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Crucial role of peripheral κ-opioid receptors in a model of periodontal disease in rats

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Background and Objective: Periodontal disease is a chronic inflammatory condition of the tooth supporting tissues, the periodontium. Opioids have been shown to account for the relief of various chronic and acute inflammatory conditions. The aim of the present study was to investigate the participation of peripheral opioid receptors in development of periodontal disease.

Material and Methods: Morphine and selective agonists and antagonists of opioid receptors were used in an experimental model of ligature-induced periodontal disease in rats. To evaluate the development of disease, the loss of fiber attachment, alveolar bone and number of cells in periodontal tissues were assessed. Measurements of these indicators were obtained by morphometric analysis of histological sections of periodontal-diseased tissues stained with hematoxylin and eosin.

Results: Local administration of either morphine or a selective κ-opioid agonist for three consecutive days from the onset of periodontal disease reduced the loss of periodontal tissues, without changing the number of leukocytes in inflamed periodontium. Nor-binaltorphimine, a selective κ-antagonist, reversed the beneficial effects of both morphine and the compound U-50,488 in this model. The use of either an agonist or an antagonist of δ-opioid receptors, however, did not affect disease progression.

Conclusion: Our results showed that the beneficial effect of opioids in periodontal disease depended mainly on the activation of specific κ-opioid receptors located in the periphery. Activation of such receptors could be considered in the management of periodontal disease, since it would not present the classical central side-effects associated with opioid use.

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Periodontal disease is a progressive chronic inflammatory condition of the tissues responsible for anchoring teeth in the oral cavity, the periodontium (1). This chronic disease afflicts a significant percentage of individuals in different societies around the world (2), especially elder people, being responsible for most tooth losses in developed countries (2,3). The impact of periodontal disease does not seem to be restricted to tooth loss, since recent studies have demonstrated a relationship between periodontal disease and an increased risk for cardiovascular disease, diabetes and complications during pregnancy such as premature labor and low-weight newborns (4–6).

Although periodontal disease presents bacterial etiology, its progression is mostly dependent on how the host deals with the bacterial challenge (7). For instance, the recruitment of

leukocytes, brought to periodontal tissues by the presence of bacterial products, triggers an array of inflammatory mediators in order to eliminate the pathogenic microorganisms. Inflammatory mediators in excess may, however, cause tissue damage, which leads to disease progression.

Accumulating experimental evidence in the literature has shown an important role for opioids in the pathophysiology of chronic and acute inflammatory conditions (8,9). Moreover, exogenous systemic as well as local (peripheral) opioid administration has presented anti-inflammatory activity using different models of inflammation (8,10). Consistently, synthesis and expression of endogenous opioids and their receptors, especially those present in the periphery, are increased during inflammation (11–13).

In line with such studies, we recently demonstrated a beneficial effect of systemic and local morphine administration in decreasing the loss of periodontal tissues in a model of periodontal disease in rats (10). Morphine is a prominent opioid agonist at μ-receptors, but it can bind to κ- and δ-opioid receptors as well (14). A well-conducted study showed that morphine reduced the signs and symptoms of chronic inflammation associated with experimental arthritis through activation of κ-opioid receptors (15). Most importantly, the beneficial effects of κ-agonists in this model were shown to derive from a peripheral effect (16). Notwithstanding, the third important type of opioid receptor, the δ-opioid receptor, has also been associated with relief of the signs of acute and chronic inflammation (17).

The aim of the present study was, therefore, to evaluate whether κ- and δ-opioid receptors could participate in the pathophysiology of periodontal disease in rats. Additionally, we sought to verify whether the beneficial effects of opioids in this model were due to activation of opioid receptors in the periphery. To assess this, selective agonists and antagonists of κ- and δ-opioid receptors were injected locally into the gingival tissue close to the inflamed periodontium.

Material and methods

Animals

Male Holtzman rats, weighing 260–300 g, from the Animal House of the Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Brazil, were used throughout the experiments. The rats were maintained under a 12 h–12 h light–dark cycle (lights on at 07.00 h) at 23–25°C with water and food *ad libitum*. The Animal Ethics Committee of the UFMG approved the handling of the rats throughout this study.

Induction of periodontal disease

The model for experimental periodontal disease in rats was based on an earlier publication (18) and published elsewhere (10). Briefly, rats were anaesthetized intramuscularly with a mixture of ketamine and xylazine (90 and 15 mg/kg, respectively). A sterile silk ligature (4–10) was tied around the cervix of the second left superior molar and served as a retention device for subgingival oral microorganisms. The ligature remained fixed until the end of the experiments, when the rats were killed. The contralateral right side was used as the unligated control. The impaction of materials other than indigenous flora in rats' periodontium was avoided by keeping the animals in individual suspended cages after ligature fixation.

