Periodontal disease reduces water and sodium intake induced by injection of muscimol into the lateral parabrachial nucleus

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Abstract

Objective: Gamma-aminobutyric acid A (GABA<sub>A</sub>) receptor activation with muscimol in the lateral parabrachial nucleus (LPBN) induces water and 0.3 M NaCl intake. The purpose of this study was to investigate whether a local inflammatory event, such as periodontal disease (PD), is able to alter the effects of muscimol on water and 0.3 M NaCl intake in fluid-replete rats and in rats treated with furosemide (FURO) combined with captopril (CAP) injected subcutaneously.

Design: Male Wistar rats were divided into two groups: with PD and those without PD (control condition). Fifteen days after PD, both groups had cannulas implanted bilaterally into the LPBN.

Results: In fluid-replete rats without PD, injections of muscimol (0.5 nmol/0.2 µl) into the LPBN induced 0.3 M NaCl and water intake and a pressor response. In fluid-replete rats with PD, a decrease was observed in water intake and pressor response but not in 0.3 M NaCl intake. In control rats with FURO + CAP treatment, injections of muscimol into the LPBN increased 0.3 M NaCl and water intake. In PD rats with FURO + CAP treatment, a decrease was observed in 0.3 M NaCl and water intake after muscimol in the LPBN. Alveolar bone loss and interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) plasmatic concentration were higher in PD rats in comparison with controls.

Conclusion: These results suggest that PD is able to reduce the pressor response and the dipsogenic and natriorexigenic effects induced by the activation of GABA<sub>A</sub> receptors in the LPBN, probably due to the elevation of the plasmatic concentration of pro-inflammatory cytokines IL-6 and TNF-α.

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1. Introduction

The lateral parabrachial nucleus (LPBN), a pontine structure located dorsolaterally to the superior cerebellar peduncle (SCP), is an important hindbrain area involved in the inhibitory control of ingestive behaviour. 1,2

The LPBN might also regulate central responses produced by systemic immune stimuli. 3,4 It has been demonstrated that the LPBN plays a critical role in cytokine-induced Fos expression in the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST) and ventrolateral medulla (VLM) neurons. 5 Thus, the LPBN is a site in which...
systemic inflammation could potentially modulate or mediate its functions.

Periodontal disease is a chronic inflammatory disease characterised as a reaction to bacterial infection, which involves both the innate and the adaptive arms of the immune system. Elevated circulating levels of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8 from the inflamed periodontal tissues are related to critical events that occur during periodontal disease, such as loss of attachment, alveolar bone loss and periodontal pocket formation. In rats, different models to induce periodontal disease have been proposed such as intra-peritoneal (i.p.) injection of an endotoxin for example, lipopolysaccharide (LPS), or by placement of a ligature in the dentogingival area, which acts as a source of pathogenic micro-organism species that colonise the tooth surface (dental plaque) in close contact with the gingival margin which stimulates host-mediated tissue destruction.

A dense group of gamma-aminobutyric acid (GABA) immunoreactive varicosities has been described in the parabrachial complex and Kölliker-Fuse nucleus, suggesting that the neuronal process of this area is under GABAergic influence, particularly the gustatory and visceral portion of the parabrachial nucleus. Previous studies have shown that the activation of GABA<sub>A</sub> receptors by bilateral injections of muscimol into the LPBN induced a large 0.3 M NaCl intake and also a slight ingestion of water and pressor response in fluid-replete rats. In addition, injections of muscimol into the LPBN increased FURO + CAP- and 24-h sodium depletion-induced NaCl intake, suggesting that a GABAergic mechanism present in the LPBN is involved in the control of sodium intake.

Several reports have shown that immune-response mediators, such as pro-inflammatory cytokines, may modulate GABAergic neurotransmission. For example, the application of IL-1β and IL-6 reduced the frequency of spontaneous inhibitory post-synaptic currents (sIPSCs) and GABA-induced currents in dorsal horn neurons and amygdala neurons.

