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Participation of the endocannabinoid system in lipopolysaccharide-induced inhibition of salivary secretion

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ABSTRACT

Objective: The aim of the present paper was to assess whether lipopolysaccharide (LPS)-induced inhibition of salivary secretion involves the activation of the endocannabinoid system and the participation of tumor necrosis factor (TNF)α in the submandibular gland.
Design: Pharmacological approaches were performed by using CB1 and/or CB2 cannabinoid receptor antagonists, AM251 and AM630, respectively, injected into the submandibular gland, to study the participation of the endocannabinoid system in LPS inhibitory effects on metacholine-induced salivary secretion. To assess the participation of TNFα on LPS inhibitory effects, salivary secretion was studied in LPS treated rats after the intraglandular injection of etanercept, a soluble form of TNF receptor which blocks TNFα action. Finally, to evaluate the possible interplay between endocannabinoids and TNFα on the submandibular gland function reduced during LPS challenge, the salivary secretion was studied after the intraglandular injection of this cytokine alone or concomitantly with AM251 and AM630.
Results: AM251 and AM630, injected separately or concomitantly, partially prevented LPS-induced inhibition of salivation. Also, anandamide synthase activity was increased in submandibular glands extracted from rats 3 h after LPS injection, suggesting that the endocannabinoid system was activated in response to this challenge. On the other hand, etanercept, prevented the inhibitory effect of LPS on salivary secretion and moreover, TNFα injected intraglandularly inhibited salivary secretion, being this effect prevented by AM251 and AM630 injected concomitantly.
Conclusion: The present results demonstrate the participation of the endocannabinoid system and TNFα on salivary responses during systemic inflammation induced by LPS.

1. Introduction

Lipopolysaccharide (LPS), an integral part of the outer membrane of gram-negative bacteria, is the main pathogenic factor that leads to endotoxemia. Macrophages are the primary target for LPS, where the endotoxin interacts with the CD14 protein/toll-like receptor-4 complex to activate multiple signalling pathways.[1,2] This signalling pathways lead to the activation of a variety of transcription factors,[3] that regulate gene expression encoding inflammatory mediators.[4] Lipopolysaccharide induces the expression of cytokines such as tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1), IL-6, and IL-8, which have been implicated in the pathophysiology of sepsis.[1] Additionally,

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LPS induces the production of different lipid mediators in macrophages, such as prostaglandins,[5] leukotrienes[6] and endocannabinoids.[7] The best-known endocannabinoids are arachidonoyl ethanolamide (anandamide) and arachidonoyl glycerol, both derivatives of arachidonic acid.[8] Mammalian tissues contain, at least two types of cannabinoid receptors, CB_1 and CB_2, concentrated in the nervous system[9–11] and peripheral tissues, respectively.[12] Both receptors are coupled to Gi/o proteins and respond by inhibiting the activity of adenylyl cyclase.[13]

The submandibular gland is one of the major salivary glands, together with the sublingual and the parotid glands. End secretory units, called acini, are continuous with a ductal system that leads the saliva to the oral cavity.[14] Previously, we have described the presence of CB_1 and CB_2 receptors in the ductal system of the submandibular gland and, additionally, the expression of CB_2 receptors in the periphery of acinar cells.[15] We also have shown that anandamide (10 ng/50 μl) injected into the submandibular gland decreases salivary secretion through the activation of both cannabinoid receptors, since AM251 and AM630, CB_1 and CB_2 receptor antagonists respectively, block this inhibitory effect.[15]

Salivary secretion is altered in different pathological states. Moreover, we have previously demonstrated that LPS (5 mg/kg/3 h) injected intraperitoneally inhibits salivary secretion by increasing the production of prostaglandins, that are derivatives of arachidonic acid like endocannabinoids.[16] Also, TNFα is known to be released after LPS administration and mediates a number of effects attributed to LPS; therefore it could be involved in LPS-induced inhibition of salivary secretion. In addition, anandamide content is rapidly increased in different tissues in response to LPS intraperitoneal or intravenous injection.[7,17] Furthermore, anandamide is able to inhibit proinflammatory cytokines production, including TNFα in LPS-stimulated monocytes[18] and rat microglial cells,[19] suggesting that endocannabinoids modulate inflammatory responses.