Measurements of alveolar bone and fiber attachment loss by morphometric analysis

The rats were killed by cervical dislocation. The left and right maxillae halves were excised, fixed in 10% buffered formalin solution, pH 7.2, for 48 h, washed in water and demineralized in 10% EDTA for 30 days. At the end of demineralization period, each hemi-maxilla was washed in tap water for 24 h, dehydrated in serial alcohols, cleared and embedded in paraffin. The blocks were cut in serial 4 μm sections in a mesiodistal direction. Hematoxylin and eosin staining was performed on the most central section of each tooth, i.e. the one showing the center of the dental pulp. Images from experimental (ligated) and control (unligated) sites were obtained at 10-fold magnification through a JVC TK-1270/RGB camera (Victor Company of Japan, Yokohama, Japan) adapted to a microscope. The distance from the cemento-enamel junction (CEJ) to the most coronal level of fiber attachment (FA) and alveolar bone crest (B) were measured using KS300 software (CarlZeiss, Oberkochen, Germany) built into a Kontron Elektronick/CarlZeiss image analyzer. The alveolar bone and fiber attachment loss are reported (in mm) as the difference between values at the experimental and unligated control sites. An observer who was unaware of the nature of the tissue sample made the measurements.

Assessment of cell number in gingival tissue

After excision of the maxillae, gingivomucosal tissue samples (~5 mm^2) were removed and fixed in 10% buffered formalin solution, pH 7.2, for 48 h. These samples underwent the same procedures as the hemi-maxilla for tissue cutting and staining. Six images from different locations of the gingival tissue section were obtained at 40-fold magnification. An automatic macro-recorder assembler (an algorithm of the KS300 software) was elaborated for capture, image processing and segmentation, definition of morphometrical conditions and counts of all the nuclei contained in each image. Image processing techniques were applied in order to highlight the nucleus of the cells. Segmentation permitted the separation of these nuclei from the cell cytoplasm and from other structures in the section, such as blood vessels and extracellular space, enabling the creation of a binary image containing these two locations, nucleus and other spaces. The nuclei from resident cells in the gingivae as well as newly recruited leukocytes were then counted (19). An observer who was unaware of the nature of the tissue sample made the measurements. For each gingival tissue sample, six differ-

ent fields were counted. The result of the six fields counted was totaled and represented the total number of cells present in that tissue sample.

Drug treatment of rats

With the exception of morphine, which was supplied by Merck (Darmstadt, Germany), all selective κ- and δ-opioid agonist and antagonist compounds were supplied by Tocris (Bristol, UK). The κ-agonist U-50,488, nor-binaltorphimine (nor-BNI, selective κ-antagonist), morphine and naltrindole (selective δ-antagonist) were diluted in sterile physiological saline (NaCl; 0.9% w/v; vehicle 1). The selective δ-opioid receptor agonist SNC80 was diluted in 10% DMSO (vehicle 2).

Previous work in our laboratory showed that preventive administration of drugs (from 3rd to 5th day after ligation) in rats with periodontal disease produced better results than when drugs were administered symptomatically (from 9th to 11th day after ligation). Furthermore, local administration (injection of the drugs into gingival tissue surrounding the ligated tooth) was shown to be as effective as systemic administration (20). Therefore, all drugs used in this work were administered locally (0.1 ml per site) using the preventive schedule, during three consecutive days. Control animals received drug-vehicles by the same route and at the same time. The animals were killed on the eleventh day after ligature fixation by cervical dislocation. Nor-binaltorphimine (200 μg/day per site) was administered 15 min before administration of either morphine (1 mg/day per site) or U-50,488 (250 μg/day per site), and naltrindole (NTD, 100 μg/day per site) was administered 15 min before morphine (1 mg/day per site). Control animals were given the same volume of vehicle alone. In order to verify whether the lower effective dose of U-50,488 had a local rather than systemic effect, in one group of animals the κ-agonist was administered in the contralateral side (injection of the drugs into gingival tissue surrounding the non-ligated tooth), the ipsilateral (ligated tooth) site being evaluated.

Assessment of effectiveness of δ-opioid agonist and antagonist using an experimental model of pain

To demonstrate that SNC80 and naltrindole were effective throughout the experiments, δ-agonist (80 μg per site) and antagonist (30 μg per site) were injected locally in rats whose paws had been injected with PGE_2 and tested for development of mechanical hyperalgesia using the paw pressure test, a well-established model to detect drugs with analgesic effects (21,22).