Considering the involvement of GABAergic mechanisms in the LPBN in the control of hypertonic NaCl and water intake and that pro-inflammatory cytokines may modulate GABAergic neurotransmission, we investigated whether ligature-induced periodontal disease would change the effects of GABA<sub>A</sub> receptor activation into the LPBN in ingestive behaviour and pressor response in fluid-replete rats and in rats submitted to sodium depletion (treated with the diuretic furosemide (FURO) combined with a low dose of the angiotensin-converting enzyme inhibitor captopril (CAP) injected subcutaneously). In addition, alveolar bone loss and levels of TNF-α and IL-6 stimulated by periodontal disease were also investigated.

2. Methods

2.1. Animals

All experiments conducted in this study were approved by the Institutional Animal Research Ethics Committee (CEEA) (process number 2010-00516). Male Wistar rats weighing 290-310 g were used. The animals were housed in individual stainless steel cages with free access to a standard sodium diet (Guabi Rat Chow, Paulinia, SP, Brazil), water and 0.3 M NaCl solution. The positions of the bottles containing water and 0.3 M NaCl were rotated daily to avoid place preference. Rats were maintained in a room whose temperature was controlled at 23 ± 2 °C and humidity at in a 12-h light/dark cycle with lights on 7:30 a.m.

2.2. Experimental periodontal disease

The animals were randomly divided into two groups: the control group (CN) and the periodontal disease group (PD). Under general anaesthesia (a mixture of ketamine (80 mg/kg of body weight (b.w.), Cristália, Brazil) and xylazine (7 mg/kg of b.w., Agener, Brazil)) injected subcutaneously, a sterile silk ligature (strength 4/0) was tied in the cervical region of the mandibular first molars teeth bilaterally in the PD group using a technique that was previously described. The ligatures served as a retention device for oral micro-organisms. Ingestion of 0.3 M NaCl and water (ml/24 h) was measured 3 and 16 days after experimental ligature-induced periodontal disease in order to verify the systemic conditions of the animals.

2.3. Brain surgery

On the 15th day after ligature placement, control rats (without ligature) and rats with PD were anaesthetised with i.p. injection of ketamine (80 mg/kg of b.w.) combined with xylazine (7 mg/kg of b.w.) and placed in a stereotaxic instrument (Kopf, Tujunga, CA, USA). The skull was levelled between bregma and lambda. Stainless steel guide-cannulas (12 mm × 0.6 mm outer diameter (o.d.)) were implanted bilaterally into the LPBN using the following coordinates: 9.2 mm caudal to bregma, 2.2 mm lateral to the midline and 3.8 mm below the dura mater. The tips of the cannulas were positioned 2 mm above each LPBN. The cannulas were fixed to the cranium using dental acrylic resin and jeweller screws and were filled with 30-gauge metal obturators between tests. After the surgery, only control rats received a prophylactic dose of the antibiotic penicillin (30,000 IU). All animals were allowed to recover for 5 days before starting ingestion tests and during this period they had free access to standard sodium diet, water and 0.3 M NaCl solution.

2.4. LPBN injections

Bilateral injections into the LPBN were made using 5-μl Hamilton syringes connected to 30-gauge injection cannulas by means of polyethylene tubing (PE-10). At the time of testing, the obturators were removed and the injection cannula (2 mm longer than the guide cannula) was carefully inserted into the guide cannula. For bilateral injections, the first injection was performed on one side, the needle was removed and repositioned on the contralateral side and then the second injection was given. Therefore, injections were given ~1 min apart. The injection volume into the LPBN was 0.2 μl on each site. The obturators were replaced after the injections, and the rats were put back into their cages.
2.5. **Drugs**

FURO (Sigma–Aldrich, Saint Louis, MO, USA) was dissolved in alkaline saline (pH adjusted to 9.0) and administered subcutaneously (s.c.) at a dose of 10 mg/kg of b.w. CAP (Sigma–Aldrich, Saint Louis, MO, USA) was dissolved in 0.15 M NaCl and administered s.c. at the dose of 5 mg/kg of b.w.