Based in the evidences described, the aim of the present work was to assess whether LPS-induced inhibition of salivary secretion involves the participation of TNFα and the activation of the endocannabinoid system in the submandibular gland.

2. Materials and methods

2.1. Chemicals

Anandamide, Forskolin and LPS from *Escherichia coli* were purchased from Sigma Chemicals (St. Louis, MO, USA). Chloralose and methacholine were obtained from FLUKA (Laborchemikalien, Berlin, Germany). TNFα was purchased from Promega Corporation (Madison, WI, USA). AM251 [N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] and AM630 6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl) methanone were obtained from Tocris™ (Ellisville, MO, USA). Etanercept was purchased from Amgem and Wyeth Pharmaceuticals (Philadelphia; USA).

2.2. Animals

Adult male Wistar rats (250–300 g) from our own colony (Department of Biochemistry, Dental School, University of Buenos Aires) were kept in group cages in an animal room having a photoperiod of 12 h of light (07:00–19:00), room temperature of 22–25 °C and free access to rat chow and tap water. The animals were divided into several experimental groups (6–8 animals/group). The experimental procedures reported here were approved by the Animal Care Committee of the Center for Pharmacological and Botanicals Studies of the National Council of Scientific and Technical Research of Argentina and were carried out in accordance with the National Institute of Health of USA guidelines.

2.3. "In vivo" studies

2.3.1. Salivary secretion studies

Salivary responses were determined in anesthetized rats (chloralose 100 mg/kg, 0.5 ml NaCl 0.9% iv). The submandibular ducts were cannulated with a fine glass cannula, and salivary secretion was induced by different doses of methacholine (1, 3 and 10 μg/kg in saline) administered sequentially via the right femoral vein. The saliva was collected, for 3 min after the injection, on aluminum foil and weighed as previously described.[20] Resting flow of saliva (unstimulated) was not observed in rats. There were 5–6 rats per group and results were expressed as mg of saliva/3 min.

In the first group of experiments, the rats received intraperitoneal injections of LPS (5 mg/kg) or saline as vehicle. To evaluate the participation of the endocannabinoid system in salivary responses to LPS, AM251 (15 μg in 50 μl of 1% dimethyl sulfoxide), a selective antagonist for CB_1 receptors,[21] AM630 (15 μg in 50 μl of 1% dimethyl sulfoxide), a selective antagonist for CB_2 receptors[22] or vehicle were injected into the submandibular gland. The injections were performed with a 30 G × needle and the substances were injected very slowly between the capsule that covers the gland and the parenchyma. In order to be sure that the substances reached the entire gland, the injections were performed at 30 min, 1.5 and 2.5 h post-LPS injection. Three hours after LPS injection, dose–response curves to methacholine were performed to evaluate salivary secretion. The doses of cannabinoid receptor antagonists employed were obtained from our previous reports.[15,23]

To assess whether TNFα is involved in LPS-induced inhibition of salivary secretion, we injected etanercept (800 μg in 50 μl), a TNFα antagonist, or saline as vehicle, into the submandibular gland 2.5 h after LPS intraperitoneal injection and 30 min after, dose–response curves to methacholine on salivary secretion were performed.

Therefore, the rats were divided in six groups: (1) vehicle, (2) LPS (5 mg/kg, intraperitoneal), (3) LPS + AM630 (15 μg/50 μl, intraglandular), (4) LPS + AM251 (15 μg/50 μl, intraglandular), (5) LPS + AM630 + AM251 and (6) LPS + etanercept (800 μg/50 μl, intraglandular).

