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Desipramine changes salivary gland function, oral microbiota, and oral health in rats

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Abstract

Tricyclic antidepressants are still a dominating group of psychotherapeutic drugs used in the treatment of depression. Oral dryness is one of their major side-effects, leading in humans to increased oral disease and dysfunction of speech, chewing, swallowing and taste. We previously reported that the tricyclic antidepressant desipramine desensitizes β-adrenergic signal transduction in salivary glands. In this study, we evaluated the effects of this treatment on parotid and submandibular gland function, oral microbiota, and oral health in rats. Total protein secretion and salivary α-amylase was not affected by treatment, while cellular α-amylase and the content of epidermal growth factor was depressed. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis revealed increased secretion for proline-rich proteins and glycoproteins. Surprisingly, flow rates were temporarily increased. These alterations in salivary gland function may partially explain the observed changes in oral microbiota and the increased incidence of gingivitis. Under other nutritional conditions, desipramine might have more severe impacts on oral health.

Keywords: Desipramine; Gingivitis; Microbiota; Salivary protein; Salivary flow rate; Tricyclic antidepressant

1. Introduction

Saliva components are critical in maintaining oral health and supporting other oral functions. It is well known that sympathetic stimulation via neurotransmitters norepinephrine, neuropeptide Y, adenosine triphosphate (ATP), α- and β-adrenoceptors leads to high levels of protein secretion, whereas high rates of fluid output occur in response to parasympathetic stimulation via the neurotransmitters acetylcholine, substance P, vasoactive intestinal polypeptide, ATP and acetylcholine receptors (Looms et al., 1998). The α_1-adrenergic signal transduction system is involved in the flow of saliva as well.

Besides systemic diseases and radiation therapy, prescription and non-prescription drugs are important causes of salivary gland disturbances (Mandel, 1980). Many subjective, e.g. xerostomia (Remick, 1988), and objective findings, e.g. increased susceptibility to dental caries and opportunistic oral yeast infections (Atkinson and Fox, 1992), have been related to salivary gland dysfunction. Specific salivary proteins, such as mucins and proline-rich proteins, play a major role in bacterial colonization on tooth surfaces (Schenkels et al., 1995). Tricyclic antidepressants may indirectly (central nervous system), and/or directly (salivary glands) reduce salivary flow rate and the secretion of salivary proteins as a result of their action on modulating α-, β-adrenergic and muscarinic–cholinergic neural transmission. It has been shown (Scarpace et al., 1992, 1993) that in rats, after long-term administration of

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desipramine, salivary glands demonstrated desensitization of the isoproterenol-stimulated activity, and parotid glands revealed decreased post-receptor signal transduction as well.

Based on these studies, we assessed the effects of chronic desipramine administration on salivary gland function and oral health. As desipramine is a relatively selective inhibitor of the re-uptake of norepinephrine, and has weak effects on serotonergic neuronal activity, on acetylcholine and histamine H_1 receptors, as well as on α_1-adrenoceptors (Frazer, 1997; Potter et al., 1991), we hypothesized (a) that saliva flow rate would decrease during treatment; (b) that protein secretion of parotid and submandibular glands, α-amylase activity and epidermal growth factor (EGF) concentration would be depressed temporarily; (c) that these changes would influence total cultivable microbiota and its composition present in young female NIA Fischer 344 rats; and (d) that this would interfere with the animal's gingival health status.

2. Materials and methods

2.1. Animals

Pathogen free 3-month old female Fischer 344 NIA rats were obtained from Harlan Sprague–Dawley (Indianapolis, USA) under contract with the National Institute of Aging. Upon arrival, rats were examined and housed individually in 7×10 in. stainless steel micro-isolated cages at 25°C. All animals were maintained on Purina rat chow and water ad libitum and exposed to a 12-h light/dark cycle. Animals were acclimatized for a minimum of 10 days before use.

Animals were cared for in accordance with the principles of the "Guide to the Care and Use of Experimental Animals", and all protocols were approved by the University of Florida committee for animal studies (IUCAC approval #8112 to M.H.B.).

2.2. Experimental design

Saline or desipramine (10 mg/kg each, i.p.) was administered at 9 a.m. for 28 days. The beginning of the treatment period was staggered. Two animals per day were used for the experiments. One half of the animals given desipramine or saline were sacrificed after saliva collection on the third day after the last dose (desipramine and saline groups), and the remainder after a washout period of 15 days (washout groups). Six animals were assigned to the desipramine and desipramine-washout groups, five rats to the saline and saline-washout groups. The operator did not know to what group the animals belonged to. Body weight was measured weekly, the final weight at the day of the experiment.