Muscimol HBr (Sigma–Aldrich, Saint Louis, MO, USA) was dissolved in 0.15 M NaCl. The muscimol dose used in the present study was the same as that used in previous studies\(^\text{12,13}\) that investigated the effects of muscimol injected into the LPBN on water and 0.3 M NaCl intake. This dose of muscimol produces a long-lasting action (at least for 1 h) when injected into the LPBN.\(^\text{12}\)

2.6. **Water and 0.3 M NaCl intake by fluid-replete rats**

The rats were tested in their home cages. Water and 0.3 M NaCl were provided from burettes with 0.1-ml divisions and were fitted with metal drinking spouts. Food was not available to the rats during the tests. Cumulative intake of 0.3 M NaCl and water (two-bottle test) was measured at every 30 min during a 180-min period, starting 10 min after bilateral injections of muscimol (0.5 nmol/0.2 µl) or saline (0.2 µl) into the LPBN. Rats with ligature-induced periodontal disease (PD) and without PD were submitted to two tests. In each test, the group of rats was divided into two. In the first test, half of the group received saline and the other half received muscimol into the LPBN. In the next test, the rats received the same treatments in a counterbalanced design. All tests began between 13:00 and 15:00. A recovery period of at least 2 days was allowed between tests.

2.7. **Water and 0.3 M NaCl intake by FURO + CAP-treated rats**

The same group of rats (with PD and without PD) were used to test water and 0.3 M NaCl intake induced by treatment with FURO + CAP s.c. On the day of the test, food, water and 0.3 M NaCl were removed and the cages were rinsed with water. Rats received injections of the diuretic FURO (10 mg/kg b.w.) plus CAP (5 mg/kg b.w.) as described previously.\(^\text{12,16}\) One hour after FURO + CAP-treatment, burettes with water and 0.3 M NaCl solution were returned to the cages, and measurements were taken at 30-min intervals for 180 min (sodium appetite test). Ten minutes before access to water and 0.3 M NaCl, rats received bilateral injections of muscimol (0.5 nmol/0.2 µl) or saline into the LPBN. The rats were submitted to two tests. In each test, the group of rats was divided into two. In the first test, half of the group received saline and the other half received muscimol injection into the LPBN. In the next test, the rats received the same treatments in a counterbalanced design. All tests began between 13:00 and 15:00. A recovery period of at least 2 days was allowed between tests. The order of treatments was randomised because repeated FURO + CAP injections enhanced stimulated and spontaneous NaCl intake.\(^\text{17}\)

2.8. **Arterial pressure and heart rate recordings**

Rats were anaesthetised with ketamine (80 mg/kg of b.w.) + xylazine (7 mg/kg of b.w.) and a piece of polyethylene tubing (PE 10 connected to a PE 50) was inserted into the abdominal aorta through the femoral artery. The cannula was tunnelled subcutaneously and exteriorised on the back of the rat to record mean arterial pressure (MAP) and heart rate (HR). Immediately after recovery from the surgery, the rats were moved into individual cages with wood-chip bedding and given free access to food and water for 24 h, after which they were transferred to the cardiovascular recording room. On the next day, food and water were removed and the arterial catheter was connected to a F23 Db pressure transducer (Statham Gould, Madison, WI, USA) coupled to a pre-amplifier (model ETH-200 Bridge Bio Amplifier, CB Sciences, Dover, NH, USA) that was connected to a PowerLab computer data acquisition system (model PowerLab 16SP, ADInstruments, Colorado Springs, CO, USA) to record MAP and HR in unanaesthetised and unrestrained rats. A period of 15–20 min was necessary for MAP and HR readings to stabilise.

The effects of injections of saline or muscimol (0.5 nmol/0.2 µl) into the LPBN were tested in control rats and with ligature-induced PD only after 20 min of stable MAP and HR recordings. MAP and HR were recorded for the next 180 min after muscimol or saline injections into the LPBN and the maximum changes were analysed. During MAP and HR recordings, water and food were not available to the rats.