Additionally, to confirm whether TNFα (300 ng in 50 μl, intraglandular) alters salivary secretion by activating the endocannabinoid system, we studied its effect on salivary secretion when it was injected alone or 10 min after the injection of AM251 (15 μg/50 μl) and AM630 (15 μg/50 μl) into

the submandibular gland. Thirty minutes after TNFα injections, dose–response curves to methacholine on salivary secretion were performed.

Additionally, 3 h after LPS injection (5 mg/kg, intraperitoneal), submandibular glands were extracted from anesthetized rats and homogenized in 1 ml of H_2O and centrifuged at 6000 × *g* for 10 min at 4 °C. Supernatants were collected and stored at −20 °C until cyclic adenosine monophosphate (cAMP) measurements.

2.4. "In vitro" studies

The animals were killed by decapitation, and submandibular glands were removed. Submandibular glands (5–6 per group) were cut in halves to enhance the penetration of the different substances into the tissue and preincubated in 500 μl of Krebs–Ringer bicarbonate buffer medium (pH 7.4) containing 0.1% glucose in a Dubnoff metabolic shaker (50 cycles per min, 95% O_2/5% CO_2) for 15 min before replacement with fresh medium containing the compounds to be tested. The incubation was continued for another 30 min with forskolin (80 μM), an adenylyl cyclase stimulator, forskolin plus TNFα (3×10^{-9} M); forskolin plus TNFα plus AM251 (10 μM), AM251 alone; forskolin plus TNFα plus AM630 (10 μM); AM630 alone; forskolin plus TNFα plus AM251 plus AM630; and finally, AM251 plus AM630. After the incubations, the submandibular glands were homogenized in 1 ml of distilled H_2O and centrifuged at 6000 × *g* for 10 min at 4 °C. Supernatants were collected and stored at −20 °C until cAMP measurements.

2.5. Radioimmunoassay

cAMP was measured by radioimmunoassay, using the highly specific cAMP antibody kindly provided by Dr. A.F. Parlow (National Hormone & Peptide Program, CA, USA). The assay sensitivity was 0.061 pmol/ml. Intra- and inter-assay coefficients of variation were 8.1 and 10.5%, respectively.

2.6. Anandamide production

The production of anandamide was assayed as described by Paria et al. 1996 with minor modifications.[24] The submandibular glands were removed 3 h after the injection of LPS (5 mg/kg, intraperitoneal), homogenized in 800 μl of buffer [20 mM Tris–HCl/1 mM EDTA (pH 7.6)] and centrifuged at 2000 × *g* for 15 min. Supernatant protein (150–100 μg) was incubated in a total volume of 200 μl of 50 mM Tris–HCl (pH 9.0) with 40 μM (0.1 μCi) of [1-^{14}C]arachidonic acid (40–60 μCi/mmol, PerkinElmer, Waltham, MA, USA) and 20 mM ethanolamine (Sigma Chemicals, St. Louis, MO, USA) during 5 min at 37 °C. The reaction was terminated by the addition of 400 μl of chloroform–methanol (1:1) mixture. Two additional washes of the aqueous phase with 400 μl of chloroform were performed. Organic phases were evaporated to dryness under nitrogen gas and dissolved in 40 μl of chloroform–methanol (1:1). Samples and standards were applied on Silica Gel 60 plates (Merck, Darmstadt, Germany). The synthesized [^{14}C]anandamide was resolved by using the organic layer of an ethyl acetate–hexane–acetic acid–water (100:50:20:100) mixture. The plate was exposed to X-ray film at −70 °C. After autoradiography, distribution of radioactivity on the plate was counted in a scintillation counter by scraping off the corresponding spots of the plate. The retardation factor values of anandamide and arachidonic acid were 0.33 and 0.78, respectively.