2.3. Saliva collection and flow rates

Saliva collections were carried out between 9 and 11 a.m., always by the same person. Rats were deprived of food at 4 a.m., the day before the experiment. Animals were anesthetized (sodium pentobarbital; 50 mg/kg, i.p.) and tracheotomized. The parotid and submandibular ducts of the animal's left side were canulated with calibrated (lumen and length) drawn PE-10 plastic tubes. Total saliva from the animal's right side was collected with a 20 μl pipette for the estimation of epidermal growth factor (EGF) content and electrophoresis. To stimulate salivary flow, pilocarpine HCl (Sigma, USA; 5 mg/kg, i.p.) was administered; to induce discharge of stored proteins isoproterenol (Sigma, USA; 5 mg/kg, i.p.) was chosen. These agents were dissolved in 37°C isotonic saline. The initial drop of saliva was discarded and subsequent flow was collected for 30 min into pre-weighed plastic tubes kept on ice. Flow rate was calculated as μl/min/gland and μl/min/g gland wet weight. One percent of sodium ethylene diamine tetraacetate (EDTA, pH 8.6) was added to the submandibular and total saliva (100 μl/ml) to prevent the formation of a calcium–protein precipitate (Abe et al., 1980). Saliva was stored at −80°C until analyzed.

2.4. Salivary proteins

For each analysis, all samples were aliquoted to avoid repetitive freezing and thawing, assayed at one time, and in duplicate, to minimize error. Proteins were analyzed by the method of Bradford (1976), using bovine plasma gamma globulin as the standard. Amylase activity was determined by activity assays (Bernfeld, 1955) using starch as a standard. One unit of amylase was defined as the amount that hydrolyzed 1 mg starch per minute and per milliliter saliva (Us) and per milligram protein (Up), respectively at 37°C.

Total saliva (30 μg of protein) was subjected to electrophoresis, using a modified Tris–glycine system of Laemmli, as described by Pugsley and Schnaitman (1979). Gels were fixed and stained by a modification of the method of Fairbanks, as described by Humphreys-Beher and Wells (1984).

For EGF estimation, the volume of total saliva was brought up to 1 ml with sterile water. Fifty eight of glacial acetic acid were added, the solution cooled on ice for 30 min and then centrifuged at 4°C for 30 min. The supernatant was transferred to a new tube, frozen at −80°C and then lyophilized. The remaining proteins were resuspended in 200 μl sterile water. One hundred microliters were added to 100 μl of human placental microvilli membranes and [^{125}I]-labeled human EGF. Following incubation at 37°C for 2 h, the membrane was diluted in phosphate buffered saline, centrifuged for 20 min at 7000 g and the radiolabel associated with the membrane determined in a

gamma counter. This method of membrane binding competition is independent of species origin for the EGF source (Booth et al., 1980).

2.5. Membrane preparation, DNA synthesis, cellular α-amylase

In vivo DNA synthesis was determined in parotid and submandibular glands by the incorporation of [^{3}H]thymidine into DNA by the injection of radiolabel (50 μCi/100 g) 1 h before pentobarbital anesthesia and 4 h prior to killing. At sacrifice, parotid and submandibular glands were carefully excised and dissected free of lymph nodes, fat and fascia, the sublingual gland removed, and the wet weight determined. Total membrane fractions were prepared at 4°C by homogenization of intact parotid and submandibular tissues in 0.25 M sucrose, 1 mM $MgCl_2$, and 5 mM Tris–HCl (pH 7.4). To avoid protein degradation, protein inhibitors were added to the homogenate (1 μM leupeptin, 100 μM benzamidine, 100 μM phenylmethylsulfonyl fluoride (PMSF) final concentrations). Preparations were minced with a mechanical tissue chopper, the cells dispersed with two 15 s bursts using a polytron tissue homogenizer and then lysed with 10 strokes of a motor driven teflon-tipped pestle at moderate speed. The homogenate was passed through two layers of cheese cloth. Samples (100 μl) for [^{3}H]thymidine incorporation were analyzed by scintillation counting using 10 ml of Fisher premixed non-aqueous scintillation cocktail (Sci Vers 5X20-4).

The rest of the slurry was centrifuged at 48 000 g for 30 min to recover total membrane. The supernatant was saved and frozen until analysis for cellular α-amylase with the above mentioned activity assay (Bernfeld, 1955). The total membrane pellet was resuspended in 20 volumes of 8-mM $MgCl_2$, 0.08-mM ascorbic acid, and 50-mM HEPES (*N*-hydroxyethyl piperazine-N′-2-athane sulfonic acid, pH 7.4) and the above mentioned proteinase inhibitors added. Protein assays for total membrane (structural proteins) and supernatant (soluble proteins) were determined at one time by the above mentioned method of Bradford (1976).