2.9. **Assay of serum TNF-α and IL-6**

Control rats and rats with ligature-induced PD were submitted to median laparotomy and blood samples (4 ml) were taken via inferior vena cava puncture, followed by perfusion. The samples were then distributed into tubes containing heparin (Hemofol, Cristália, Brazil). Plasma was prepared by centrifugation of blood at 3000 × g for 15 min at 4 °C and then stored in aliquots at −70 °C until used. Plasmatic concentrations of IL-6 and TNF-α were quantified by means of enzyme-linked immunosorbent assay techniques using commercial kits (IL-6, BD Biosciences, San Diego, CA, USA and TNF-α, Invitrogen, Camarillo, CA, USA). The limits of detection of the TNF-α and IL-6 were <4 and <0.7 pg ml⁻¹, respectively.

2.10. **Radiographic evaluation of alveolar bone loss**

At the end of the experiments (water and sodium intake and blood pressure recording), on the 28th day after periodontal disease induction, the animals were euthanised. The right and left hemi-mandibles were dissected and fixed in 10% formaldehyde for 24 h. Radiographic images were acquired using 70 kvp, 10 mA, 0.10 s time exposure. The source-to-film distance was always set at 40 cm. The digital image was obtained directly with the optical digital plate (Digora, Soredex, Tuusula, Finland). The optical plate readings were performed in sensitised laser scanner equipment, and the images were analysed by Digora 1.51 for Windows (Soredex, Tuusula, Finland). Radiographic analyses were performed to detect alveolar bone loss as previously described\(^\text{18}\) and to show that the induction of periodontal disease was effective. The distance between the cemento-enamel junction (CEJ) and the height of alveolar bone was determined for mesial root surfaces of the left and right mandible first molars with the aid of the software. The distances were measured in millimetres.
2.11. Histology

At the end of the experiments, the animals also received bilateral injections of 2% Evans blue dye solution (0.2 μl/injection site) into the LPBN. They were then deeply anaesthetised with sodium thiopental (CRISTALIA, Itapira, SP, Brazil, 80 mg/kg of b.w.) and perfused transcardially with saline followed by 10% formalin. The brains were removed, fixed in 10% formalin, frozen, cut coronally into 60 μm sections and stained with Giemsa stain. Only animals with injections into the LPBN were considered for statistical analysis.

2.12. Statistical analysis

The results are reported as means ± standard error of the mean (SEM). Statistical analysis was performed using two-way analysis of variance (ANOVA) with repeated measures followed by Student-Newman–Keuls post hoc tests to determine significant differences between groups. IL-6 and TNF-α levels and alveolar bone loss were analysed by the Student t-test. Differences were considered significant at P < 0.05. The software used to analyse the data was SigmaStat for Windows, version 2.03 from SPSS Inc.

3. Results

3.1. Alveolar bone radiographic analysis

Alveolar bone analysis revealed that rats with periodontal disease had more bone loss than rats in the control group (1.29 ± 0.04 vs. CN group: 0.50 ± 0.02 mm, P < 0.001, Fig. 1), showing that the induction of periodontal disease was effective.

3.2. Daily sodium and water intake

There were no statistically significant differences between the ingestion (ml/24 h) of water and 0.3 M NaCl for both groups (control and PD rats) in any of the evaluation periods (3 and 16 days) after ligature-induced periodontal disease (Fig. 2).

3.3. Water and 0.3 M NaCl intake by fluid-replete rats

ANOVA showed significant differences among treatments and times for water intake (F(18,126) = 17.4; P < 0.001, Fig. 3C and D) and 0.3 M NaCl intake (F(18,126) = 5.4; P < 0.001, Fig. 3A and B) when fluid-replete rats had simultaneous access to water and 0.3 M NaCl. Compared with saline injections into the LPBN, the cumulative ingestion of 0.3 M NaCl and water significantly increased after injections of muscimol (0.5 nmol/0.2 μl at each site, n = 8) into the LPBN after 120 min until the end of the test in control and PD rats (Fig. 3A and C). Post hoc tests showed that ligature-induced PD attenuated the effects of muscimol on water intake (Fig. 3C and D) without changing 0.3 M NaCl intake (Fig. 3A and B).

