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Upregulation of cannabinoid receptor gene expression in oral tissues subjected to hyposalivation and periodontitis

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ABSTRACT

Cannabinoid receptors (CBr) 1 and 2 (CB1r and CB2r) are present in periodontal tissues and salivary glands and play a significant role in the pathogenesis of oral diseases. ***Aim:*** *To study the gene expression of CB1r and CB2r in gingival tissue and submandibular glands (SMG) of rats subjected to experimental periodontitis (EP) and/or hyposalivation (H), and discuss their possible implication in oral health.* ***Material and methods:*** *Rats were divided into controls, rats with EP induced by application of lipopolysaccharide (1 mg/ml), rats with H induced by bilateral submandibulectomy, and rats with both EP and H. Alveolar bone loss was measured with a digital caliper, mRNA expression of CBr and bone parameters were determined by endpoint RT-PCR, PGE_2 glandular content was assessed by radioimmunoassay, and stimulated salivary secretion was collected and weighed.* ***Results:*** *CB1r and CB2r mRNA in gingival tissue were upregulated in groups EP, H and EP+H as compared to controls (CB1r (AU): C 0.27±0.06^A, EP 0.46±0.05^B, H 0.51±0.08^B, EP+H 0,49±0,1^B; CB2r (AU): C 0.38±0.04^A, EP 1.22±0.08^B, H 0.98±0.06^C, EP+H 1,041±0,1B^C). Groups EP, H and EP+H showed alveolar bone loss as opposed to controls, while only groups EP and EP+H evidenced RANKL/OPG imbalance measured in the dental attachment tissue. Likewise, CB1r and CB2r mRNA in SMG showed upregulation in group EP as compared to controls (CB1r (AU): C 0.52±0.09^A, EP 0.95±0.13^B; CB2 (AU): C 0.23±0.12^A, EP 1.48±0.22^B). In group EP, salivary secretion was lower and the content of PGE_2 (inhibitory mediator of said function) was higher than in controls.* ***Conclusion:*** *The upregulation of CB2r in oral tissues under the pathophysiological conditions studied suggests that they participate actively in the response to oral diseases.*

Keywords: *cannabinoid receptors - submandibular gland excision - lipopolysaccharides - periodontium - inflammation.*

Regulación positiva de la expresión génica de los receptores cannabinoides en los tejidos bucales sometidos a hiposalivación y periodontitis

RESUMEN

Los receptores cannabinoides (CBr) 1 y 2 (CB1r y CB2r) están presentes en los tejidos periodontales y en las glándulas salivales, y desempeñan un papel importante en la patogénesis de las enfermedades orales. ***Objetivo:*** *Estudiar la expresión génica de CB1r y CB2r en tejido gingival y glándulas submandibulares (GSM) de ratas sometidas a periodontitis experimental (PE) y/o hiposalivación(H) y discutir su posible implicancia en la salud oral.* ***Materiales y método:*** *Las ratas se dividieron en controles, ratas con PE inducida por aplicación de lipopolisacáridos (1 mg/ml, grupo LPS), ratas con H inducida por submandibulectomía bilateral y ratas con EP y H. Se evaluó la pérdida ósea alveolar mediante un calibre digital, se determinó la expresión de ARNm de CBr y los parámetros óseos mediante RT-PCR de punto final, se evaluó el contenido glandular de PGE_2 mediante radioinmunoanálisis y se recogió y pesó la secreción salival estimulada.* ***Resultados:*** *el ARNm de CB1r y CB2r en el tejido gingival mostró regulación positiva en los grupos EP, H y EP+H en comparación con los controles (CB1r (UA): C 0,27±0,06A, EP 0,46±0,05^B, H 0,51±0,08^B, EP+H 0,49±0,1^B; CB2r (UA): C 0,38±0,04^A, EP 1,22±0,08^B, 0,98±0,06^C, EP+H 1,041±0,1B^C). Los grupos EP, H y EP+H mostraron pérdida ósea alveolar en comparación con los controles, mientras que sólo los grupos EP y EP+H evidenciaron un desequilibrio RANKL/OPG medido en el tejido de inserción dentaria. Asimismo, los ARNm de CB1r y CB2r en la GSM mostraron regulación positiva en el grupo EP en comparación con el grupo control (CB1r (UA): C 0,52±0,09^A, EP 0,95±0,13^B; CB2 (UA): C 0,23±0,12^A, EP 1,48±0,22^B). En el grupo EP, la secreción salival fue menor y el contenido de PGE_2 (mediador inhibidor de dicha función) aumentó en comparación con el grupo control.* ***Conclusión:*** *la regulación positiva de los CB2r en los tejidos orales en las condiciones fisiopatológicas estudiadas sugiere su participación activa en la respuesta a enfermedades de la cavidad bucal.*