2.6. Microbiological analysis

For the cultivation of tooth- and mucosa-associated microbiota, upper right and lower left teeth were aseptically removed with the surrounding alveolar bone. Mucosa was obtained from the tongue and palate. The samples were collected in skim milk and lyophilized, resuspended in 2 ml GAM (Gifu anaerobic medium) broth (Nissui, Japan), crushed and homogenized for 30 s, and diluted serially to 10^{-3} with 0.1 ml of each dilution. Aliquots (0.1 ml) were spread on 5% sheep blood agar plates, supplemented with Trypticase Soy base (Difco Laboratories, USA), and incubated anaerobically in Anaero Box AZ-125 (Hirasawa, Japan) for 5 days. The isolates were characterized by colony morphology, microscopic observation with Gram stain, catalase reaction, and identified using API 20E strips. All API strips were purchased from Bio Mérieux (France). Aliquots (0.1 ml) were cultured on Candida GS agar plates (Eiken Chemical, Japan).

2.7. Gingival health

Gingival health was estimated microscopically (magnification factor 7) by means of the rat gingival bleeding index (Hefti, 1979) on the lingual surface of the upper right and lower left first and second molars with a fine tooth probe. One score value per side was taken 10 s after probing. Two animals were used per day and the operator calibrated each time (20p).

2.8. Statistical analysis

Body weight was analyzed by repeated measures ANOVA (analysis of variance test). The Bonferroni/Dunn multiple comparison procedure was carried out to determine significant differences among the means following a significant one way ANOVA. Microbiology data (n = colony forming units; CFU) were analyzed after $\log_{10}(n+1)$-transformation. The unpaired t-test showed no significant differences between the two saline groups for all these data. The respective data were pooled for the final analysis (saline group).

Non-parametric statistics were performed for rat gingival bleeding index data. The Friedman test showed no significant differences between the two dental locations and the Kruskal–Wallis test showed no significant differences between the two saline control groups. Therefore, the respective data were pooled for the final analysis (Kruskal–Wallis test; saline group).

3. Results

3.1. Body weight, gland weight, DNA synthesis

There were substantial differences in body weight throughout the experimental period between control and desipramine treated animals. Recovery was nearly complete during the washout period. In the treatment group, body weight at the day of sacrifice (day 31) was significantly lower compared to the controls ($P = 0.006$). The wet weight of parotid and submandibular glands was not affected by desipramine treatment. The histosomatic index, (body weight/gland weight) × 100, showed no treatment-related changes. With regard to the general state of the glandular cells, we found that the DNA synthesis, as measured by the incorporation of tritiated thymidine, was not influenced by drug treatment either.

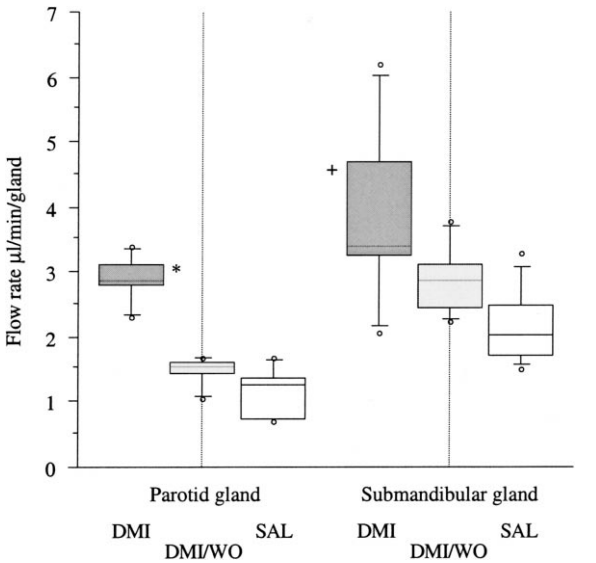

Fig. 1. Box-plot of parotid ($P < 0.0001$) and submandibular ($P < 0.0062$) flow rates with treatment, expressed as µl/min/gland. Saline (saline group) or desipramine was administered once a day for 28 days, followed by a 3- (desipramine group) or 15-day (washout group) recovery period. The results express the values of six animals (desipramine and washout groups) and 10 animals (saline group). Significant differences (5%) compared to the washout and the saline group are marked with (*), compared only to the saline group with (+) by one-way ANOVA.

