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Dexamethasone-mediated androgen metabolism in human gingival and oral periosteal fibroblasts

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Abstract

Dexamethasone modulates the effects of other hormones and mediates cell function; the periodontium is a target tissue for androgens. It was therefore relevant to investigate the modulation of androgen metabolism by dexamethasone in cultured human gingival (HGF) and oral periosteal fibroblasts (HPF). Each cell line was incubated in Eagle minimum essential medium with [^{14}C]testosterone/[^{14}C]4-androstenedione as substrates and serial concentrations of dexamethasone (0.5–50 μg/ml), for 24 h; the medium was solvent-extracted, analyzed and quantified for steroid metabolites. In response to dexamethasone, both HGF ($n = 6$) and HPF ($n = 4$) showed up to two-fold increases in the formation of 5α-dihydrotestosterone and 4-androstenedione ($P < 0.01$, one-way ANOVA), and 3.6- to 5-fold increases in the formation of testosterone ($P < 0.001$), from [^{14}C]4-androstenedione, with some inhibition at higher concentrations. Dexamethasone stimulated the formation of physiologically active androgen metabolites in a dose-dependent manner. These metabolites might therefore contribute to dichotomous effects in connective tissues of the periodontium, dependent on effective concentrations of dexamethasone.

Keywords: Androgen action; Dexamethasone; Gingival; Periosteal fibroblasts; Repair

1. Introduction

Periodontal disease is characterized by episodic bursts of inflammation, triggered by plaque antigens, often prolonged and damaging even after removal of the antigenic stimulus. Physiological concentrations of circulating androgens influence the formation of connective tissue and bone matrices (Fujita et al., 2001). Glucocorticoids modulate the effects of other hormones and also mediate cell function. They enhance the mitogenic activity of fibroblast growth factor (Holley and Kiernan, 1974) and insulin-like growth factor-1 (Conover et al., 1986), but inhibit epidermal growth factor in human osteoblast-like cells (Otto et al., 1981). Dexamethasone has dichotomous effects on tissue matrices: it reduces endothelial cell fibronectin, resulting in weak endothelial cell–substratum adhesion and also the incorporation of fibronectin into the extracellular matrix (Romer and Polin, 1995). In vivo, some of these actions may be mediated by hormones and it was therefore relevant to study the interactions of androgens and dexamethasone in human gingival and oral periosteal fibroblasts derived from a chronically inflamed source. Androgens can also regulate anabolic activity by increasing the expression of transforming growth factor-β (Benz et al., 1991). In view of these actions, it was relevant to investigate the modulation of the anabolic action of androgens by dexamethasone in cultured human gingival and oral periosteal fibroblasts in the context of a possible adjunctive regenerative therapy; this aspect of glucocorticoid function has not been reported previously.

When osteogenic cells derived from fetal calvaria and bone marrow were treated with dexamethasone in vitro, there were increases in alkaline phosphatase activity and elevated bone-specific marker proteins such as osteopontin and osteocalcin (Kasugai et al., 1991; Leboy et al., 1991; Nagata et al., 1991). Treatment with dexamethasone also increased the number of mineralized bone nodules (Bellows et al., 1987). When dexamethasone was omitted from osteogenic cell cultures there was a moderate increase in alkaline phosphatase mRNA, low amounts of type 1 collagen mRNA and little production of osteopontin mRNA (Leboy et al., 1991). Dexamethasone appears to play an important part not only

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in early but also in late stages of osteogenic cell differentiation (McCulloch and Tenenbaum, 1986; Leboy et al., 1991). If an androgen-responsive element to dexamethasone could be identified in cultured gingival and oral periosteal fibroblasts, it might be a useful marker for regenerative potential in the inflamed periodontium. The influence of dexamethasone on steroid hormone metabolism in fibroblasts has not been investigated previously.

Oral periosteal fibroblasts as used here are a useful model, in view of the inductive effects of dexamethasone on osteogenesis by triggering osteoprogenitor cells (Bellows et al., 1987) and inducing adult bone-marrow cells to differentiate into osteoblasts (Kasugai et al., 1991). Some of these actions may be mediated by hormonal mechanisms. Oral periosteal fibroblasts represent cells with a high turnover of tissue matrices. Periosteal tissue is a source of osteoprogenitor cells associated with bone repair (Zhang et al., 1997). It has been demonstrated that mineralized tissue formation can be induced in periodontal ligament and periosteal cells cultured in direct contact with bovine dentine slices (Groenveld et al., 1994). The regulation of osteoblastic differentiation by cytokines and bone morphogenetic protein has been documented (Wozney et al., 1989). Similarly, proanabolic therapeutic agents could have similar effects, via hormone mediation. It was therefore relevant to use cultured oral periosteal fibroblasts, which have considerable capacity for matrix induction, in order to demonstrate hormone-mediated anabolic effects of dexamethasone. In addition to being a novel concept, gingival and oral periosteal fibroblasts have not previously been used in this context.