Statistical analysis

The measurements are presented in millimetres as means ± SEM obtained from groups of five to 10 animals. Differences between means were evaluated by one-way ANOVA followed by the Student–Newman–Keuls test. Probabilities less than 5% ($p < 0.05$) were considered to be statistically significant.

Results

Reversal of morphine effects by κ-opioid antagonist in the periodontal disease model

Previous work from our laboratory (10) showed that local administration of morphine decreased the loss of both fiber attachment and alveolar bone crest in a dose-dependent manner (0.5, 1.0 and 4.0 mg/day per site). Here, we reproduced these effects of morphine. Moreover, the selective κ-opioid antagonist nor-BNI reversed the beneficial effect of the lower effective dose of locally administered morphine in relation to fiber attachment loss but not in relation to alveolar bone loss (Fig. 1).

Effect of κ-opioid agonist and antagonist following local administration in the periodontal disease model

To confirm that κ-opioid receptors would be involved in periodontal disease development, the κ-opioid receptor agonist U-50,488 was administered locally in a range of doses (100–500 μg per site). As shown in Fig. 2 and illustrated in Fig. 3, the compound U-50,488 was able to reduce both fiber attachment (250 μg/day per site) and

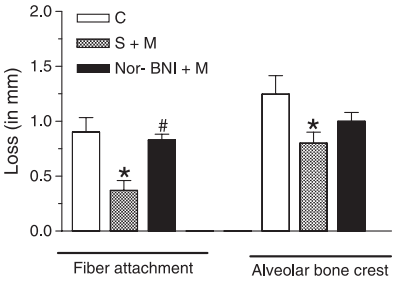

Fig. 1. Reversal of the beneficial effect of morphine by the selective κ-opioid receptor antagonist nor-binaltorphimine in experimental periodontal disease in rats. Nor-binaltorphimine (Nor-BNI, 200 μg/day per site) was locally administered 15 min before morphine (M, 1 mg/day per site). Control animals (C) received two administrations of saline (S) by the same route and at the same time as drug-treated animals. * and # indicate a significant difference ($p < 0.05$, $n = 6$–10 animals per group) from the control and morphine-treated group, respectively, using one-way ANOVA followed by Student–Newman–Keuls *post hoc* test.

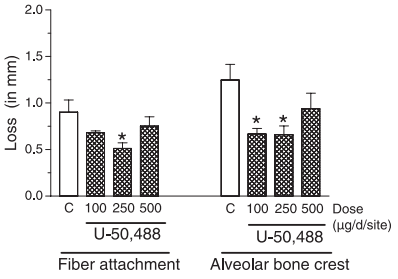

Fig. 2. Effect of local administration of different concentrations of U-50,488 on experimental periodontal disease in rats. The specific κ-opioid receptor agonist U-50,488 was injected into the gingival tissue surrounding the ligated tooth. Drug treatment (once a day) was made for three consecutive days from the 3rd to the 5th day, and the animals were killed on the 11th day after ligature placement. Control animals (C) were administered with the same volume of vehicle in accordance with the route of administration (0.1 ml per site). * Indicates a significant difference ($p < 0.05$, $n = 6$–8 animals per group) from the control group, using one-way ANOVA followed by Student–Newman–Keuls *post hoc* test.

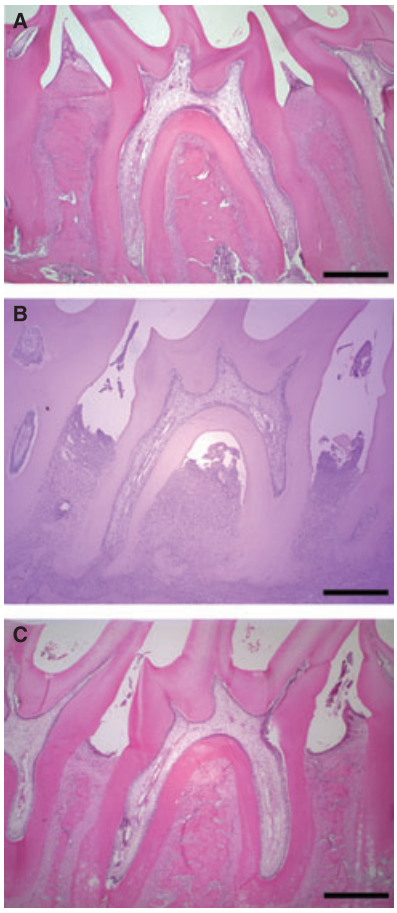

Fig. 3. Photomicrographs from transverse histological sections of maxillary rat molar teeth. (A) A healthy tooth with its supporting tissues. (B and C) Experimental teeth from animals treated with saline and κ-opioid agonist U-50,488, respectively. Observe in (C) a smaller retraction of periodontal attachment and alveolar bone crest when compared with (B). All photomicrographies were captured at fourfold magnification. The black bars at the bottom of each picture indicate 300 μm.