2.7. Determination of TNFα

TNFα was determined by the sandwich enzyme immunoassay technique using a rat TNFα Kit provided by Quantikine M, R&D Systems (Minneapolis, MN, USA). The optical density was determined at 450 nm with a wavelength correction set at 540 using a microplate reader (Bio-Rad model 550). These assays have been shown to be specific for rat TNFα. The sensitivity of the assay was 12.5 pg/ml per tube and the curve was linear up to 400 pg/ml of TNFα. The inter- and intra-assay variation was 3.5 and 9.4%, respectively.

2.8. Statistics

Data are presented as the mean ± SE. Comparisons between more than two groups were performed by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparison test or by two-way ANOVA followed by the Bonferroni post-test. When two groups were compared, Student's *t*-test was used. All analyses were performed with the Graph-Pad Instat software. Differences with p values <0.05 were considered statistically significant.

3. Results

3.1. Effect of cannabinoid receptor antagonists and etanercept on LPS-induced inhibition of salivary secretion

Since the inhibitory actions of LPS and anandamide on salivary secretion are similar, we hypothesized that the effect of LPS might be mediated, at least in part, by the endocannabinoid system. For that reason, we assessed the effect of selective antagonists for CB_1 and CB_2, AM251 and AM630, respectively, on salivary secretion reduced by LPS. The inhibitory effect of LPS (5 mg/kg/3 h) on methacholine (3 and 10 μg/kg)-induced salivary secretion was prevented by intraglandular injections of AM251 (15 μg/50 μl) at 3 μg/kg of methacholine and partially blocked at 10 μg/kg of methacholine ($P < 0.01$ vs. vehicle) (Fig. 1). Additionally, the inhibitory effect of LPS on methacholine-induced salivary secretion was partially blocked by injecting AM630 (15 μg/50 μl) at 10 μg/kg of methacholine ($P < 0.001$ vs. vehicle). Furthermore, the simultaneous injection of both antagonists, AM251 and AM630, completely prevented the inhibitory effect of LPS on salivary secretion induced by 3 μg/kg of methacholine and partially blocked it at 10 μg/kg of methacholine ($P < 0.05$ vs. vehicle).

Since TNFα is one of the well-known mediators of LPS actions, we studied LPS effect on salivary secretion after intraglandular injection of etanercept, a TNFα antagonist. Etanercept (800 μg/50 μl) prevented completely LPS inhibitory effect on salivary secretion induced by 3 μg/kg of methacholine and partially when 10 μg/kg of methacholine was used ($P < 0.001$ vs. vehicle) (Fig. 1).

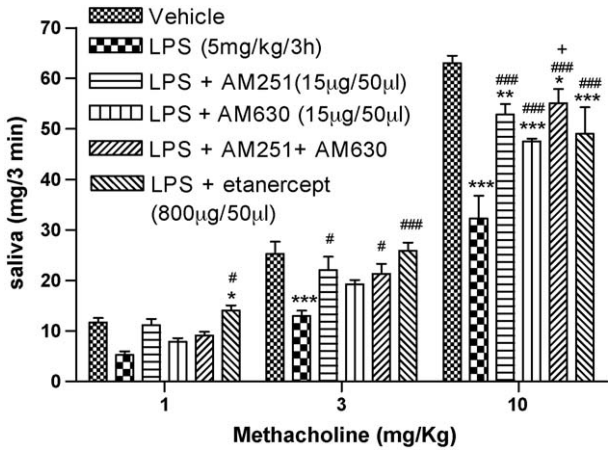

Fig. 1 – Effect of intraglandular injection of AM251 (15 μg/50 μl), AM630 (15 μg/50 μl), AM251 plus AM630 and etanercept (800 μg/50 μl) on LPS (5 mg/kg/3 h, intraperitoneal)-induced inhibition on methacholine-stimulated salivary secretion. Values are mean ± SE (5–6 rats per group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ vs. LPS and $^{+}P < 0.05$ vs. LPS plus AM630 (two-way ANOVA followed by Bonferroni post-test).