Palabras clave: *receptores cannabinoides - submandibulectomía-lipopolisacáridos - periodonto - inflamación*

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INTRODUCTION

Cannabinoid (CB) receptors 1 and 2 (CB1r and CB2r) are central elements of the endocannabinoid system (ECS). They are both involved in a range of signaling pathways that influence physiological processes. CB1r is primarily present in the central nervous system (CNS) and influences neurotransmission, while CB2r is mainly present in peripheral tissues, participating in the modulation of immune and inflammatory responses[1]. The signaling mechanisms of both receptors contribute to the wide range of effects attributed to cannabinoids. CBrs are activated by endocannabinoids such asanandamide and 2-arachidonoylglycerol, as well as by exogenous cannabinoids such as Δ-9-Tetrahydrocannabinol. These receptors are primarily coupled with Gi/o proteins, which inhibit adenylyl cyclase enzyme and, subsequently, affect various downstream cellular processes. They also promote MAPK activity, thus influencing cellular processes, including gene expression, cell proliferation, and survival. Only CB1r has been shown to signal via ion channels. It inhibits voltage-gated calcium channels, reducing calcium influx, and activates inwardly rectifying potassium channels, causing hyperpolarization. CB1r and CB2r also influence other second messenger systems such as phosphatidyl inositol 3-OH kinase (PI3K)-Akt pathway, through which CB1r has a role in cell survival and apoptosis, whereas CB2r takes part in cell survival, migration and immune responses. Extracellular signal-regulated kinase (ERK) is another pathway activated by CB1r, becoming involved in cellular growth and differentiation. Furthermore, NF-κB and JAK/STAT pathways can be activated by CB2r, playing a role in immune and inflammatory responses[1,2].

CBrs are present in periodontal tissues and play a significant role in the pathogenesis of oral diseases[3]. Periodontitis is a chronic inflammatory disease generated mainly by the accumulation of dental biofilm, inflammation of the dental insertion tissues and formation of periodontal pockets. It causes the resorption of alveolar bone and the loss of insertion periodontal tissue, and can even lead to tooth loss if untreated[4]. Recent studies have demonstrated the involvement of the ECS in the modulation of immune responses in periodontitis[5-7]. Activation of CB2r exerts anti-inflammatory effects, bringing the potential capability to reduce the severity of inflammation and tissue destruction associated with the disease[8]. Destruction of periodontal tissues results from the release of harmful molecules by periodontopathogenic bacteria and host-derived proinflammatory mediators released during the immune response[4,9]. The inflammatory environment leads to an imbalance between alveolar bone resorption and formation, favoring the former[10]. Bone metabolism is regulated by the receptor activator of nuclear factor-kappa B (RANK), its ligand (RANKL) and osteoprotegerin (OPG), a decoy receptor[11,12]. Thus, controlling inflammation by CBrs activation could be a useful tool for avoiding bone metabolism imbalance, as reported in preclinical studies[13].

Cannabinoid receptors are also present in salivary glands. In addition to their role in inflammation, CBrs are involved in the regulation of saliva production[14,15]. Saliva is composed of water as solvent and electrolytes, proteins and other molecules as solutes. It is crucial in the mouth to maintain the integrity of the mucous membranes and hard tissues[16-18].Reduction in saliva production affects the integrity of oral structures, increasing the risk of infection by microorganisms and the development of dental caries[19]. Furthermore, the submandibular gland has been shown to play an immunomodulatory role, controlling oral homeostasis[20]. Hyposalivation (H) and the subjective sensation of dry mouth, called xerostomia, are health problems that affect millions of people around the world[21,22].