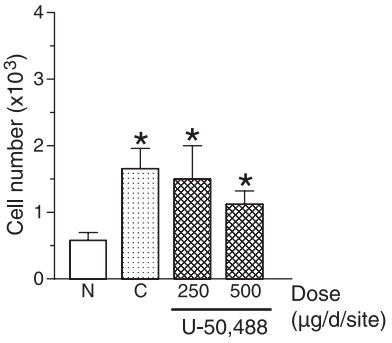

Fig. 4. Cell number in the gingival tissue surrounding the ligated molar tooth after local administration of different concentrations of U-50,488. Cell number was obtained by morphometric analysis as described in the Material and methods section. The naive (N) group was left unligated. Drug treatment (once a day) was made for three consecutive days from the 3rd to the 5th day, and the animals were killed on the 11th day after ligature placement. Control animals (C) were administered with the same volume of vehicle in accordance with the route of administration (0.1 ml per site). * Indicates a significant difference ($p < 0.05$, $n = 6$–8 animals per group) from the naive group, using one-way ANOVA followed by Student–Newman–Keuls *post hoc* test.

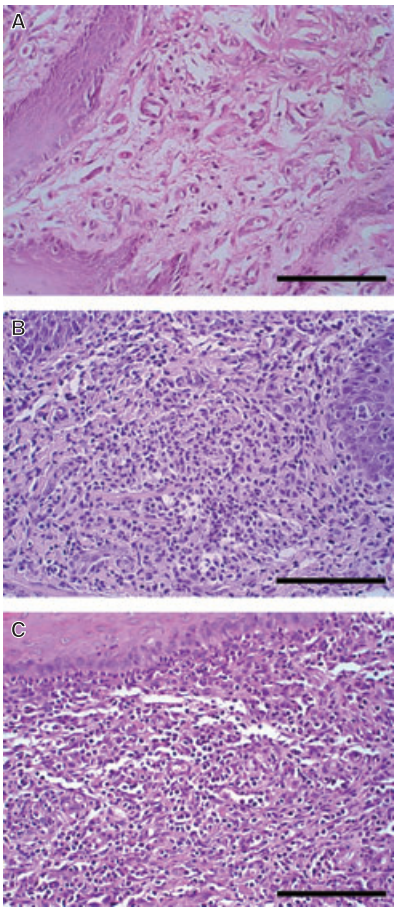

Fig. 5. Photomicrographs from histological sections of gingival connective tissue of naive rats or rats with periodontal disease treated locally with either saline or κ-opioid receptor agonist U-50,488. (A) Gingival connective tissue from a naive animal (non-ligated), showing the predominance of fibroblasts. (B) Gingival tissue from a control animal (ligated and saline-treated), showing an intense inflammatory infiltrate of mixed polymorpho-mononuclear cells. (C) Experimental gingival tissue from animal treated locally with U-50,488 (250 μg/day per site, 0.1 ml), showing no decrease in inflammatory infiltrate when compared with (B). All photomicrographies were captured at 40-fold magnification. The black bars at the bottom of each picture indicate 50 μm.

alveolar bone loss (100 and 250 μg/day per site) on day 11 of periodontal disease. However, histopathological analysis of gingivomucosal tissue surrounding the ligated tooth revealed that neither of the doses of U-50,488 used (250 and 500 μg/day per site) was related to changes in cell number in these tissues, as shown in Fig. 4 and illustrated in Fig. 5. In addition, prior treatment with nor-BNI (200 μg/day per site), a selective κ-opioid receptor antagonist, completely reversed the effect of U-50,488 administration in relation to both fiber attachment and alveolar bone loss. The same dose of antagonist did not induce any change in the loss of periodontal tissues when given alone (Fig. 6).

Periodontal disease effects following contralateral administration of U-50,488

The medium dose of U-50,488 (250 μg/day per site) was administered in two different groups of animals, either ipsi- or contralateral to the ligated tooth, and the evaluation of periodontal tissue loss was made in the ligated tooth. Only the ipsilateral administration of U-50,488 reduced the loss of both fiber attachment and alveolar bone crest when compared with administration of the same dose in the contralateral side (Fig. 7). These results confirmed a local effect of U-50,488 in periodontal disease, at least for the dose used in these experiments.