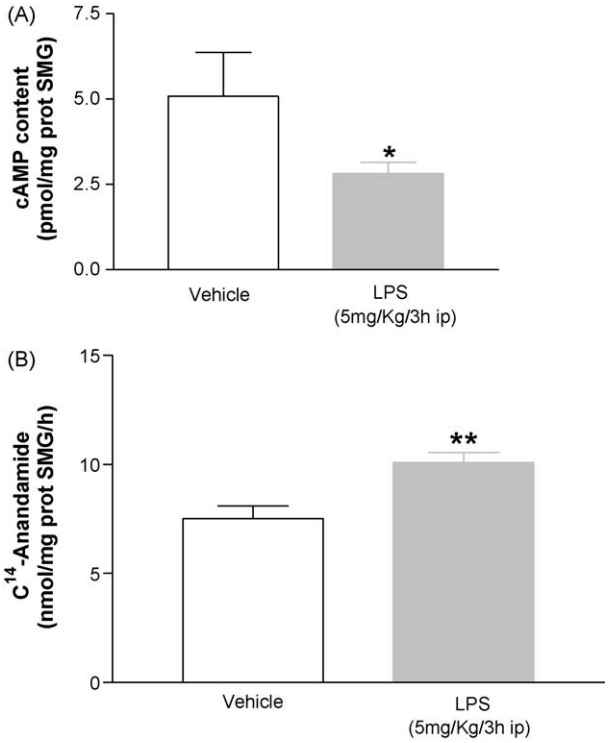

Fig. 2 – Effect of LPS (5 mg/kg/3 h, ip) on (A) cyclic AMP content and (B) production of AEA in the submandibular gland (SMG). Values are means ± SE (7–8 rats per group). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle (Student's t-test).

3.2. *Effect of LPS administration on cAMP content and anandamide production in the submandibular gland*

Lipopolysaccharide (5 mg/kg/3 h) injected intraperitoneally decreased significantly ($P < 0.05$) cAMP content in the submandibular gland. This effect could be due to activation of Gi coupled cannabinoid receptors by increased levels of endocannabinoids (Fig. 2A). To confirm this hypothesis, we studied the effect of LPS injected intraperitoneally on anandamide production in submandibular glands *ex vivo*. Fig. 2B shows that LPS, 3 h after its injection, increased significantly ($P < 0.01$) anandamide production.

3.3. *Effect of LPS on TNFα plasma levels and content in the submandibular gland*

Since it is well known that LPS stimulates TNFα production, we studied the *in vivo* effect of LPS on TNFα plasma concentration and content in the submandibular gland. Lipopolysaccharide (5 mg/kg/, intraperitoneal) increased TNFα plasma concentration 30 min after its injection, showing a peak at 1 and 3 h post-injection (Fig. 3A). Also, TNFα content was increased in submandibular glands 3 and 6 h after LPS injection as compared to control values (Fig. 3B).

3.4. *Inhibitory effect of TNFα on cAMP content in submandibular glands in vitro*

Cyclic AMP content was increased ($P < 0.01$) by forskolin (8×10^{-5} M), an activator of AC, in submandibular glands *in vitro* (Fig. 4). This increase in cAMP was significantly blocked ($P < 0.01$) by TNFα (3×10^{-9} M). To investigate whether the inhibition of TNFα on cAMP content is mediated by cannabinoid receptors, AM251 (10^{-5} M) and AM630 (10^{-5} M) were added to the media together with TNFα and forskolin or alone. AM251 as well as AM630 blocked ($P < 0.05$) the inhibition of TNFα on forskolin stimulated cAMP content. When AM251 and AM630 were used together, the blockade of TNFα inhibitory effect was higher ($P < 0.01$). Neither AM251 nor AM630 alone nor AM251 and AM630 together had any effect on cAMP content as compared to control values as can be seen in Fig. 4.