Periodontitis-induced hyposalivation, or reduced saliva flow, can exacerbate oral health problems by impairing the natural cleansing mechanisms of the oral cavity and increasing the risk of infection. Studies have indicated that activation of CBrs in gingival tissues and submandibular glands can differentially influence salivary gland function, which could lead to changes in saliva secretion[14,23,24]. Furthermore, the anti-inflammatory effect induced by CB2r activation may also help to alleviate the inflammation of oral tissues, thus improving saliva production[24]. This dual role of cannabinoid receptors in both inflammation and salivary gland function highlights their potential as therapeutic targets for managing periodontal disease and its associated complications, such as hyposalivation.

In line with these findings, an increasing number of studies have highlighted the therapeutic potential of cannabinoid-based interventions for various oral

pathologies. Evidence from preclinical and clinical research supports the use of cannabinoids for their anti-inflammatory, analgesic, antimicrobial, and immunomodulatory properties in conditions such as periodontitis, oral mucositis, temporomandibular joint disorders, and even oral cancer[6,25,26]. These studies underscore the relevance of the endocannabinoid system as a promising target in oral medicine, paving the way for the development of novel cannabinoid-based therapeutic strategies aimed at improving oral health outcomes.

In a recently published article, using the same experimental model as in the current study, we showed an increase in gingival inflammatory parameters and bone loss in rats subjected to hyposalivation (H) compared to controls. Furthermore, we observed higher levels of inflammatory mediators in the experimental periodontitis (EP) group than in the H group, resulting in greater damage to periodontal tissues. However, we concluded that concomitant exposure to EP and H did not show clear aggregate effects[27]. The aim of the current work was to study the gene expression of CB1r and CB2r in periodontal tissue and submandibular glands of rats subjected to experimental periodontitis and/or hyposalivation, and to discuss their possible implications for oral health.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (weighing 300-350 g) from the laboratory's own colony were housed in group cages in a controlled environment with a 12-hour light/dark cycle (0800-2000). Room temperature was kept at 22±2ºC, and the animals had unlimited access to standard rat food and tap water. The experimental protocols were approved by the Animal Care Committee of the Dental School at the University of Buenos Aires, Argentina (CICUAL-ODON/FOUBA N° 013/2016), and adhered to the European Communities Council Directive 2010/63/UE. All animal experiments followed the ARRIVE guidelines 2.0.

Design

Twenty-four rats were divided into four groups of six: 1) control rats, 2) rats subjected to experimental periodontitis (EP), 3) rats subjected to hyposalivation (H), and 4) rats concomitantly subjected to EP and H. Groups 2 and 4 received 20 µl injections of LPS (1 mg/ml) derived from *Escherichia coli* (serotype 055-B5, Sigma-Aldrich) into the vestibular and lingual gingiva of both the right and left first molars, and into the interdental space between the first and second molars (a total 60 µl of LPS per tooth, 120 µl per rat per treatment) while under sevoflurane inhalation anesthesia. This injection regimen, commonly used as an experimental periodontitis model, was carried out over six weeks, with injections administered on days 1, 3, and 5 of each week, following a previously established method. The gingival injections were delivered using a 13-mm 27-gauge microfine insulin syringe. Groups 3 and 4 underwent bilateral submandibulectomy surgery under anesthesia seven weeks before euthanasia, utilizing intraperitoneal injections of ketamine hydrochloride (Holliday-Scott SA, 70 mg/kg) and 2% xylazine hydrochloride (König Laboratories SA, 10 mg/kg), as described in previous studies. A 15-mm midline incision was made on the ventral neck, allowing the excretory ducts and major blood vessels to be ligated. The submandibular–sublingual salivary glands were carefully excised from surrounding connective tissue, preserving key neural structures, such as the marginal mandibular branch of the facial nerve, along with the hypoglossal and facial nerves. All experiments were repeated at least twice, with the graphs showing the outcomes of one of these trials.

Macroscopic assessment of periodontal bone loss: distance and width techniques

Immediately following euthanasia, hemimandibles and hemimaxillae were dissected, cleaned, and stained with 1% aqueous methylene blue to highlight the cementoenamel junction (CEJ) and the alveolar crest (AC)[28]. A stereomicroscope and a stainless-steel digital caliper were used to measure three buccal and three lingual/palatal distances (mesial, central and distal) between the CEJ and the AC. The sum of these three measurements on both sides of the upper and lower molars served as an indicator of alveolar bone loss (ABL), expressed in millimeters. The width of the mandibular alveolar process was determined at the level of the first mandibular molar. Using the digital caliper, the distance between two points located at the central root level on the buccal and lingual surfaces was measured in millimeters.