Human and animal studies show that androgens are actively metabolized in gingival tissues; the presence of inflammation can increase the activity of the enzymes concerned with the metabolic conversion of these hormones. When testosterone was incubated with homogenate, mitochondrial, microsomal and soluble fractions of healthy and inflamed gingiva from humans of both sexes, the metabolic activity was higher in the preparation from inflamed tissue than in the samples from healthy gingiva (Ojanotko et al., 1980; Sooriyamoorthy and Gower, 1989b). In both types of tissue, testosterone was converted to 5α-dihydrotestosterone, suggesting that gingiva might be a target tissue for androgens.

Our aim now was to demonstrate the modulatory effects of dexamethasone on the androgen metabolic pathway in cultured gingival and periosteal fibroblasts, because of its possible applications in regenerative therapy of the chronically inflamed periodontium.

2. Materials and methods

2.1. Chemicals

Radiolabeled androgens, [^{14}C]testosterone and [^{14}C]4-androstenedione (spec. act. 58 μCi/μmol), were obtained from Amersham International, Amersham, Bucks, UK. Organic solvents (benzene, acetone) for thin-layer chromatography, ethyl acetate for extraction of metabolites and chloroform to redissolve the dried extract were all provided by Merck Ltd., Dagenham, Essex, UK. The incubation medium was Eagle's minimum essential medium, L-glutamine, antibiotic solution (penicillin and streptomycin) and sodium bicarbonate, which were all provided by Gibco Ltd., Paisley, Scotland. Dexamethasone used in the incubations were obtained from Sigma Chemicals Co., Poole, Dorset, UK.

2.2. Culture techniques and analysis for androgen metabolites

Gingival tissue was obtained from six periodontal patients aged 30–50 years attending the Department of Periodontology, King's College (GKT) Dental Institute, London, UK. Local ethical approval and patient consent were obtained. The patients presented with periodontal pocket probing depths of 6–8 mm and chronically inflamed gingiva that bled on probing. Following initial periodontal treatment comprising complete debridement of root surfaces, gingival tissue was obtained during surgical procedures for the elimination of deep pockets. Oral periosteal tissue was taken from four patients aged 20–40 years during mucogingival surgery after the completion of initial treatment for chronically inflamed gingiva, as described above. The tissue (2 mm^3) was taken from the surface of the bone during periosteal fenestration.

Although gingival tissues from healthy males metabolized testosterone better than those of healthy females, chronically inflamed gingiva from both sexes showed no difference in testosterone metabolism (Ojanotko et al., 1980; Sooriyamoorthy and Gower, 1989a). Based on this evidence, the present study sample was not categorized by sex, but the samples were not pooled, maintaining individual cultures; sample numbers include males and females.

Gingival and oral periosteal tissues were minced into small fragments, approx. 1 mm^3, and gingival/periosteal fibroblasts were established in primary culture in 25 cm^2 tissue-culture flasks. Primary cultures were serial passaged by partial digestion with 0.25% trypsin. Fibroblasts of the fourth–ninth passage in confluent monolayer culture were used in the experiments. The contents of a fully confluent 25-cm^2 flask (2.2×10^6 cells) were distributed into 24 wells of a multiwell dish for each cell line of gingival ($n = 6$) and oral periosteal ($n = 4$) fibroblasts. The cells were allowed to become fully confluent in the dishes before any experimentation to overcome mitogenic effects (Kahari et al., 1987).

Cell lines were not pooled and duplicate incubations were performed for each individual gingival and periosteal fibroblast cell line in Eagle minimum essential medium, using [^{14}C]testosterone/[^{14}C]4-androstenedione as substrate (0.025 and 0.01 μCi/ml, respectively) and serial concentrations of dexamethasone (0.5–50 μg/ml). After an incubation period of 24 h in a humidified cell-culture incubator at 37°C, the medium was solvent-extracted with ethyl acetate (2 ml × 2) with added cold steroid standards. The extract was

evaporated to dryness, redissolved in chloroform, spotted on thin-layer chromatographic plates and the metabolites were separated in a benzene:acetone solvent system (4:1 v/v). The separated metabolites were then quantified using a radioisotope scanner.