3.5. *Effect of TNFα and cannabinoid receptor antagonists on salivary secretion*

To ensure that TNFα mediates LPS-induced inhibition of salivary secretion by activating the endocannabinoid system we studied the effect of intraglandular injections of TNFα and cannabinoid receptor antagonists on salivary secretion. We observed that TNFα (300 ng/50 μl) inhibited methacholine-induced salivary secretion ($P < 0.05$ at 1 μg/kg and $P < 0.001$ at 3 and 10 μg/kg vs. vehicle) and that previous intraglandular injection of AM251 (15 μg/50 μl, ig) plus AM630 (15 μg/50 μl) prevented almost totally this inhibitory effect (Fig. 5).

4. Discussion

During the last years the number of studies on the effects of endocannabinoids in physiological and pathophysiological conditions has been increased. Moreover, the participation of endocannabinoids in the cytokine network of various organs

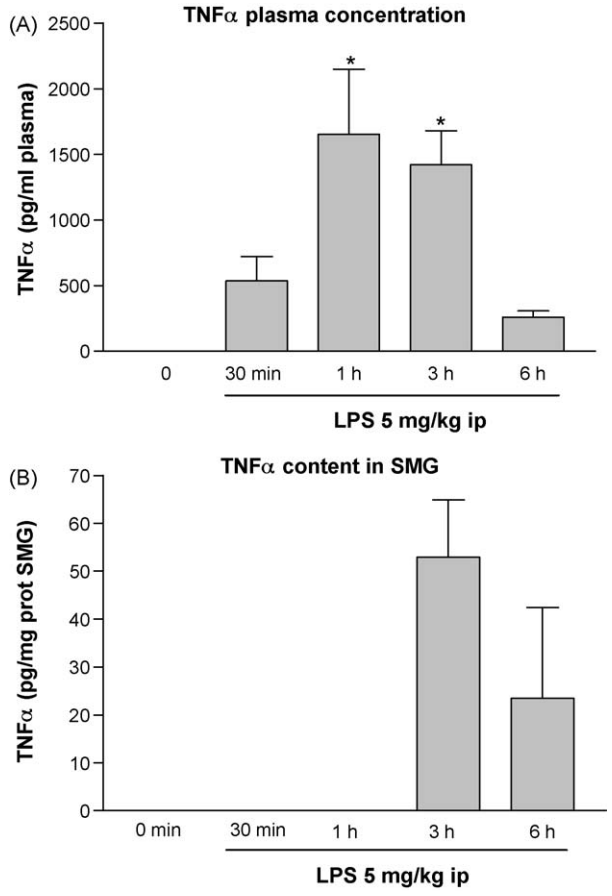

Fig. 3 – Effect of LPS (5 mg/kg, ip) on time course of TNFα concentration in (A) plasma and (B) submandibular gland (SMG). Values are means ± SE (6 rats per group). *P < 0.05 vs. 30 min and 6 h (one-way ANOVA followed by Student–Newman–Keuls multiple comparison test).

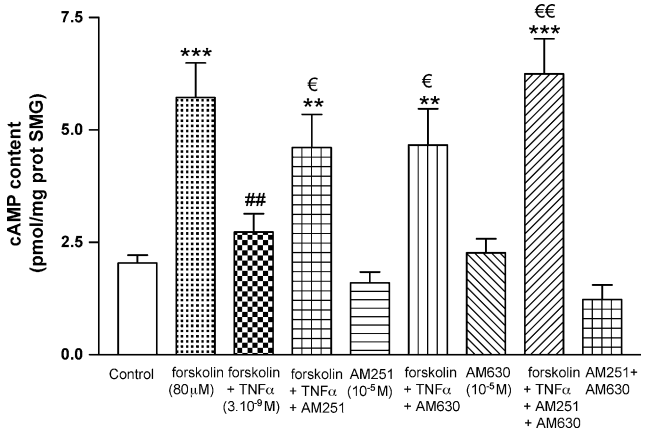

Fig. 4 – Effect of TNFα (3×10^{-9} M), AM251 (10^{-5} M), AM630 (10^{-5} M) and the combinations of them on cAMP content increased by forskolin in submandibular glands (SMG) *in vitro*. Values are means ± SE (6 rats per group). **P < 0.01 and ***P < 0.001 vs. control. ##P < 0.01 vs. forskolin. €P < 0.05 and €€P < 0.01 vs. forskolin + TNFα (one-way ANOVA followed by Student–Newman–Keuls multiple comparison test).

