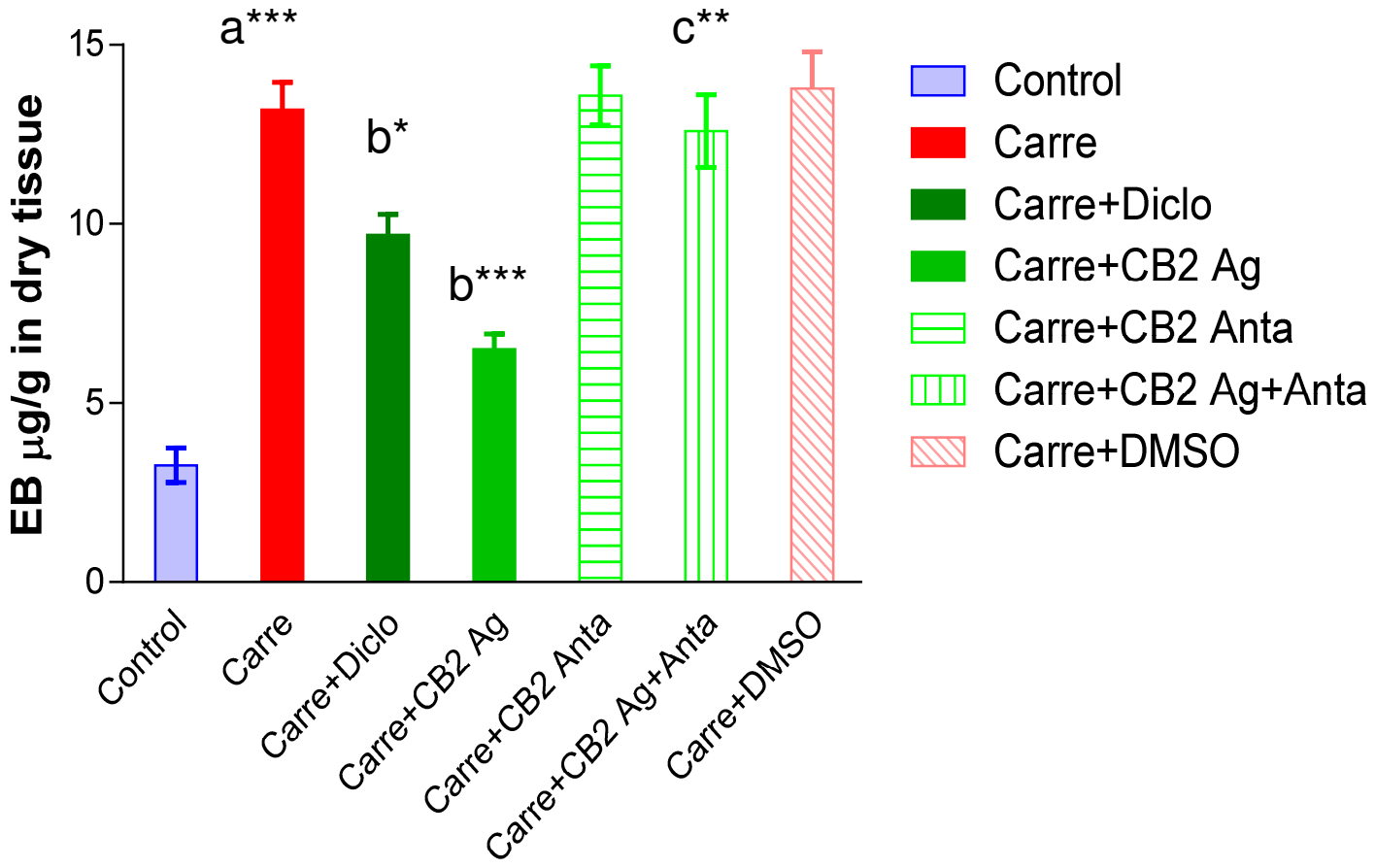


RESULTS

- III. **The effect of CB2 agonist on plasma extravasation in carrageenan-induced paw oedema**

- The GW405833 significantly inhibited plasma extravasation in carrageenan-induced paw oedema ($P < 0.001$) . Diclofenac inhibited also plasma extravasation, but has effect as more week ($P < 0.05$)
- CB2 receptor antagonist, AM630, significantly reversed the effect of CB2 agonist ($P < 0.01$)

Figure 3. **The carrageenan-induced inflammation in paw tissue and the effect of CB2 agonist on plasma extravasation**