Gene expression by semi-quantitative RT-PCR

For the gene expression analysis of CB1r and CB2r mRNA, tissue samples were obtained from the free and attached gingiva of the first molars and submandibular glands. For the gene expression analysis of RANKL and OPG mRNA, dental insertion tissues (including alveolar bone, periodontal ligament and root cementum) were collected by alveolar scraping performed after post-mortem extraction of the first molars. To ensure an adequate yield of total mRNA, tissue was harvested bilaterally from the maxilla and right hemimandible, and collected the equivalent of tissue from three teeth of the same animal. Total messenger RNA was isolated using RNAzol Reagent according to the manufacturer's protocol (Molecular Research Center Inc., Cincinnati, OH, USA). RNA concentration and purity were determined by measuring absorbance at 260 and 280 nm using a Pico200 Microliter UV/Vis spectrophotometer (Spectra Services Inc.). Subsequently, 2 µg of total RNA was reverse-transcribed into cDNA using the Improm-II™ system (Promega Corporation, Madison, USA) with Oligo(dT) primers (Invitrogen™) and a ribonuclease inhibitor (Promega, USA). Semi-quantitative PCR was carried out using GoTaq® DNA Polymerase (Promega, USA) on a GenePro thermal cycler (BIOER). The PCR protocol began with an initial denaturation at 94 °C for 2 minutes, followed by 25 to 30 cycles of 1 minute at 94 °C (denaturation), 1 minute at 60 °C (annealing), and 2 minutes at 72 °C (extension), with a final extension at 72 °C for 5 minutes. Primers specific to the target genes, listed in Table 1, were designed using the NCBI Primer-BLAST tool and synthesized by Biodynamics®. All gene expression results were normalized to β-actin, which was used as the reference gene. The PCR products were analyzed through electrophoresis on a 2% agarose gel, stained with GelRed™ nucleic acid stain (Biotium, Inc., Fremont, CA, USA), visualized with a Gel Doc XR+ imaging system (BioRad, CA, USA), and quantified using Image Lab software. The data were presented in arbitrary units (AU) of relative optical density.

Salivary secretion assessment

One week before euthanasia, rats from the control and EP groups were anesthetized via intraperitoneal injection of ketamine hydrochloride (Holliday-Scott SA, 70 mg/kg) and 2% xylazine hydrochloride (König Laboratories, 10 mg/kg). Then, pilocarpine (Sigma-Aldrich, 0.5 mg/kg) was administered intraperitoneally to induce salivation, and a cotton ball was promptly placed under the tongue to collect the entire salivary output. The amount of saliva was determined by weighing the cotton ball before and after the collection. The saliva was gathered over a 90-minute period after pilocarpine administration. The rats and the saliva were weighed, and salivary production was reported as milligrams of saliva secreted per gram of body weight.

PGE_2 assessment by radioimmunoassay

To measure PGE_2 levels, submandibular gland samples were homogenized in 1 mL of absolute ethanol. After centrifugation, the supernatants were dried using a centrifugal vacuum concentrator (Speed Vac, Thermo Fisher Scientific) at room temperature. The resulting residues were resuspended in buffer, and antiserum (Sigma-Aldrich) was added according to the method outlined by Mohn et al., 2011[29]. The assay had a sensitivity of 12.5 pg per tube, with 100% cross-reactivity for PGE_2 and PGE_1, and only 0.1% for other prostaglandins. The intra-assay and inter-assay variation coefficients for PGE_2 were 8.2% and 12.0%, respectively. The results were reported as picograms of PGE_2 per milligram of wet tissue weight.

Statistical analysis

Data were presented as the mean of six determinations ± SEM for each group. Statistical

Table 1. Primer sequence for specific markers.