3.2. Salivary flow rates

Salivary flow rates (Fig. 1), expressed as µl/min/gland, were highly affected by treatment, and showed significantly higher levels with desipramine (parotid gland 2.46-fold increase, $P = 0.0001$; submandibular gland 1.79-fold increase, $P = 0.0062$) compared to the control group. The treatment effect was not completely reversed during the 15-day washout period in both glands. As wet gland weights were not significantly affected by treatment, these desipramine-induced changes were the same when correcting the fluid production for 1 g gland wet weight (µl/min/g gland).

3.3. Proteins

No significant treatment-related changes in protein concentrations (µg/µl) were detected for total membrane (structural proteins) or supernatant (soluble proteins) in both glands. The salivary protein concentrations (µg/µl) showed significant changes between the three groups (Fig. 2). There was a 59% decrease in protein concentration in parotid saliva, and a 44% decrease in submandibular saliva in the drug treated animals compared to the controls. Recovery was almost complete for both glands (parotid gland 88%, submandibular gland 87%) following the washout period. As the flow rate had demonstrated a treatment-related increase, we calculated the total amount of protein secreted over 30 min. There remained no significant drug-related effect in total salivary protein secretion.

Total salivary protein was qualitatively analyzed on a sodium dodecyl sulfate–polyacrylamide gel (SDS/PAGE)

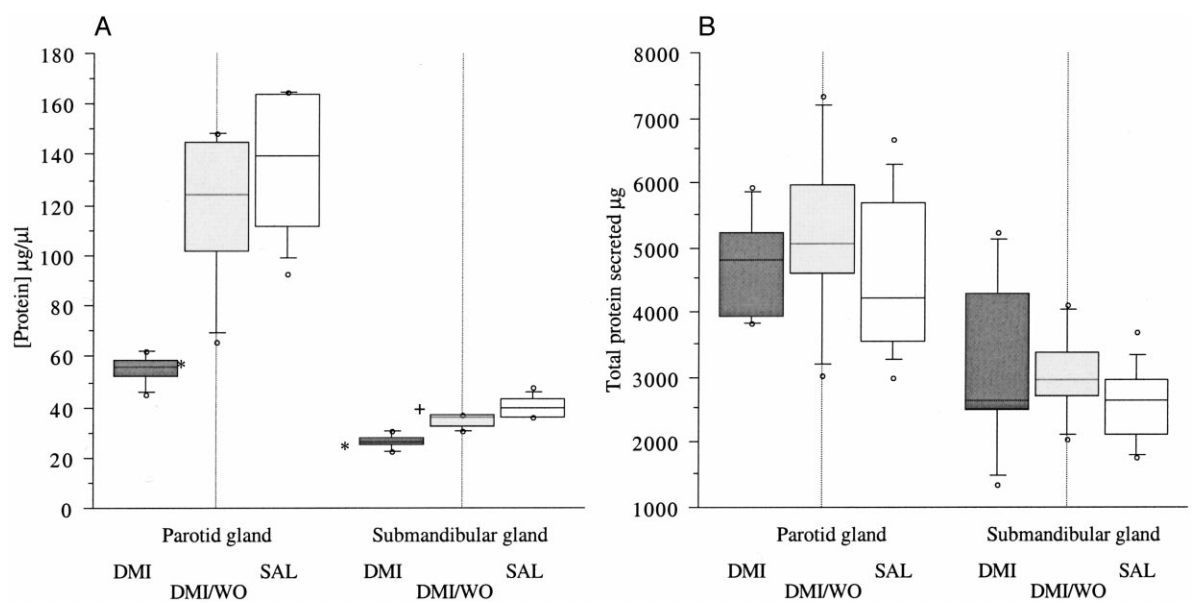

Fig. 2. Box-plot of (A) parotid ($P < 0.0001$) and submandibular ($P < 0.0001$) protein concentrations with treatment, expressed as µg/µl; and of (B) parotid ($P = 0.6653$) and submandibular ($P = 0.4631$) total protein secreted (µg). Saline (saline group) or desipramine was administered once a day for 28 days, followed by a 3- (desipramine group) or 15-day (washout group) recovery period. The results express the values of six animals (desipramine and washout groups) and 10 animals (saline group). Significant differences (5%) compared to the washout and the saline group are marked with (*), compared only to the saline group with (+) by one-way ANOVA.