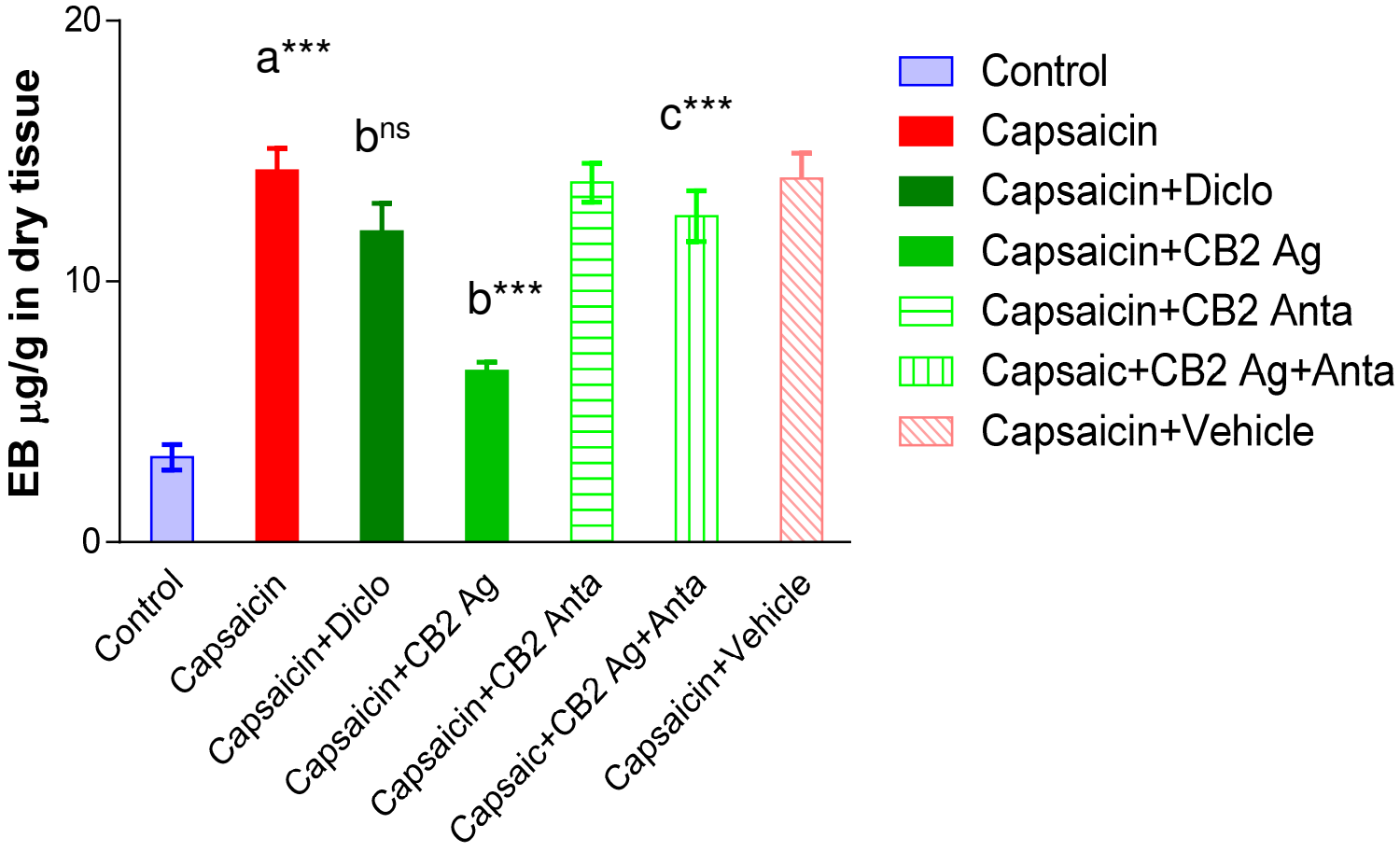


RESULTS

- IV. **The effect of CB2 agonist on plasma extravasation in capsaicin-induced paw inflammation**

- The GW405833 strongly inhibited plasma extravasation in capsaicin-induced the neurogenic inflammation in paw tissue ($P < 0.001$).
- Diclofenac not inhibited plasma extravasation ($P > 0.05$)
- CB2 receptor antagonist, AM630, significantly reversed the effect of CB2 agonist ($P < 0.001$)

Figure 4. The capsaicin-induced neurogenic inflammation and the effect of CB2 agonist on plasma extravasation



DISCUSSION

- GW405833 significantly decreased the plasma extravasations in both carrageenan-induced mix type inflammation and capsaicin-induced neurogenic inflammation of rat paw.
- The pretreatment with AM630 clearly reversed the effects of GW405833, which suggests a significant interaction between GW405833 and AM630.
- So CB2 receptors mediate the anti-oedematous and anti-plasma extravasations effects of GW405833.

Discussion

- The present study increases the understanding that pharmacological level of CB2 agonist plays on anti-inflammatory effects by demonstrating that **GW405833** reduces capsaicin or carrageenan-induced paw edema.

- These effects were similar in magnitude to those produced by the CB2 agonist **GW405833**, as well as the nonselective COX inhibitor diclofenac.

- The anti-edematous effects of **GW405833** were mediated through CB2 receptors.
- CB2 antagonist, AM630, reversed these anti-edema effects.

- These results suggest that the GW405833 reduces inflammation through the activation of CB2 receptors when administered after carrageenan, and that effect seems to be related to the suppression of neurogenic inflammation.

FINALLY

- The stimulation of CB2 receptors induces anti-inflammatory effects in several experimental conditions

- **Our experimental studies provide evidence that supports the hypothesis for the activation of CB2 receptors may have beneficial effects against inflammatory processes, maybe via and related the control of neurogenic inflammation**

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