GENE	ACCESSION NUMBER	FOWARD PRIMER (5´-3´)	REVERSE PRIMER (5´-3´)
β-ACTINA	NM_031144.3	ACCCGCCGAGTACAACCTTC	ATGCCGTGTTCAATGGGGTA
RANKL	NM_057149.1	ACCAGCATCAAAATCCCAAG	TTTGAAAGCCCCAAAGTACG
OPG	NM_012870.2	GTTCTTGCACAGCTTCACCA	AAACAGCCCAGTGACCATTC
CBr1	NM_001429314.1	AGGAGCAAGGACCTGAGACA	TAACGGTGCTCTTGATGCAG
CBr2	NM_009924.4	AGGTTGCATTCCCAACAGAC	TTAGTTCCTCTGGGCAATGG

analysis was performed using Student's t-test for comparisons between two groups, and Two-Way ANOVA followed by Tukey's post-hoc test for unequal replicates. The sample size was selected to ensure an 80% power to detect differences between treatments comparable to the experimental error. All statistical tests were conducted using Prism software (GraphPad Software), with significance defined as P values less than 0.05.

RESULTS

Assessments in periodontal tissues

Messenger RNA expression of CB1r and CB2r

The expression of CB1r mRNA in gingival tissue homogenates was almost two times higher in groups EP, H and EP+H than in controls, while the expression of CB2r mRNA was more than three times higher in group EP and two and a half times higher in groups H and EP+H than in controls (Table 2). Additionally, CB2r mRNA expression was significantly higher in group EP than in group H.

Table 2. Messenger RNA expression of CB receptors in gingival tissue measured by RT-PCR.

Group/ parameter	CB1r/β-actin mRNA expression (AU)	CB2r/β-actin mRNA expression (AU)
Control	0.27±0.06^{A}	0.38±0.04^{A}
EP	0.46±0.05^{B}	1.22±0.08^{B}
H	0.51±0.08^{B}	0.98±0.06^{C}
EP+H	0.49±0.1^{B}	1.041±0.1^{BC}

EP: experimental periodontitis. H: hyposalivation.
Values are expressed as media ± standard error.
Different letters represent significant statistical differences analyzed by One Way ANOVA followed by Tukey's multiple comparisons test ($p<0.05$).

Macroscopic assessment of periodontal bone loss

Distance and width methods

Gingival administration of LPS (group EP) and SM (group H) increased alveolar bone loss evaluated by both distance and width methods. Bone loss determined as the increased distance between the CEJ and the AC was evidenced in groups EP, H and EP+H compared to controls, in both maxilla and mandible (Fig. 1A and Fig. 1B). Even in the mandible, bone loss was greater in group EP+H than in EP and H alone. However, the width method, which evaluates alveolar bone levels by measuring in the buccal-lingual direction on the mandibular first molars, confirmed that EP, H and EP+H conditions induced bone loss compared to the control group. However, no significant differences were observed among the three experimental groups (Fig. 1C).

Messenger RNA expression of RANKL and OPG

RANKL mRNA expression in homogenized dental insertion tissues was significantly higher in groups EP and EP+H compared to the control group, while OPG mRNA expression was notably lower (Table 3). In contrast, group H did not differ significantly from the control group in RANKL or OPG mRNA expression. The RANKL/OPG ratio was similar between the H and control groups but was twice as high in groups EP and EP+H than in the other two groups.

Assessments in submandibular glands

Messenger RNA expression of CB1r and CB2r

The expression of CB1r mRNA in SMG homogenates was almost two times higher in group EP than in controls (Table 4). It was noteworthy that even though the basal expression of CB2r mRNA was lower than that of CB1r, it was more than six times higher in group EP than in controls.

Salivary secretion assessment

After a 90-minute post-stimulation period, secreted saliva was significantly reduced in animals that received LPS gingival administration (group EP) than in controls (Fig. 2A).

Radioimmunoassay of PGE_2

PGE_2 content level in submandibular glands, a known mediator of salivary flow reduction, was higher in the EP group than in controls (Fig. 2B).

DISCUSSION

The relationship between oral diseases and cannabinoid receptors is an area of growing scientific interest. Previous studies by our group have demonstrated that the administration of cannabinoid receptor agonists exerts a therapeutic effect in experimental models of periodontitis, resulting in decreased alveolar bone loss and gingival inflammation[24,30-32]. *In vitro* studies using human periodontal cells further support these beneficial effects[33].