Lack of effect of naltrindole on the beneficial effect of morphine in the periodontal disease model

As for the κ-agonist and antagonist, the hypothesis that the beneficial effect of morphine in periodontal disease was due to activation of δ-opioid receptors was studied. To that end, naltrindole

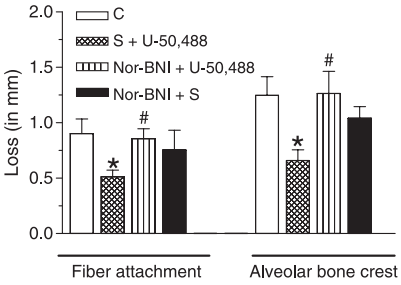

Fig. 6. Reversal by nor-BNI of U-50,488-induced inhibition of indicators in periodontal diseased rats. Nor-binaltorphimine (200 μg/day per site) was locally administered 15 min before U-50,488 (250 μg/day per site). Control animals (C) received two administrations of saline (S) by the same route and at the same time as drug-treated animals. * and # indicate a significant difference ($p < 0.05$, $n = 6$–10 animals per group) from the control and U-50,488-treated group, respectively, using one-way ANOVA followed by Student–Newman–Keuls *post hoc* test.

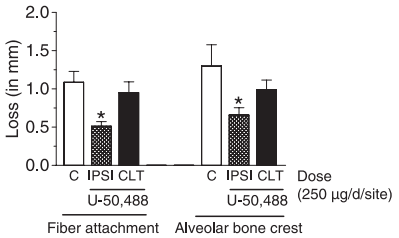

Fig. 7. Comparison between ipsi- and contralateral administration of U-50,488 on experimental periodontal disease in rats. The U-50,488 (250 μg/day per site) was administered locally in the right (CLT, non-ligated) side, while no treatment was made in the left (IPSI, ligated) side. * Indicates a significant difference ($p < 0.05$, $n = 5$–10 animals per group) in relation to group C, using one-way ANOVA followed by Student–Newman–Keuls *post hoc* test.

was administered 15 min prior to morphine (1 mg/day per site). The results showed that δ-receptor antagonist did not reverse the beneficial effect of morphine in this periodontal disease model. Moreover, the antagonist administered alone was not able to affect the loss of periodontal tissues (Fig. 8). These results suggested that δ-opioid receptors were not involved in periodontal disease development.

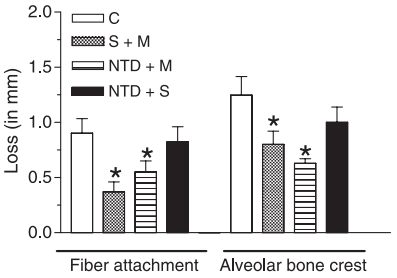

Fig. 8. Effect of selective δ-opioid receptor antagonist on the beneficial effect induced by morphine in experimental periodontal disease in rats. Naltrindole (NTD, 100 μg/day per site) was locally administered 15 min before morphine (M, 1 mg/day per site). Control animals (C) received two administrations of saline (S) by the same route and at the same time as drug-treated animals. * Indicates a significant difference ($p < 0.05$, $n = 6$–10 animals per group) from the control group using one-way ANOVA followed by Student–Newman–Keuls *post hoc* test.

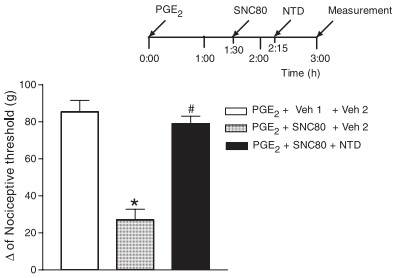

Fig. 9. Complete antagonism induced by intraplantar administration of naltrindole on the antinociception produced by SNC80 in hyperalgesic paws (PGE_2, 2 μg). Naltrindole (30 μg per paw; intraplantar) was administered intraplantarly 45 min after SNC80 (80 μg per paw; intraplantar), as shown in the schematic diagram above the bar graph. The antinociceptive response was measured by the paw pressure test as described previously. * and # indicate a significant difference ($p < 0.05$, $n = 5$ animals per group) compared with (PGE_2 + Vehicle 1 + Vehicle 2) and (PGE_2 + SNC80 + Vehicle 2)-injected controls, using one-way ANOVA followed by Student–Newman–Keuls *post hoc* test. Veh = vehicle.