and tissues was reported and its therapeutic potential was proposed.[25,26]

In the present work, we demonstrate that LPS injected intraperitoneally reduced salivary secretion 3 h after its injection and also increased anandamide production and decreased cAMP content, a second messenger whose reduction is linked to cannabinoid receptors activation,[13] in the submandibular gland. In concordance, other authors reported that LPS stimulates anandamide production in human peripheral lymphocytes and mouse macrophages.[7,27] Different hypotheses were proposed about the increases of anandamide in response to LPS: (1) down-regulation of fatty acid amide hydrolase expression in immune cells,[27] the enzyme implicated in anandamide degradation; (2) direct increase of anandamide production through a phospholipase C/phosphatase pathway.[7] In addition, we have previously reported that LPS-induced inhibition of salivary secretion is associated to the increase of prostaglandin E_2, another derivative of arachidonic acid.[16] Therefore, we hypothesized that the endocannabinoid system could mediate the inhibitory effect of LPS on salivary secretion. The present results demonstrate that both CB_1 and CB_2 antagonists blocked, at least partially, the inhibitory effect of LPS on methacholine-induced salivary secretion when were injected intraglandularly. This effect may be due to an increase in the availability of cAMP by the blockade of cannabinoid receptors, probably resulting in elevated cytosolic calcium levels and the consequent high salivary flow in response to methacholine. As was mentioned in introduction, cannabinoid receptors are mainly coupled to $G_{i/o}$ proteins and their activation produce an inhibitory effect of adenylyl cyclase activity and the consequent decrease of cAMP production.[13] Although calcium is the main second messenger that mediates salivary secretion after muscarinic cholinergic receptor activation, an emerging body of evidence indicates that, a cross-talk exist between

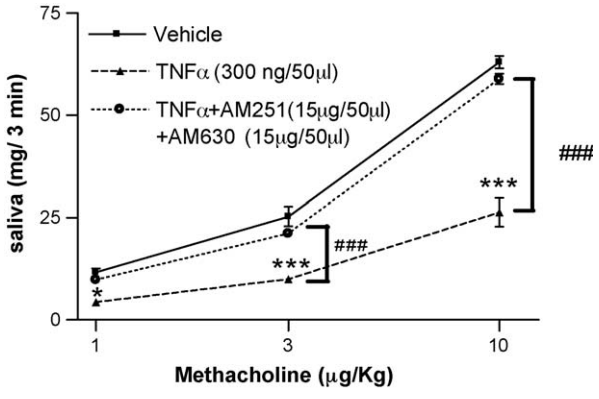

Fig. 5 – Effect of intraglandular injection of TNFα (300 ng/50 μl) alone and after the injection of AM251 (15 μg/50 μl) plus AM630 (15 μg/50 μl) on methacholine-stimulated salivary secretion. Values are mean ± SE (5–6 rats per group). *P < 0.05 and ***P < 0.001 vs. vehicle. ###P < 0.001 vs. TNFα (two-way ANOVA followed by Bonferroni post-test).

phospholipase C/inositol triphosphate/calcium and adenylyl cyclase/cAMP pathways that regulates the calcium transport processes.[28,29] Moreover, it was suggested that adenylyl cyclase/cAMP is the intracellular signalling pathway resulting in elevated cytosolic calcium levels. In fact, acetylcholine-evoked secretion from the parotid gland is substantially enhanced by cAMP-raising agonist.[30]