2.3. Confirmation of the identity of metabolites

The identity of the formed metabolites was confirmed by using the mobility of cold standards added to the samples and disclosing them in iodine. Further confirmation of the authenticity of steroid metabolites was established by gas chromatography–mass spectrometry.

2.4. Characterization of dihydrotestosterone by gas chromatography–mass spectrometry

As 5α-dihydrotestosterone is the most significant biologically active metabolite in stimulating fibroblast matrix synthetic activity (Normington and Russell, 1992), it was characterized as follows. Several incubations were performed with human gingiva and unlabelled testosterone (10^{-6} mol/l). After extraction, the identity of 5α-dihydrotestosterone as a metabolite in the dried extracts was confirmed by combined gas chromatography–mass spectrometry (courtesy of Professor A.I. Mallet, St. Thomas' Hospital, London, UK). The derivatized biological material as the pentafluorobenzyloxime trimethylsilylether had a molecular ion (557) and mass-spectral fragmentation pattern identical to those of the authentic pentafluorobenzyloxime trimethylsilylether ether of 5α-dihydrotestosterone, but at lower levels, due to smaller concentrations of steroid.

Characteristic ions were noted: for example, at *m/z* values of 542 $[M\text{-}15]^+$ due to loss of a methyl group; 467 $[M\text{-}90]^+$ due to loss of trimethylsilylether; 452 $[M\text{-}90\text{-}15]^+$ due to loss of trimethylsilylether plus a methyl group 360, due to loss of the pentafluorobenzyloxime group. All these procedures have been described in detail elsewhere (Soory, 1995).

2.5. Statistical analysis

Means were obtained for each of the analytes isolated from incubations of six gingival and four oral periosteal cell lines in duplicate. The data represent means and S.D. One-way ANOVA was used for significance testing.

3. Results

3.1. Effects of serial concentrations of dexamethasone on the metabolism of testosterone by gingival fibroblasts

The main metabolites formed from [^{14}C]testosterone were 5α-dihydrotestosterone and 4-androstenedione. Dexamethasone stimulated dihydrotestosterone synthesis from [^{14}C]testosterone in gingival fibroblasts at most concentrations (Fig. 1). The increases were more marked in response to the lower concentrations. The maximum increase in dihydrotestosterone synthesis was 60% ($n = 6$; $P < 0.01$) at dexamethasone concentrations of 1, 3, 5 and 8 μg/ml, declining to control values at 40 and 50 μg/ml.

Fig. 1 also shows 4-androstenedione synthesis from [^{14}C]testosterone in gingival fibroblasts in response to the same concentrations of dexamethasone. There were 52–60% increases in the formation of 4-androstenedione in response to dexamethasone at concentrations of 1 and 3 μg/ml ($n = 6$; $P < 0.005$, one-way ANOVA), reaching control values at concentrations of 10 and 15 μg/ml. There was 36–44% inhibition at 40 and 50 μg/ml.

3.2. Effects of serial concentrations of dexamethasone on the metabolism of 4-androstenedione by gingival fibroblasts

Fig. 2 shows the metabolic conversion of 4-androstenedione to dihydrotestosterone and testosterone in response to

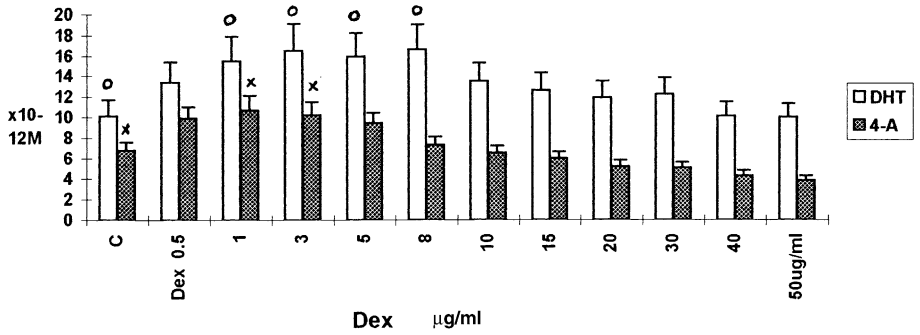

Fig. 1. Effects of serial concentrations of dexamethasone (Dex) on the metabolic conversion of [^{14}C]testosterone to dihydrotestosterone (DHT) and 4-androstenedione (4-A) in gingival fibroblasts. Duplicate incubations were performed with radiolabeled testosterone and serial concentrations of Dex; the means derived from duplicate control and test incubations of six gingival cell lines are shown. C, control; significant increases in the two metabolites between control and test incubations are shown with symbols (○), (×).