Effect of δ-opioid agonist SNC80 in the periodontal disease model

To confirm that δ-opioid receptors were not involved in periodontal disease, local administration of the δ-agonist SNC80 (100 and 250 μg/day per site) was unable to reduce all three parameters of periodontal disease evaluated: loss of fiber attachment, alveolar bone and cell infiltrate to gingival tissue surrounding the ligated tooth (Table 1). However, despite their lack of effect in periodontal disease, SNC80 and naltrindole were effective to reduce the hypersensitivity to the algogenic substance PGE_2 and to reverse this effect, respectively, as shown in Fig. 9.

Discussion

The first question investigated in the present study referred to whether the beneficial effect of locally administered morphine in decreasing the destruction of periodontal tissues was due to

Table 1. Effect of local administration of different concentrations of SNC80 on experimental periodontal disease in rats

Groups ($n = 6$–9 animals per group)	Loss (mm; means ± SEM)		Cell number ($\times 10^3$) (means ± SEM)
	Fiber attachment	Alveolar bone crest	
N	—	—	0.58 ± 0.12
C	0.9 ± 0.1	1.25 ± 0.17	1.66 ± 0.3*
SNC80 (100 μg per site)	1.2 ± 0.24	1.72 ± 0.55	1.34 ± 0.2*
SNC80 (250 μg per site)	0.75 ± 0.15	1.28 ± 0.30	1.2 ± 0.35*

The specific δ-opioid receptor agonist SNC80 was injected into the gingival tissue surrounding the ligated tooth. Drug treatment (once a day) was made for three consecutive days from the third to the fifth day, and the animals were killed on the eleventh day after ligature placement. Control animals were administered with the same volume of vehicle in accordance with the route of administration (0.1 ml per site). The naive (N) group was left unligated. *$p < 0.05$ ($n = 5$–8 animals per group) from the naive group, using one-way ANOVA followed by Student–Newman–Keuls *post hoc* test. —, absence of effect.

activation of either κ- or δ-opioid receptors. It is shown here, at least for the loss of fiber attachment, that activation of κ-receptors was important, as indicated by the antagonism of morphine effects seen when a selective κ-antagonist was administered. Our data suggested, however, that different mechanisms seem to underlie the loss of both bone and fiber tooth supporting tissues, since only the beneficial effect of morphine on the fiber attachment loss was reversed by the selective κ-opioid antagonist. In line with this, another study has shown that a selective cyclo-oxygenase-1 inhibitor (the compound SC560) was able to decrease fiber attachment loss without affecting alveolar bone loss (20). In contrast, activation of δ-opioid receptors does not seem to mediate the actions of morphine in periodontal disease; naltrindole was uneffective in this respect.

The present study also demonstrated inhibition of fiber attachment and alveolar bone loss following local administration of the κ-opioid receptor agonist U-50,488 in this experimental model of periodontal inflammation. In agreement with our results, opioid agonists that act through κ-receptor activation have been viewed as having more potent anti-inflammatory properties than those acting via μ- and δ-opioid receptors (23,24). In our experiments, a lower dose of a κ-opioid agonist (U-50,488) decreased alveolar bone loss, whereas higher doses were required to decrease fiber attachment loss. These effects were specific, since a selective antagonist (nor-BNI) reversed them.

Opioid receptors are widely distributed in the central nervous system (25), while in the periphery they can be found in leukocytes (26) and vascular endothelial cells (27) and were even identified in a human osteoblast cell line (28). Binding of opioid agonists with opioid receptors, especially in leukocytes, causes a lower release of inflammatory mediators such as cytokines. In this regard, it has been shown that the compound U-50,488 inhibited the production of cytokines such as tumour necrosis factor (TNF-α) and interleukin-1 (IL-1) by macrophages *in vitro* (29). In contrast, those cytokines have been strongly implicated in the pathogenesis of periodontal disease (30–32). In the present study, it was noteworthy that the selective κ-opioid agonist did not affect the number of cells in periodontal tissues in a similar manner to what was observed when the agonist used was morphine (10). Taken together, these results suggest that κ-receptors may be located in resident rather than in circulating cells. Between resident cells expressing opioid receptors are endothelial cells and fibroblasts, which are also able to release cytokines such as IL-1 and TNF-α. Another possible location of opioid receptors in the periphery is the primary afferent fibers; the activation of such receptors decreases the release of neuropeptides such as substance P (33). Substance P has been also implicated in the progression of periodontal disease (34).