In the present study, we investigated the expression levels of cannabinoid receptors in the periodontal tissues and salivary glands of rats subjected to

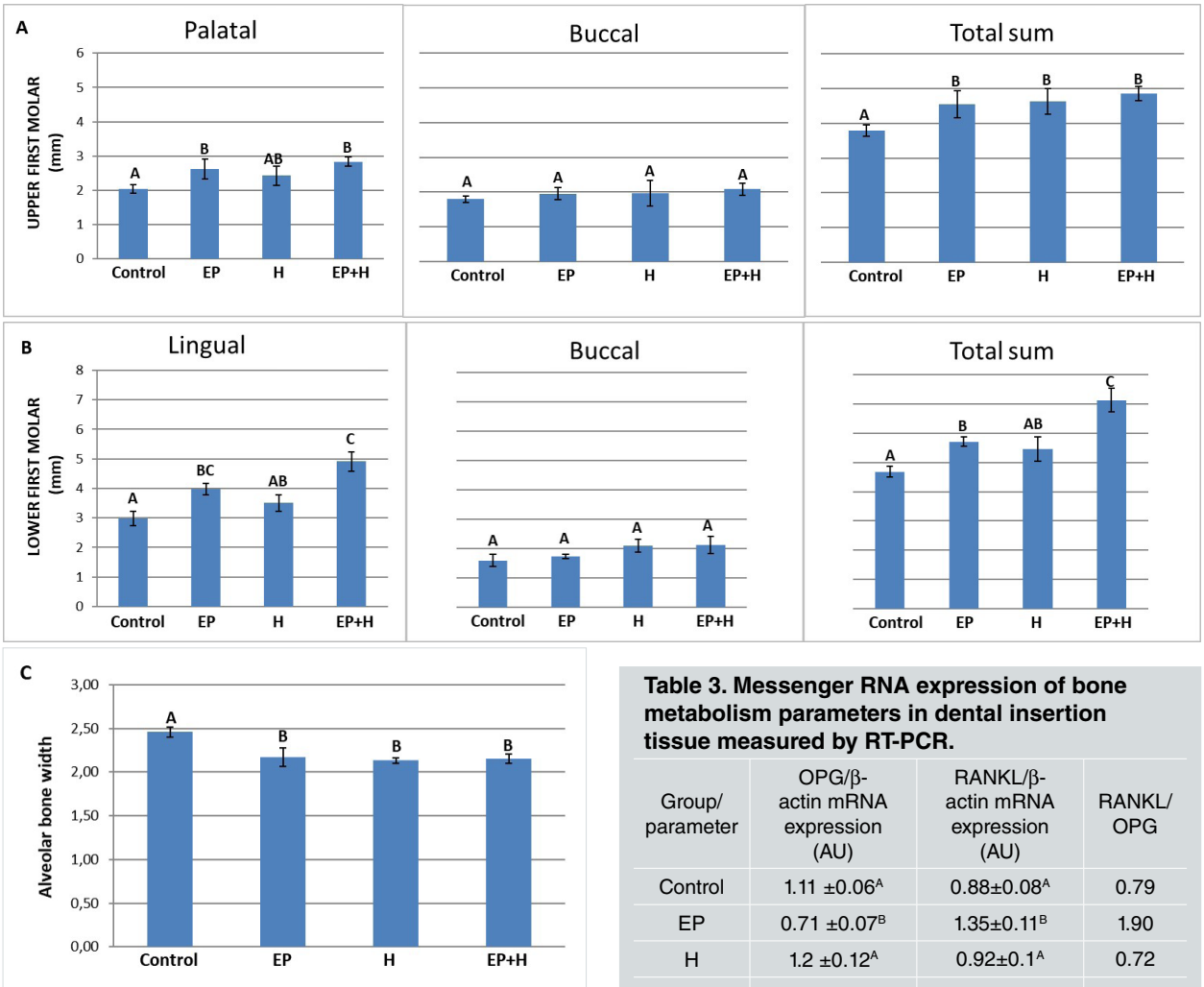

Fig. 1: Morphometric evaluation of alveolar bone loss in cortical plates: A) Palatal face, buccal face and total sum in the upper first molar. B) Lingual face, buccal face and total sum in the lower first molar. C) Width of the alveolar bone measured in the buccal-lingual direction in the lower first molars. EP: experimental periodontitis. H: hyposalivation. Results are presented as mean ± SEM. Different letters represent significant statistical differences analyzed by One Way ANOVA followed by Tukey's multiple comparisons test (p<0.05).