3.4. Water and 0.3 M NaCl intake by FURO + CAP-treated rats

ANOVA showed significant differences among treatments and times for water intake (F(18,126) = 6.9; P < 0.001, Fig. 4C

Fig. 1 – (A) Measurement of the distance from the cement-enamel junction (CEJ) to the alveolar bone crest; (B) individual measurement of the distance from the CEJ to alveolar bone crest; (C1) radiographic images showing normal alveolar bone; (C2) radiographic images showing bone loss in the interproximal and interradicular regions (white arrowhead) 28 days after ligature-induced periodontitis. Values are means ± S.E.M. n = number of rats. * Different from control group (Student’s t test).
and D) and 0.3 M NaCl intake (F(18,126) = 4.7; P < 0.001, Fig. 4A and B) when FURO + CAP-treated rats (control and PD) that received muscimol or saline in the LPBN had simultaneous access to water and 0.3 M NaCl. In control rats, the cumulative ingestion of 0.3 M NaCl after injections of muscimol (0.5 nmol/0.2 μl at each site, n = 8) into the LPBN was significantly different from ingestion after saline injections into the LPBN from 90 to 180 min of the test, with P values ranging from P < 0.05 at 90 min to P < 0.005 from 120 to 180 min (Newman–Keuls post hoc test) (Fig. 4A and B). However, FURO + CAP-induced water intake after muscimol was significantly different from the intake after saline injections into the LPBN from 150 to 180 min (P < 0.001) (Fig. 4C).

For all the times tested, FURO + CAP-induced water and 0.3 M NaCl intake after saline injection into the LPBN in rats with PD did not differ from the control group with saline injections into the LPBN (P > 0.5, Newman–Keuls post hoc test) (Fig. 4A and C). However, FURO + CAP-induced water and 0.3 M NaCl intake after muscimol injection into the LPBN in PD rats was significantly different from the intake after muscimol injections into the LPBN in control rats from 90 to 180 min of the test, with P values ranging from P < 0.05 at 90 min to P < 0.001 from 120 to 180 min (Newman–Keuls post hoc test) (Fig. 4A and C).

3.5. Changes in arterial pressure and HR in fluid-replete rats treated with injections of muscimol into the LPBN

In normotensive fluid-replete rats (MAP: 101 ± 3.4 mmHg and HR: 327 ± 0.9 beats per minute (bpm)) without ligature, bilateral injections of muscimol (0.5 nmol/0.2 μl, n = 5) into the LPBN increased MAP (15.2 ± 3.3 mmHg, vs. saline: 0.6 ± 1.3 mmHg/180 min) and HR (36 ± 6.8 vs. saline: 4.1 ± 2.0 bpm/180 min). Experimental ligature-induced PD alone produced no change in MAP and HR. However, post hoc tests showed that ligature-induced PD reduced the increase in MAP (F(3,12) = 21.0; P < 0.05) and HR (F(3,12) = 61.7; P < 0.05) from 30 to 180 min after treatment with muscimol into the LPBN.

3.6. IL-6 and TNF-α plasmatic concentration

The IL-6 and TNF-α plasmatic concentration values were higher in PD rats compared with controls (Table 1).

4. Discussion

Similar to a previous study, the present study shows that bilateral injections of muscimol (GABA<sub>A</sub> receptor agonist) into the LPBN induce a pressor response and hypertonic NaCl and water ingestion in fluid-replete rats and increase hypertonic...
NaCl and water intake in FURO + CAP-treated rats. The new finding of the present study is that periodontal disease (PD) induced by ligature placement, confirmed by radiographic analysis, caused a significant amount of bone loss, increased plasmatic concentration of pro-inflammatory cytokines IL-6 and TNF-α and reduced water intake and the pressor response induced by muscimol injected into the LPBN in fluid-replete rats and reduced water and hypertonic NaCl intake induced by muscimol injected into the LPBN in FURO + CAP-treated rats. Experimental ligature-induced PD produced no change in 0.3 M NaCl and water intake, suggesting that a local inflammatory event, such as PD, alone does not inhibit or facilitate these behaviours.