It was relevant in the present work that the κ-agonist had a local (ipsilateral) rather than a systemic effect, as shown by the lack of effect following its contralateral administration. Thus, side-effects derived from opioid stimulation in the central nervous system may be avoided by administering low doses of the drug locally at the site of inflammation. It is important to highlight that peripheral effects of opioids are observed early during inflammation (35) and it has been demonstrated that opioid receptors are increased in such conditions (36,37), which can lead to an increase in the action mediated by them. In fact, it had already been demonstrated that U-50,488 had potent peripheral anti-arthritic effects in a model of rheumatoid arthritis in rats (38). In that study, early administration of κ-agonist close to the site of inflammation prevented not only edema but also later destruction of joint tissues seen in that model.

Differently from κ-opioid receptors, however, either activation or blockade of δ-opioid receptors did not alter the indicators of periodontal disease used in the present study, at least in the chosen dose range. In contrast, activation of such receptors has already been implicated in decreasing the signs of inflammation in another experimental model of inflammation (13).

In conclusion, this study demonstrated a beneficial effect of locally administered opioids in a chronic model of inflammation, namely periodontal disease in rats, which was particularly dependent on activation of κ-opioid receptors. This unique finding should be explored further in therapeutics, since peripheral activation of κ-opioid receptors not only reduces the side-effects classically associated with opioids but might also be strongly effective to reduce the loss of tooth supporting tissues associated with chronic periodontitis.

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References

1. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontol 2000* 1997;**14:**216–248.
2. Fox CH. New considerations in the prevalence of periodontal disease. *Curr Opin Dent* 1992;**2:**5–11.
3. Van Der Velden U. Effect of age on the periodontium. *J Clin Periodontol* 1984;**11:**281–294.
4. Johnson-Leong C, Patel G, Messieha Z. The relationship between coronary artery disease and periodontal disease. *Dent Today* 2003;**22:**100–105.
5. Matthews DC. The relationship between diabetes and periodontal disease. *J Can Dent Assoc* 2002;**68:**161–164.
6. Boggess KA, Moss K, Madianos P, Murtha AP, Beck J, Offenbacher S. Fetal immune response to oral pathogens and risk for preterm birth. *Am J Obstet Gynocol* 2005;**193:**1121–1126.
7. Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with anti-proteinase, anti-inflammatory, and bone-sparing agents. A systematic review. *Ann Periodontol* 2003;**8:**12–37.
8. Walker JS, Wilson JL, Binder W, Scott C, Carmody JJ. The anti-inflammatory