Table 3. Messenger RNA expression of bone metabolism parameters in dental insertion tissue measured by RT-PCR.

Group/ parameter	OPG/β-actin mRNA expression (AU)	RANKL/β-actin mRNA expression (AU)	RANKL/ OPG
Control	1.11 ±0.06^{A}	0.88±0.08^{A}	0.79
EP	0.71 ±0.07^{B}	1.35±0.11^{B}	1.90
H	1.2 ±0.12^{A}	0.92±0.1^{A}	0.72
EP+H	0.87±0.09^{B}	1.47±0.15^{B}	1.68

EP: experimental periodontitis. H: hyposalivation.
Values are expressed as mean ± standard error. Different letters represent significant statistical differences analyzed by One Way ANOVA followed by Tukey's multiple comparisons test (p<0.05).

Table 4. Messenger RNA expression of CB receptors in submandibular gland measured by RT-PCR.

Group/ parameter	CB1r/β-actin mRNA expression (AU)	CB2r/β-actin mRNA expression (AU)
Control	0.52±0.09^{A}	0.23±0.12^{A}
EP	0.95±0.13^{B}	1.48±0.22^{B}

EP: experimental periodontitis.
Values are expressed as mean ± standard error. Different letters represent significant statistical differences analyzed by Student's T-test (p<0.05).

experimental periodontitis and hyposalivation induced by submandibulectomy. Our results demonstrated upregulation of both CB1r and CB2r under these pathological conditions. These findings provide additional insight into the involvement of the endocannabinoid system (ECS) in oral disease pathogenesis and support its potential as a therapeutic target.

The upregulation of cannabinoid receptors in inflamed tissues, as shown in this study, may reflect a compensatory anti-inflammatory mechanism aimed at mitigating tissue damage during the inflammatory process. This interpretation aligns with previous findings in human tissues. For instance, Navarro-Saiz et al.[34] reported that both CB1r and CB2r are expressed in human odontoblasts and gingival fibroblasts, and that CB2r expression increases significantly under inflammatory conditions.

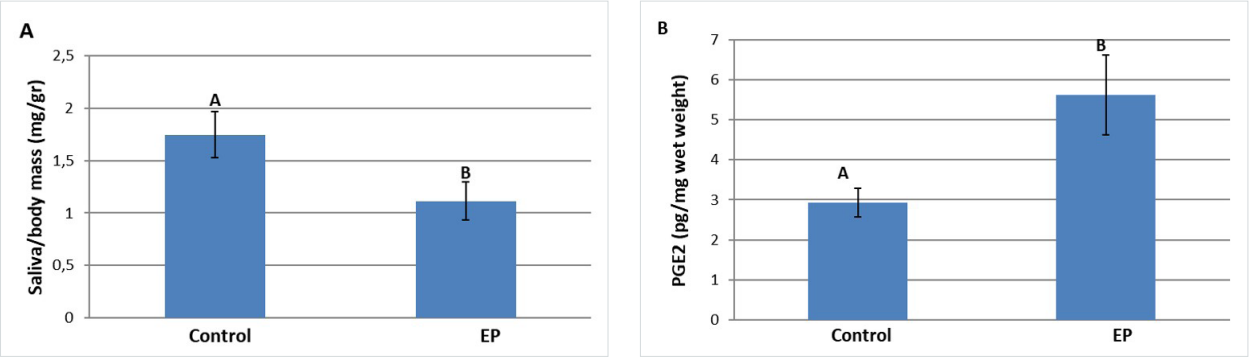

Fig. 2: A) Salivary secretion (mg of saliva/body mass) after administration of pilocarpine to rats in the control and EP groups. B) PGE_2 content (pg/mg wet weight) in the submandibular glands of rats from the control and EP groups. EP: experimental periodontitis. Values are expressed as mean ± standard error. Different letters represent significant statistical differences analyzed by Student's t-test ($p<0.05$).