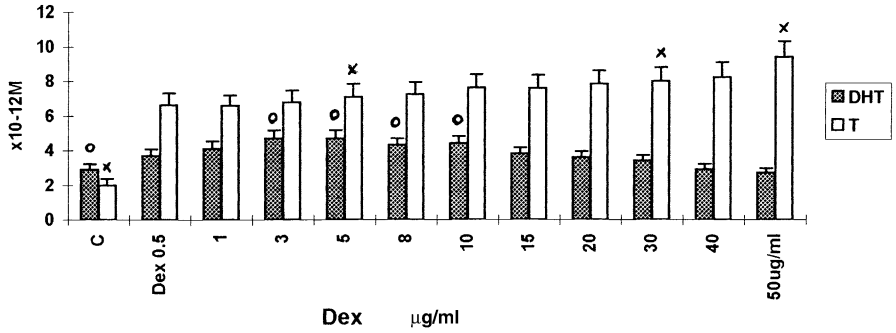

Fig. 2. The metabolic conversion of [^{14}C]4-androstenedione to dihydrotestosterone (DHT) and testosterone (T) in response to serial concentrations of dexamethasone (Dex) in gingival fibroblasts. Similar experiment to that described in Fig. 1, using [^{14}C]4-androstenedione as substrate. C, control; (○), (×), significant increases in the two metabolites between control and test incubations.

dexamethasone in gingival fibroblast cultures. There were increases in dihydrotestosterone synthesis in response to most concentrations of dexamethasone studied, decreasing to control values at 40 and 50 μg/ml. The above increases in dihydrotestosterone synthesis were maximal at 3, 5, 8 and 10 μg/ml dexamethasone (52–62% increases over the control value, $P < 0.01$; one-way ANOVA).

Testosterone synthesis from [^{14}C]4-androstenedione was very markedly increased in response to all concentrations of dexamethasone. There were 3.6-, 4- and 4.8-fold increases at 5, 30 and 50 μg/ml, respectively ($n = 6$; $P < 0.001$; one-way ANOVA).

3.3. Effects of serial concentrations of dexamethasone on the metabolism of [^{14}C]testosterone by oral periosteal fibroblasts

When periosteal fibroblasts were incubated with radiolabeled testosterone, the main metabolites formed were 5α-dihydrotestosterone and 4-androstenedione. There was a two-fold increase in the formation of dihydrotestosterone at a dexamethasone concentration of 3 μg/ml (Fig. 3; $n = 4$; $P < 0.01$; one-way ANOVA), declining to values similar to those of controls at 50 μg/ml. There was a 20% increase in the formation of 4-androstenedione at 0.5 and 1 μg/ml ($P < 0.01$), decreasing to greater than two-fold inhibition at 30, 40 and 50 μg/ml.

3.4. Effects of serial concentrations of dexamethasone on the metabolism of [^{14}C]4-androstenedione by oral periosteal fibroblasts

When [^{14}C]4-androstenedione was used as substrate with oral periosteal fibroblasts, there was a 20% increase in the formation of 5α-dihydrotestosterone in response to dexamethasone at 3 and 5 μg/ml ($n = 4$; $P < 0.01$), with 28% inhibition at 40 and 50 μg/ml (Fig. 4; $n = 4$; $P < 0.01$). Dexamethasone significantly stimulated the synthesis of testosterone at all concentrations tested, with a four-fold increase at 8, 10, 15, 20, 30, 40 and 50 μg/ml ($n = 4$; $P < 0.001$; one-way ANOVA).