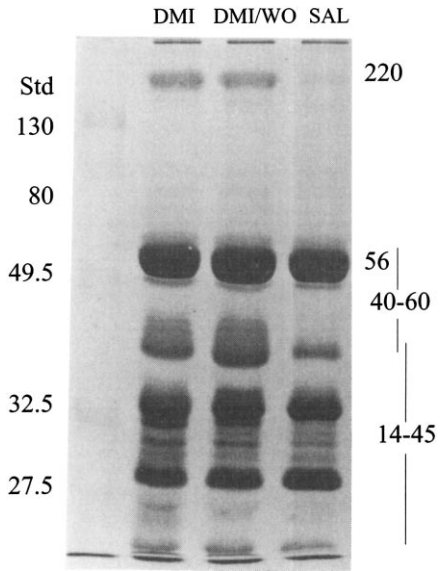

Fig. 3. Total saliva proteins analyzed on a 10% SDS polyacrylamide gel with a representative sample of each of the three treatment groups (30 μg of protein; Coomassie blue staining). Saline (saline group) or desipramine was administered once a day for 28 days, followed by a 3- (desipramine group) or 15-day (washout group) recovery period. The migration of glycosylated proline-rich protein was determined to be 200 kDa, of α-amylase 56 kDa, of acidic proline-rich proteins 40–46 kDa, and of basic proline-rich proteins 14–45 kDa. Pre-stained molecular standards (Std) were: phosphorylase B, 130 kDa; bovine serum albumin, 80 kDa; ovalbumin, 49.5 kDa; carbonic anhydrase, 32.5 kDa; soybean trypsin inhibitor, 27.5 kDa.

with a representative sample of each of the three treatment groups (Fig. 3). Protein composition from the two experimental groups differed from that of the control animals. Specifically, there was an increase of the glycosylated proline-rich protein (band 220 kDa), an increase in the metachromatically stained ladder of acidic proline-rich proteins (bands 40 to 46 kDa), and an increase in basic proline-rich proteins (bands ranging from 14 to 45 kDa) in the Coomassie blue-stained gel. The mobility of rat proline-rich proteins has previously been determined (Humphreys-Beher and Wells, 1984; Bedi and Bedi, 1995).

Amylase activity (Fig. 4) was significantly decreased (65%; $P = 0.0001$) during desipramine administration, followed by an almost complete recovery (80%; $P = 0.0001$) at the end of the washout period in parotid saliva, expressed as milligram starch hydrolyzed per minute and per milliliter saliva (Us). Controlled for changes in flow rate and based on 1 mg protein (Up), the treatment induced decrease in the enzyme's activity was not significant ($P = 0.2147$) anymore. To determine whether these changes in amylase activity were due to a reduction in the biosynthesis of the enzyme, or due to a decline in secretory function after pilocarpine-isoproterenol stimulation, we analyzed the supernatant of the parotid homogenates for amylase activity. The results (mg/min/mg protein, Up) revealed a significant decrease in the desipramine group compared to the washout ($P < 0.0001$) and the saline group ($P < 0.0001$) of residual cellular amylase activity following stimulation.

To investigate whether these drug-related changes in the biosynthesis and/or the secretory process of proteins were unique for the acinar cells of the parotid gland, the secretory capacity of the submandibular granular tubule cells