Ligature-induced PD around the molar teeth acts as a bacterial retentive device and promotes the growth of microorganisms in the subgingival area. The relationship between periodontal bacterial infection and alveolar bone loss has been well established, and the roles played by inflammatory mediators in the bone loss process that develops from periodontal disease have been studied. In the present study, the statistically significant differences found between teeth with and without ligatures demonstrated that the placement of ligatures generated significant bone loss, which validates the use of ligatures in this study. The pro-inflammatory cytokines IL-6 and TNF-α are among the most frequently found active promoters of bone loss during periodontal disease. The present study also demonstrated an increase in plasmatic concentrations of IL-6 and TNF-α in ligature-induced PD rats compared with controls.

Behavioural alterations induced by infection and inflammation including decrease in food and water intake after systemic or central infusion of cytokines or administration of molecules that induce endogenous cytokine synthesis (e.g., lipopolysaccharide (LPS), the active fragment of endotoxin from Gram-negative bacteria) in experimental animals is collectively referred to as ‘sickness behaviour’. LPS administered i.p. inhibited 0.3 M NaCl intake induced by FURO + CAP treatment and abolished the intracellular thirst induced by an intragastric load of 2 M NaCl. In addition, peripheral and central administration of IL-1β, an immunoregulatory cytokine activated by LPS, inhibited water intake after dehydration, hyperosmolarity and hypovolaemia.

In the present study, we evaluated whether experimental ligature-induced PD inhibits thirst and sodium appetite induced by injection of muscimol into the LPBN. First, in order to check for general health, we monitored water and 0.3 M NaCl intake 3 and 16 days after ligature placement (Fig. 2) and evaluated body weight and food intake before the ingestive tests (data not shown). No significant differences in water and sodium intake (ml/24 h) and body weight and food intake were observed in PD rats in comparison with...
control rats. In addition, ligature-induced PD alone produced no changes in water and sodium intake in fluid-replete rats and Furo + CAP-treated rats. These findings suggest that PD rats had good systemic conditions and ligature-induced PD reduced the pressor response and water and 0.3 M NaCl intake induced only by bilateral injections of muscimol into the LPBN.

The LPBN is involved in a variety of homeostatic mechanisms such as cardiovascular regulation\textsuperscript{25,26} and control of digestive behaviour.\textsuperscript{1,2} It has been demonstrated that the neurons of the LPBN are activated by a systemic immune challenge.\textsuperscript{3,4,27} In addition, the LPBN plays a critical role in cytokine-induced Fos expression in CeA, BNST and VLM neurons.\textsuperscript{7} Lesions directed at the LPBN significantly decreased the number of Fos-positive neurons in the CeA observed after systemic administration of IL-1β, suggesting a functional role for the LPBN in the activation of CeA cells after a systemic immune challenge.\textsuperscript{27}

The CeA is also involved in the control of sodium and water intake.\textsuperscript{28} In addition, a previous study showed the existence of GABAergic connections between the CeA and the LPBN.\textsuperscript{29} Recently, a study showed that bilateral lesions of the CeA abolished 0.3 M NaCl and water intake produced by the blockade of LPBN neurons with muscimol in fluid-replete rats, suggesting that facilitatory mechanisms present in the CeA are essential for dipsogenic and natriorexigenic responses induced by muscimol injected into the LPBN.\textsuperscript{30}

According to the present study, elevated circulating levels of pro-inflammatory cytokines such as TNF-α and IL-6 released during experimental ligature-induced PD could possibly inhibit CeA-projecting neurons that block facilitatory mechanisms present in the CeA and reduce the cardiovascular, dipsogenic and natriorexigenic effects of muscimol injected into the LPBN. We do not exclude the possibility of participation by other pro-inflammatory cytokines such as IL-1β and IL-8 in the reduction of water and hypertonic NaCl intake induced by muscimol injected into the LPBN in rats with experimental ligature-induced PD. This is not surprising given the several mediators activated by PD.\textsuperscript{7}

Table 1 - IL-6 and TNF-α plasmatic concentration of control rats (CN) and ligature-induced periodontal disease rats (PD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CN</th>
<th>PD</th>
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<tbody>
<tr>
<td>IL-6 (pg/ml) (n = 7)</td>
<td>38.87 ± 0.23</td>
<td>40.41 ± 0.25*</td>
</tr>
<tr>
<td>TNF-α (pg/ml) (n = 6)</td>
<td>3.39 ± 0.33</td>
<td>14.58 ± 1.73*</td>
</tr>
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Results are expressed as means ± S.E.M. n = number of rats. \* Different from CN rats (Student’s t test).