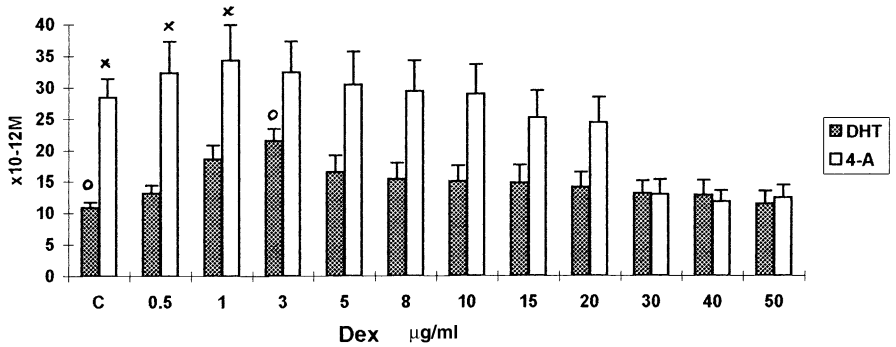

Fig. 3. The metabolic conversion of [^{14}C]testosterone in oral periosteal fibroblasts in response to serial concentrations of dexamethasone (Dex). C, control; (○), (×), significant increases in the two metabolites between control and test incubations. Other abbreviations as in Fig. 1.

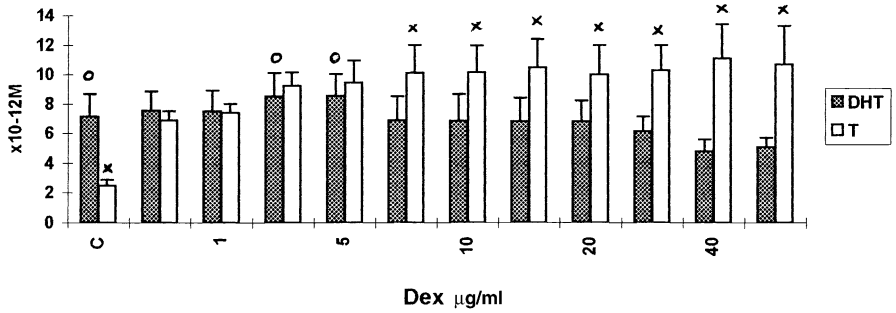

Fig. 4. The metabolic conversion of [^{14}C]4-androstenedione in oral periosteal fibroblasts in response to serial concentrations of dexamethasone. C, control; (○), (×), significant increases in the two metabolites between control and test incubations. All other abbreviations as in earlier figures.

4. Discussion

Human gingival and oral periosteal fibroblasts metabolized both the androgen substrates effectively. In gingival fibroblasts there was significant stimulation of 5α-reductase and 17β-hydroxysteroid dehydrogenase activity at lower concentrations of dexamethasone, with decreased yields of dihydrotestosterone and 4-androstenedione at higher concentrations, resulting in some inhibition of 4-androstenedione synthesis. In these cultures, significantly high yields of testosterone were obtained from 4-androstenedione as substrate.

In response to dexamethasone, periosteal fibroblasts demonstrated significantly high yields of dihydrotestosterone and testosterone from the substrates [^{14}C]testosterone and [^{14}C]4-androstenedione, respectively, while the degree of stimulation in the yields of 4-adrostenedione from [^{14}C]testosterone and dihydrotestosterone from [^{14}C]4-androstenedione was less pronounced. The role of dexamethasone in mediating hormonal mechanisms of repair appears to be associated with the formation of physiologically active androgen metabolites with anabolic effects. Although in vitro experimental results may not reproduce in vivo activity, certain trends established in vitro may be applicable to the in vivo state. A time-response kinetic study would provide more comprehensive information on the fate of some metabolites due to further enzyme action.

However, it can be suggested that the effect of dexamethasone on both 5α-reductase and 17β-hydroxysteroid dehydrogenase activity is dose-dependent in relation to different androgen substrates. The effects of dexamethasone on connective tissues have been demonstrated by others (reviewed by Sooriyamoorthy and Gower, 1989a), indicating that corticosteroids exert dichotomous effects on connective tissue in monolayer cultures. Cell growth was stimulated at low corticosteroid dosages while higher doses were inhibitory. Similar results have been reported by other workers for prednisolone, another important synthetic corticosteroid (reviewed by Sooriyamoorthy and Gower, 1989a). Our investigation demonstrates dose-dependent yields of androgen metabolites in response to dexamethasone in cultured gingival and periosteal fibroblasts, which to the best of our knowledge have not been demonstrated previously.