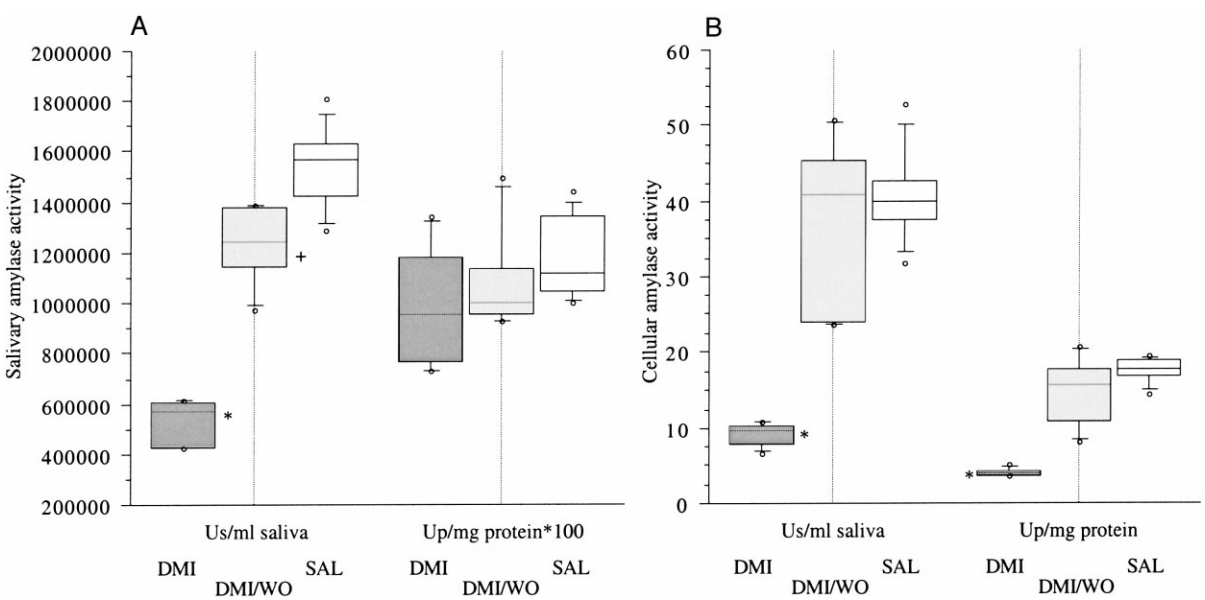

Fig. 4. Box-plot of (A) parotid salivary (Us/ml saliva, $p < 0.0001$; (Up/mg protein) × 100, $p = 0.2147$), and of (B) parotid cellular (Us/ml supernatant, $p < 0.0001$; Up/mg protein, $p < 0.0001$) α-amylase activity with treatment. Saline (saline group) or desipramine was administered once a day for 28 days, followed by a 3- (desipramine group) or 15-day (washout group) recovery period. The results express the values of six animals (desipramine and washout groups) and 10 animals (saline group). Significant differences (5%) compared to the washout and the saline group are marked with (*), compared only to the saline group with (+) by one-way ANOVA.

with treatment was tested by estimating the concentration (μg/ml) of EGF (Fig. 5). Total saliva of the washout rats contained 52% less EGF per milliliter saliva than that of the control animals ($P < 0.0001$), desipramine treated rats showed a decrease of 39% compared to the control group ($P = 0.0180$).

3.4. Oral microbiota and gingival health

The predominant bacterial groups of the cultivable microbiota on tooth surfaces in control animals were Gram-positive cocci (80%; enterococci 56%, streptococci 14%, staphylococci 30%), and Gram-positive rods (18%). There was a 6.2-fold increase in total cultivable microbiota in the treatment group ($P = 0.0074$) compared to the control animals. Recovery was not complete, as a 1.6-fold increase was found in washout animals ($P = 0.7241$). A marked change in its composition was detected with treatment (Fig. 6): streptococci increased with desipramine 29-fold, Gram-negative cocci 79-fold, and Gram-negative rods 44-fold, while all other bacteria remained quite stable. In the washout group, composition of oral microbiota was essentially the same as in the control group. Total cultivable microbiota on oral mucosa decreased significantly in both treatment groups compared to the control animals. Significant changes were observed in Gram-positive rods and Gram-positive cocci (enterococci, streptococci, staphylococci). *Candida albicans* was isolated from one sample only.

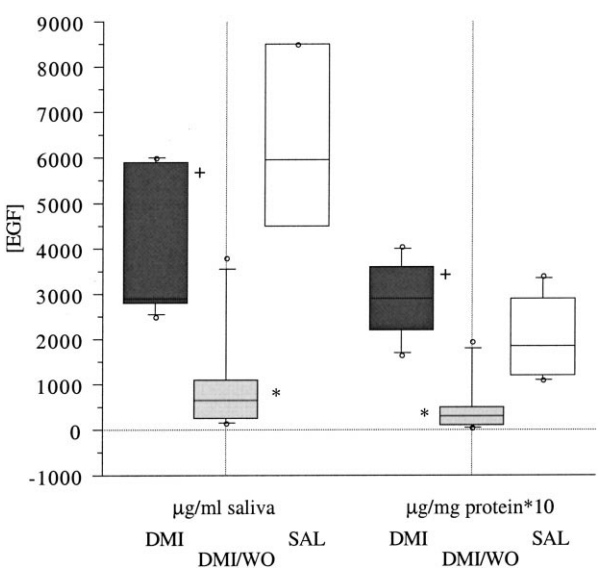

Fig. 5. Box-plot of EGF concentration (μg/ml saliva; $p = 0.0002$) and EGF content per mg protein ((μg/mg protein)×10; $p = 0.0013$) in total saliva with treatment. Saline (saline group) or desipramine was administered once a day for 28 days, followed by a 3- (desipramine group) or 15-day (washout group) recovery period. The results express the values of six animals (desipramine and washout groups) and 10 animals (saline group). Significant differences (5%) compared to the saline group are marked with (*), compared to the washout group with (+) by one-way ANOVA.

