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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