These results reinforce knowledge of target-tissue androgen metabolism in human gingiva, in relation to their ability to metabolize steroid hormones (Sooriyamoorthy et al., 1988). The biologically active metabolite 5α-dihydrotestosterone can contribute to growth and development. For example, it stimulates matrix synthesis in connective tissue and bone (Colvard et al., 1989; Kasperk et al., 1989; Normington and Russell, 1992; Dassouli et al., 1994). Such anabolic effects are more obvious when the normal synthesizing capacity of tissues is reduced; when a reparatory response is required, dihydrotestosterone can contribute to synthetic activity in fibroblasts and osteoblasts (Vittek et al., 1979; Kasperk et al., 1989; Sooriyamoorthy and Gower, 1989a). The expression of androgen receptors has been detected in a high proportion of periodontal and gingival tissues; also in fibroblasts derived from the same source (Parkar et al., 1996), implying target-tissue action in these tissues.

There could be proanabolic interactions of dexamethasone with androgens in enhancing their effects on connective tissue and bone matrices; this is a novel concept, with possible applications. Interactions of dexamethasone with cartilage-derived growth factor, stimulating DNA synthesis in Swiss mouse 3T3 cells (Levenson et al., 1985), and also synergistic actions of dexamethasone with platelet-derived growth factor, inducing human diploid periodontal ligament and gingival fibroblast proliferation in vitro (Rutherford et al., 1993), have been demonstrated. In view of our present findings, some of those actions may be hormone-mediated, with implications for wound healing and regeneration of the inflamed periodontium in vivo.

The therapeutic combination of platelet-derived growth factor, dexamethasone and a carrier matrix induces the formation of more new cementum, periodontal ligament and supracrestal bone in periodontal lesions in monkeys (Rutherford et al., 1992). In cultured skin fibroblasts from mice, a striking induction of connective tissue growth factor

expression was observed after dexamethasone treatment, and this occurred in a dose-dependent manner (Dammeier et al., 1998). Thus dexamethasone, singly or in combination with other agents that enhance repair, could play an important part in the regeneration of tissues in inflammatory periodontal lesions.

In a recent investigation we demonstrated that androgen metabolism in gingival fibroblasts derived from a chronically inflamed source was inhibited by the specific alkaline phosphatase inhibitor levamisole, indicating a link between androgen action and protein turnover mediated by alkaline phosphatase (Tilakaratne and Soory, 2000); dexamethasone also mediates protein synthesis in osteoblasts, which is characterized by increased alkaline phosphatase activity (Kasugai et al., 1991). This observation implies a link in the actions of androgens and dexamethasone in the mediation of protein synthesis.

Androgens and oestrogens have also been shown to trigger α_1(I) procollagen gene expression and enhance the expression of transforming growth factor-β mRNA (Benz et al., 1991). Dihydrotestosterone has been shown to increase trabecular bone volume in the mandibular condylar head; the differentiation of osteoblasts is associated with stimulation of collagenous protein synthesis and increased activity of creatine kinase and possible effects on alkaline phosphatase, which are not always consistent (Benz et al., 1991). It may be that, in the androgen metabolic pathway, the induction of 5α-reduction and 17β-hydroxysteroid dehydrogenase activity by dexamethasone could be partly mediated by the induction of connective tissue growth factor, transforming growth factor-β or other growth factors in these cells, resulting in a ligand-independent pathway for androgen metabolism. A ligand-dependent pathway with nuclear activation of androgen metabolism by dexamethasone via its own receptors is also a possibility.

However, the catabolic effects of dexamethasone, such as decreasing newly synthesized endothelial cell fibronectin, resulting in weak endothelial cell–substratum adhesion (Romer and Polin, 1995), could have been due to the difference in concentrations of dexamethasone used. Dexamethasone in cultured fibroblasts showed reduced collagen production in the absence, or with low concentrations, of insulin-like growth factor-1, but potentiated collagen production in cells stimulated with higher concentrations (Bird and Tyler, 1994). This finding demonstrates the influence of other factors and their concentrations on the effects of dexamethasone, which might be applicable to the chronically inflamed periodontium in vivo. The reported dose-dependent actions of dexamethasone in collagen studies reinforce our results with regard to androgen metabolism.

Thus dexamethasone could give rise to important anabolic functions in tissues of the periodontium in a dose-dependent manner, resulting from the androgen metabolites formed. This effect could have implications for the judicious topical usage of dexamethasone in adjunctive regenerative therapy of inflammatory periodontal disease.

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