Fig. 4 - (A) Cumulative 0.3 M NaCl intake; (B) individual 0.3 M NaCl intakes (180 min); (C) cumulative water intake; (D) individual water intakes (180 min) by Furo + CAP-treated rats (control, CN and periodontal disease, PD) treated with muscimol (0.5 nmol/0.2 μl) or saline injected bilaterally into the LPBN. (A and C) Values are means ± S.E.M. n = number of rats. \* Different from muscimol (PD). + Different from saline (CN) (Student–Newman–Keuls test, P < 0.05).
The precise mechanism through which ligature-induced PD inhibits the dipsogenic and natriorexigenic effects of muscimol was not addressed in the present study. A hypothesis is that pro-inflammatory cytokines may modulate GABAergic neurotransmission.\textsuperscript{14,15,31} For example, administration of IL-1β and IL-6 reduced the frequency of sPSCs and GABA-induced currents in dorsal horn neurons\textsuperscript{14} and amygdala neurons.\textsuperscript{15}

Another hypothesis to explain the present results is that the cytokines TNF-α and IL-6 released during ligature-induced PD reduce the levels of endogenous angiotensin II (ANG II) in the LPBN. Recently, we showed that pre-treatment of the LPBN with injections of the nonapeptide angiotensin II receptor type 1 (AT₁) receptor antagonist losartan reduced the dipsogenic and natriorexigenic effect of muscimol injected into the same site in fluid-replete rats and FURO + CAP-treated rats, suggesting that deactivation of LPBN inhibitory mechanisms by muscimol is facilitated by endogenous ANG II acting on AT₁ receptors in the LPBN, which drives the rats to ingest large amounts of hypertonic NaCl.\textsuperscript{32} Therefore, ANG II acting on AT₁ receptors in the LPBN facilitates the effects of muscimol injected into the LPBN on water and sodium intake.\textsuperscript{33} It is possible that the pro-inflammatory cytokines TNF-α and IL-6 released during PD reduced the effect of ANG II on AT₁ receptors in the LPBN and inhibited water and sodium intake produced by muscimol in the LPBN. Although feasible, using these hypotheses to explain the effects of muscimol injected into the LPBN in rats with periodontal disease still has to be tested.

In conclusion, the data presented suggest that activation of the immunologic system by experimental ligature-induced PD is able to reduce the cardiovascular, dipsogenic and natriorexigenic effects induced by activation of GABA\textsubscript{A} receptors with muscimol injected into the LPBN in fluid-replete rats and FURO + CAP-treated rats, probably due to the elevation of plasmatic concentrations of pro-inflammatory cytokines IL-6 and TNF-α. Further studies are necessary to gain an understanding of how periodontal disease and inflammatory processes can affect the activity of the LPBN inhibitory mechanism, the specific role of the cytokines in GABAergic neurotransmission in the LPBN and how these mechanisms interact with each other to control thirst and sodium appetite.

Authors’ contributions

Talita de Melo e Silva performed the experiment, analyzed the data and interpreted the results. Gabriela P. Bearare performed the experiments, participated in data collection and analyzed the data. Dóris H. Sumida designed the study and performed the experiments, assistance in all steps such as analyses and discussion. Supervised the study. João C. Callera designed the study and performed the experiments, analysed the data and wrote the manuscript.

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Competing interests

None declared.

Ethical approval

The procedures were approved by the Institutional Ethical Committee for Animal Care from the School of Dentistry, UNESP, Araçatuba, Brazil (protocol 2010-00516) and complied with the recommendations of the Brazilian College of Animal Experimentation (COBEA).

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