Cannabinoid receptors are expressed in different structures of the submandibular gland such as acini, ducts as well as nervous terminals[15] and their activation could probably inhibit the release of neurotransmitters from pre-synaptic terminals on the submandibular gland, consequently inhibiting salivation. In this line, it was reported that Δ^9-tetrahydrocannabinol administrated through the femoral vein decreased salivary flow from submandibular gland during electrical stimulation by a mechanism involving a decrease in the release of acetylcholine.[31]

It is well documented that LPS increases the plasma concentration of different cytokines including TNFα, one of the most important cytokine involved in the pathophysiology of sepsis.[1] Moreover, it was reported that the proinflammatory cytokines IL-1β, IL-6 and TNFα were induced by LPS via toll-like receptor type 4 in the submandibular gland of mice.[32] Particularly, TNFα has been associated with alterations of salivary secretion flow and composition.[33,34] Here we show that etanercept, a dimeric soluble form of the 75-kDa TNF receptor, injected intraglandularly, prevented the inhibitory effect of LPS on salivary secretion. This result confirmed that LPS inhibitory effect was mediated, at least partially, by TNFα. The anti-inflammatory effects of etanercept are due to its ability to bind to TNF, preventing its interaction with cell-surface receptors and rendering it biologically inactive.[35]

To investigate the possible role of TNFα in LPS induced activation of the endocannabinoid system in the submandibular gland, we first evaluated TNFα plasma concentration and content in the submandibular gland after LPS injection. We demonstrated that LPS not only increased TNFα plasma concentration from 30 min exhibiting a higher response at 1 h, but also increased TNFα content in the submandibular gland from 3 h post-LPS injection. Additionally, we showed that TNFα injected intraglandularly inhibited methacholine-induced salivary secretion. These results are supported by previous work showing that TNFα production is an important contributor to secretory dysfunction in Sjögren's syndrome by disrupting salivary epithelial cell functions necessary for saliva secretion.[33] Also, TNFα-induced inhibition of salivary secretion was prevented by AM251/AM630 injected 10 min before by the same route, confirming the participation of the endocannabinoid system in the salivation altered during the inflammatory state.

To further confirm the participation of the endocannabinoid system in TNFα-induced inhibition of salivary secretion, we measured the *in vitro* effect of TNFα on forskolin induced increase in cAMP content in submandibular glands incubated with or without cannabinoid receptor antagonists. We demonstrated that AM251 and AM630 blocked the inhibitory effect of TNFα on forskolin induced cAMP production as anandamide does in similar experimental conditions.[15] Therefore, the increase of anandamide after LPS injection in the submandibular gland could probably be due to the proinflammatory state produced by cytokines. It was also reported that anandamide could generate a negative feedback exerting inhibitory effects on proinflamatory cytokine production, confirming its role as a modulatory agent.[18,36,37] Also, there is evidence that in inflammatory conditions, cannabinoid receptor agonists down-regulate mast cells and granulocytes, and reduce cytokine release by acting on CB_1 and CB_2 receptors.[38–40]

On the other hand, since LPS injected intraperitoneally as well as TNFα injected intracerebroventricularly were shown to increase anandamide in the hypothalamus,[17,41] an area that has been shown to regulate autonomic inputs to the salivary glands via endocannabinoids,[42,43] we cannot discard the participation of the hypothalamic endocannabinoid system in LPS inhibitory effects of salivary secretion.

In summary, the present results show that the inflammatory state induced by LPS decreased methacholine-induced salivary secretion by increasing TNFα and anandamide that activates cannabinoid receptors. The participation of the endocannabinoid system during the inflammatory state involves both CB_1 and CB_2 receptors which activate signal transduction pathways whose final biological signal is the reduction of salivary secretion. Here, we report for the first time the participation of the endocannabinoid system on salivary responses during systemic inflammation indicating that cannabinoid receptors could be potential therapeutic targets for the treatment of secretory dysfunctions such as hyposialia.

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Competing interests: None declared.

Ethical approval: Not required.

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