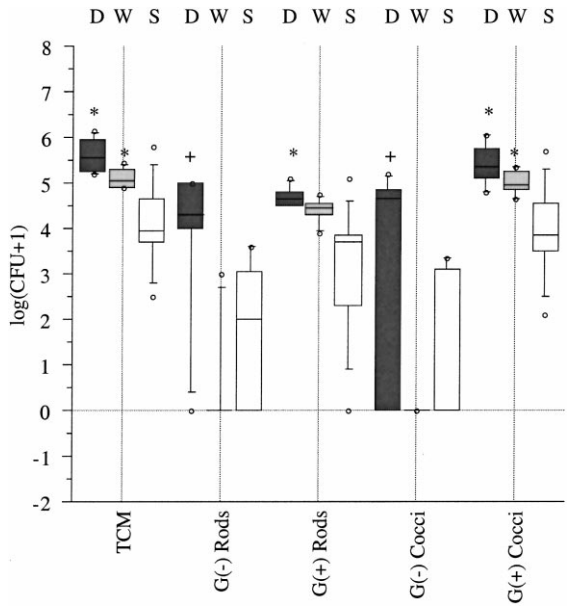

Fig. 6. Box-plot of cultivable microbiota on tooth surfaces with treatment, expressed as $\log_{10}$ (colony forming units; CFU + 1). Saline (saline group; S) or desipramine was administered once a day for 28 days, followed by a 3- (desipramine group; D) or 15-day (washout group; W) recovery period. The results express the values of six animals (desipramine and washout groups) and nine animals (saline group). Significant differences (5%) compared to the saline group are marked with (*), compared to the washout group with (+) by one-way ANOVA.

Desipramine treated animals showed a significant higher incidence of gingivitis ($P = 0.0009$, $H = 14.35$), as a comparison of the means of the rat gingival bleeding index (RGBI) with the control group ($P < 0.0001$), as well as the washout group ($P < 0.0001$). There was almost complete recovery in gingival health after the washout period (Fig. 7).

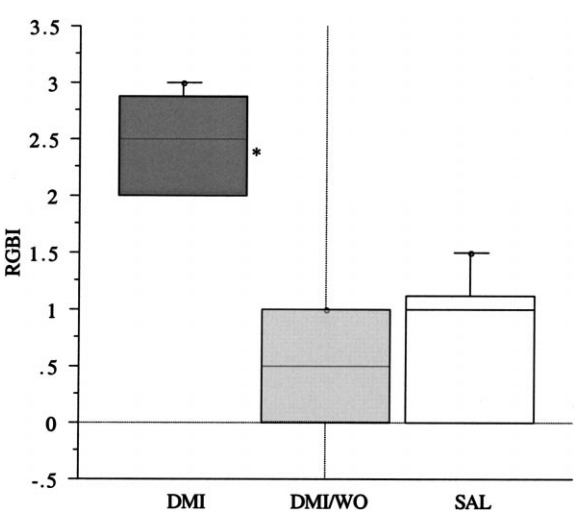

Fig. 7. Box-plot of the rat gingival bleeding index (RGBI) on tooth surfaces with treatment. Results express the values of six animals (desipramine and washout groups) and 10 animals (saline group). Significant differences (5%) compared to the washout and the saline group are marked with (*) by one-way ANOVA.

4. Discussion

As only body weight, but not the wet weight of either gland, nor cellular DNA synthesis was significantly affected by desipramine treatment, one can conclude that glandular cell proliferation was not significantly affected by prolonged administration of the antidepressant desipramine. Thyroid hormone is part of the regulatory mechanisms in salivary gland function (Johnson et al., 1987; Tumilasci et al., 1986). As nutritional changes influenced salivary protein secretions (Kurahashi and Inomata, 1999), flow rates (Johnson, 1988) and autonomic cell surface receptor density (Johnson and Cardenas, 1993), and as long-term desipramine administration decreased thyroxine production (Rousseau et al., 1998), changes in food and water intake and desipramine may have indirectly influenced salivary gland function in this experiment.