effects of opioids: their possible relevance to the pathophysiology and treatment of rheumatoid arthritis. *Rheum Arthitis ID Res Alert* 1997;**1:**291–299.
9. Mudie AS, Holland GR. Local opioids in the inflamed dental pulp. *J Endod* 2006;**32:**319–323.
10. Pacheco CMF, Queiroz-Junior CM, Maltos KLM, Rocha OA, Caliari MV, Francischi JN. Local opioids in a model of periodontal disease in rats. *Arch Oral Biol* 2007;**52:**677–683.
11. Machelska H, Schopohl JK, Mousa SA, Labuz D, Schäffer M, Stein C. Different mechanisms of intrinsic pain inhibition in early and late inflammation. *J Neuroimmunol* 2003;**141:**30–39.
12. Zöllner C, Shaquira MA, Bopaiah CP, Mousa S, Stein C, Schäfer M. Painful inflammation-induced increase in μ-opioid receptor binding and G-protein coupling in primary afferent neurons. *Mol Pharmacol* 2003;**64:**202–210.
13. Jiménez N, Puig MM, Pol O. Anti-exudative effects of opioids and expression of κ- and δ-opioid receptors during intestinal inflammation in mice: involvement of nitric oxide. *J Pharmacol Exp Ther* 2006;**316:**261–270.
14. Gutstein HB, Akil H. Opioid analgesics. In: *Goodman & Gilman*'s *The Pharmacological Basis of Therapeutics*. Brunton LL, Lazo JS, Parker KL, eds. New York: McGraw Hill, 2005:547–590.
15. Walker JS, Chandler AK, Wilson JL, Day RO. Effect of mu-opioids morphine and buprenorphine on the development of adjuvant arthritis in rats. *Inflamm Res* 1996;**45:**557–563.
16. Binder W, Walker JS. Effect of the peripherally selective κ-opioid agonist, asimadoline, on adjuvant arthritis. *Br J Pharmacol* 1998;**124:**647–654.
17. Green PG, Levine JD. Delta- and kappa-opioid agonists inhibit plasma extravasation induced by bradykinin in the knee joint of the rat. *Neuroscience* 1992;**49:**129–133.
18. Sallay K, Sanavi F, Ring I, Pharm P, Behling UH, Nowotny A. Alveolar bone destruction in the immunosuppressed rat. *J Periodont Res* 1982;**17:**263–274.
19. Maltos KL, Menezes GB, Caliari MV *et al*. Vascular and cellular responses to pro-inflammatory stimuli in rat dental pulp. *Arch Oral Biol* 2004;**49:**443–450.
20. Queiroz-Junior CM, Pacheco CMF, Maltos KLM, Caliari MV, Duarte IDG, Francischi JN. Role of systemic and local administration of selective inhibitors of cyclo-oxygenase 1 and 2 in an experimental model of periodontal disease in rats. *J Periodont Res* 2008; in press.
21. Randall LD, Sellito JJ. A method for measurement of analgesic activity on inflamed tissues. *Arch Int Pharmacol* 1957;**113:**233–249.
22. Pacheco DF, Duarte IDG. Δ-Opioid receptor agonist SNC80 induces peripheral antinociception via activation of ATP-sensitive K^+ channels. *Eur J Pharmacol* 2005;**512:**23–28.
23. Taub DD, Eisenstein TK, Geller EB, Adler MW. Immunomodulatory activity of μ- and κ-selective opioid agonists. *Proc Natl Acad Sci USA* 1991;**88:**360–364.
24. Radulović J, Miljević C, Djergović D *et al*. Opioid receptor-mediated suppression of humoral immune response *in vivo* and *in vitro*: involvement of κ opioid receptors. *J Neuroimmunol* 1995;**57:**55–62.
25. Mansour A, Fox CA, Burke S *et al*. Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. *J Comp Neurol* 1994;**350:**412–438.
26. Sharp BN, Roy S, Bidlack JM. Evidence for opioid receptors on cells involved in host defense and the immune system. *J Neuroimmunol* 1998;**83:**45–56.
27. Cadet P, Bilfinger TV, Fimiani C, Peter D, Stefano GB. Human vascular and cardiac endothelia express mu opiate receptor transcripts. *Endothelium* 2000;**7:**185–191.
28. Pérez-Castrillón JL, Olmos JM, Gómez JJ *et al*. Expression of opioid receptors in osteoblast-like MG-63 cells, and effects of different opioid agonists on alkaline phosphatase and osteocalcin secretion by these cells. *Neuroendocrinology* 2000;**72:**187–194.
29. Belkowski SM, Alicea C, Eisenstein TK, Adler MW, Rogers TJ. Inhibition of interleukin-1 and tumor necrosis factor-α synthesis following treatment of macrophages with the κ-opioid agonist U50,488H. *J Pharmacol Exp Ther* 1995;**273:**1491–1496.
30. Pfeilschifter J, Chenu C, Bird A, Mundy GR, Roodman GD. Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclast-like cells in vitro. *J Bone Miner Res* 1989;**4:**113–118.
31. Delima AJ, Karatzas S, Amar S, Graves DT. Inflammation and tissue loss caused by periodontal pathogens is reduced by interleukin-1 antagonists. *Infect Dis* 2002;**186:**511–516.
32. Oates TW, Graves DT, Cochran DL. Clinical, radiographic and biochemical assessment of IL-1/TNF-α antagonist inhibition of bone loss in experimental periodontitis. *J Clin Periodontol* 2002;**29:**137–143.
33. Barber A. μ- and κ-opioid receptor agonists produce peripheral inhibition of neurogenic plasma extravasation in rat skin. *Eur J Pharmacol* 1993;**236:**113–120.
34. Györfi A, Fazekas A, Suba Zs, Ender F, Rosivall L. Neurogenic component in ligature-induced periodontitis in the rat. *J Clin Periodontol* 1994;**21:**601–605.
35. Pol O, Puig MM. Expression of opioid receptors during peripheral inflammation. *Curr Top Med Chem* 2004;**4:**51–61.
36. Schäffer M, Imai Y, Uhl GR, Stein C. Inflammation enhances peripheral μ-opioid analgesia, but not μ-opioid receptor transcription in dorsal root ganglia. *Eur J Pharmacol* 1995;**279:**165–169.
37. Pol O, Alameda F, Puig MM. Inflammation enhances μ-opioid receptor transcription and expression in mice intestine. *Mol Pharmacol* 2001;**60:**894–900.
38. Wilson JL, Naynar V, Walker JS. The site of anti-arthritic action of the κ-opioid, U-50,488H, in adjuvant arthritis: importance of local administration. *Br J Pharmacol* 1996;**118:**1754–1760.