Similarly, Nakajima et al.[3] found higher CB2r expression in gingival fibroblasts from patients with gingivitis and periodontitis compared to healthy individuals[3]. In contrast, Ataei et al.[35] reported decreased cannabinoid receptor expression in periodontitis patients, suggesting that reduced ECS signaling might be linked to greater susceptibility to disease progression. However, this discrepancy could stem from different stages or mechanisms of disease and should be interpreted with caution.

In our study, rats with experimental periodontitis exhibited a significant increase in the RANKL/OPG ratio and notable alveolar bone loss, accompanied by elevated CB1r and CB2r mRNA levels in gingival tissues, particularly CB2r. These findings suggest that cannabinoid receptors may participate in endogenous homeostatic responses aimed at controlling inflammation and preventing further periodontal destruction. In the case of hyposalivation induced by submandibulectomy, CB1r and CB2r gingival expression was also increased, with concomitant alveolar bone loss, though without a significant change in the RANKL/OPG ratio. This suggests the activation of an alternative damage pathway, possibly indirect and different from the one triggered by LPS during periodontitis.

Although literature exploring cannabinoid receptor expression in inflamed oral tissues remains limited, evidence from other organ systems supports a similar modulatory role. For instance, CB2r upregulation has been observed in microglia following brain injury, contributing to neuroprotection by attenuating the inflammatory response[36]. Similarly, increased CB2r expression in synovial tissues after joint injury has been linked to anti-inflammatory effects mediated through fibroblasts and macrophages[37]. These parallels reinforce the hypothesis that CB2r upregulation in oral tissues may play a protective, inflammation-limiting role.

With regard to the ECS in salivary glands, previous studies have shown that CB1r and CB2r are differentially expressed in murine submandibular glands[14]. CB1r was predominantly found in the ductal structures, while CB2r was located mainly around acinar cells. In that study, activation of cannabinoid receptors by anandamide was shown to inhibit stimulated salivary secretion, an effect reversed by selective antagonists. These findings suggest that the ECS exerts a regulatory influence on salivary gland function under physiological conditions. In this context, the reduced salivary response to pilocarpine observed in our experimental periodontitis model likely reflects a similar mechanism. This reduction, together with the six-fold increase in CB2r expression in salivary glands during periodontitis, strongly suggests that the ECS may be actively involved in the pathophysiological processes leading to decreased salivary secretion. Thus, the ECS could be contributing to the development of xerostomia frequently associated with periodontal disease, not only through modulation of secretion, but also by influencing local inflammatory responses. This highlights a potential modulatory role of the ECS in the context of chronic oral inflammation.

The physiological significance of cannabinoid receptor upregulation in oral tissues under inflammatory conditions may lie in the ECS's immunomodulatory and anti-inflammatory capabilities. Endogenous cannabinoids acting through CB1r and CB2r can suppress pro-

inflammatory mediator release and influence leukocyte activity (including that of neutrophils, lymphocytes and macrophages), thus regulating immune responses and preventing excessive tissue destruction[4,5]. Given that periodontitis is a chronic bacterial infection, increased receptor expression may serve as a protective mechanism to maintain tissue homeostasis and limit disease progression.

In summary, the observed upregulation of cannabinoid receptors in response to both periodontal inflammation and hyposalivation suggests a coordinated ECS-mediated response to pathophysiological stress. In periodontal tissues, this may bean attempt to reduce inflammation and preserve tissue integrity. In salivary glands, while the situation is more complex, the ECS may contribute to both immune regulation and salivary secretion. Prior findings support the hypothesis that cannabinoid-mediated inhibition of salivary secretion in inflamed conditions could be a defense mechanism to minimize the dissemination of pathogens and inflammatory agents, particularly via CB1r[23]. Conversely, CB2r appears to mediate the anti-inflammatory and modulatory responses most prominently observed under our experimental conditions.

Cannabinoid receptor upregulation during chronic oral inflammation likely constitutes a homeostatic feedback mechanism aimed at restoring ECS balance. The present and prior findings of our group support the potential development of cannabinoid-based therapies to reduce inflammation, protect periodontal tissues and facilitate tissue repair. As research in this field is still emerging, additional studies are warranted to elucidate further the regulatory dynamics of cannabinoid receptors in oral pathologies and to explore their therapeutic potential.

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CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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