In the present study, we confirmed that long-term exposure of rats to the tricyclic antidepressant desipramine results in alteration in the pattern of salivary protein synthesis and salivary flow rates. It is well established that most exocrine protein secretion occurs subsequent to β-adrenoceptor activation (Looms et al., 1998). The biosynthesis of some specific proteins was stimulated (α-amylase, proline-rich proteins). Surprisingly, the total amount of protein secreted was not affected. Therefore, to maintain stable levels of secreted proteins, the synthesis and/or secretion rate of other proteins must have been depressed with desipramine. As serum levels of tetra- and triiodothyronine are part of the regulatory mechanisms in salivary gland function (Johnson et al., 1987; Tumilasci et al., 1986), e.g. they regulate synthesis of EGF (Dagogo-Jack, 1995), the observed decrease in salivary EGF content may have been caused by nutritional changes and drug-related effects. Differences in the induction of specific proteins with desipramine may be due to either nonspecific interaction with β-adrenergic signal transduction or differences in the induction of protein synthesis in different glands (Bedi, 1993). In addition, there may be a differential control of secretion of proteins synthesized by ductal and acinar cells (Hirata and Orth, 1980; Johnson and Cortez, 1988).

The ability of glandular cells to release secretory proteins upon stimulation, as well as the biosynthesis of secretory proteins after complete depletion, were not impaired by the chronic administration of the antidepressant for total protein in this study (stable ratio of structural and soluble glandular proteins). However, as a significant decrease of residual cellular α-amylase activity was observed with desipramine treatment, the antidepressant may actually negatively influence the biosynthesis of single proteins after complete depletion. Previous studies of desipramine in rats, applying the same drug regimen as in this study, showed desensitization and down-regulation of β-adrenergic signal transduction in salivary glands (Scarpace et al., 1992, 1993). In contrast to receptor number, desipramine attenuated G-protein linked post receptor adenylate cyclase activity persisted through the 15-day washout period (Scarpace et al., 1993). Therefore, the animals in the present study probably also experienced receptor down-regulation and adenylate cyclase desensitization, and the revealed alterations in parotid protein secretion are, at least partially, the consequences of these changes in the signal transduction cascade changes present in all animals.

Surprisingly, considering the antagonistic action of desipramine on α_1-adrenoceptors and acetylcholine receptors in the brain (Potter et al., 1991), salivary flow rate in our study showed significantly increased levels with desipramine for both glands compared to the control group, which did not completely recover during the washout period in both glands. One can speculate that the detected increase in salivary flow rate was partially caused by a positive feedback mechanism through the muscarinic–cholinergic, α_1-adrenergic and substance P signal transduction system due to decreased concentration of secreted proteins. In addition, there may have occurred an interaction of desipramine with cholinergic function at the presynaptic level (Volterra and Lecci, 1992). As we collected saliva only the third day after the last dose of desipramine, a cholinergic rebound must be considered as well (Dilsaver and Davidson, 1987). Frequently, there is a lack of predictable linear correlation between receptor activation and intracellular responses. This lack suggests that various signaling pathways intersect and cross-talk to modify and influence the biological outcome of a specific extracellular signal. The complexity of drug actions, and variations in the absorption and excretion of these compounds, make precise prediction of their effects on salivary gland function difficult, interactions among various transmitters must be considered (Hill, 1998; Looms et al., 1998).

Salivary proteins, electrolytes, and volume determine the ultimate environment of the oral microbiota, modulate bacterial colonization on oral surfaces, and maintain the integrity of oral tissues (Schenkels et al., 1995). There is ample evidence that proline-rich proteins play a major role as pellicle receptors for microbial adhesion (Scannapieco, 1994; Schenkels et al., 1995). Amylase seems to inhibit the growth of several species of bacteria (Scannapieco, 1994). In our study, besides α-amylase and proline-rich proteins, other specific salivary antimicrobial and immunological proteins have probably been diluted by the increase in saliva volume as well. These alterations in concentrations would be expected to have contributed to the observed increase in bacterial colonization, changes in plaque composition, and increase in the incidence of gingivitis.

The transition from gingival health to gingivitis and periodontitis in rats is related to an increase in total cultivable microbiota, and increased numbers of Gram-negative and Gram-positive bacteria (Weinberg and Bral, 1999). That the increased incidence of gingivitis and the changes in microbiota on tooth surfaces were interrelated, and directly caused by the chronic administration of desipramine is suggested by the facts that (a) the control

animals did not show increased bleeding on probing, and that (b) gingivitis and the increase in total cultivable microbiota and changes in bacterial composition were transient, as the animals nearly recovered after drug release for 2 weeks.

In summary, the chronic administration of the tricyclic antidepressant desipramine led to marked changes in salivary protein secretion and flow rates, alterations in the oral microbiota and increased incidence of gingivitis. With the introduction of a cariogenic diet, desipramine might have a more severe impact on the oral health status (caries, periodontal disease), as observed in humans (Gråhn et al., 1988), where salivary gland derived health problems pose a significant disease burden.

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