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METABOLISM OF PROGESTERONE BY HEALTHY AND INFLAMED HUMAN GINGIVA *IN VITRO*

ANITA OJANOTKO-HARRI
Department of Physiology, University of Turku, Kiinamyllynkatu 10, SF-20520 Turku, Finland

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Summary—[4-^{14}C]Progesterone was incubated with homogenate and mitochondrial, microsomal and soluble fraction preparations of healthy and inflamed gingiva from human subjects of both sexes. The subcellular preparations were supplemented with an NADPH-regenerating system and incubated for 2 h at pH 7.4 and 37°C. The metabolites were identified by column, multiple TLC and radioautography and quantified with liquid scintillation counting. In inflamed tissue the metabolic activity was higher than in healthy gingiva. On the basis of the identified metabolites it can be concluded that the human gingiva of both sexes contains marked 3α-, 3β- and 20α-hydroxysteroid dehydrogenase, Δ^4-5α- and Δ^4-5β-steroid hydrogenase activities, and less 20β-hydroxysteroid dehydrogenase activity.

INTRODUCTION

The role of steroid hormones in the physiology of gingival tissue has been only partially elucidated. It is known that physiological changes in the amounts of steroid hormone affect the gingiva, for instance during puberty and pregnancy [1, 2]. Gingival inflammations are known to be aggravated during these physiological conditions. Changes in the concentrations of steroids also influence the vascular permeability of healthy gingivae [3]. Different opinions about the action of ovarian hormones on the gingiva after the menopause have been reported [4–6]. Many women complain of having gingival troubles of uncertain nature, possibly similar to the changes in other mucosal surfaces (the esophagus, female reproductive tract, etc.).

Gingivae have also been shown to be target organs for androgens and estrogens. Southern *et al.*[7] found dihydrotestosterone receptors, and Bashirelahi *et al.*[8] and Vittek *et al.*[9] 17β-estradiol receptors in gingivae. Gingival inflammations are known to enhance the metabolism of androgens and estrogens [10–12] in this tissue 2 to 3-fold as compared to healthy gingiva.

Mohamed[13] has shown that intramuscularly injected radioactive progesterone concentrates in the stroma of the gingival connective tissue, less in the cytoplasm of fibroblasts, occasionally in endothelial cells and not at all in the epithelium. Later Vittek *et al.*[14] in their preliminary work reported the existence of putative progesterone receptors in human gingiva. The metabolism of progesterone is also reported to be enchanced by gingival inflammations, as has been shown by El Attar *et al.*[15] with frozen gingival slices, and Harri and Ojanotko[16] with healthy and inflamed female gingiva.

The aim of the present study was to elucidate the exact metabolism of progesterone in homogenate and subcellular fractions of the human gingiva and the role of gingival inflammations in its metabolism.

EXPERIMENTAL

Tissues and their preparation

Samples of healthy gingiva were excised from the soft tissue covering unerupted teeth. The samples were obtained from the gingivae of 7 women aged 14–39 years, and 4 men aged 24–57 years. Samples of inflamed gingiva from 10 women aged 10–64 years and 6 men aged 15–51 years, were excised from patients operated on for mild-to-severe periodontitis or gingival hyperplasia after hydantoin medication. Before the samples were excised, the subjects rinsed their mouths for 1 min with a 0.2% solution of chlorhexidine gluconate. The degree of inflammation of the samples was determined by inspection and histologically. Only samples of clinically healthy gingivae with no or little histological inflammation were used as healthy control samples. The female samples were not grouped according to menstrual phases. Pregnant females or persons with ongoing hormonal therapy were excluded. The samples which weighed from 30 to 500 mg were placed in cold buffer solution (pH 7.4) containing 0.067 M KH_2PO_4–Na_2HPO_4, 1.0 mM EDTA and 0.25 M sucrose. The subcellular preparations (homogenate, mitochondria, microsomes, soluble fraction) were obtained by homogenization and differential centrifugation as described earlier [10].

Radioactive steroids

[4-^{14}C]Progesterone (sp. act. 51 Ci/mol) was purchased from New England Nuclear, Boston, MA, U.S.A. It was purified before use with bidimensional TLC on silica gel using methyl acetate–dichlorethane

Table 1. Metabolism of progesterone (sum of all metabolites) by homogenate and subcellular fractions of healthy and inflamed female and male gingiva (nmol of metabolized substrate/h/g of tissue ± SEM)

	Healthy		Inflamed	
Cellular preparation	Female	Male	Female	Male
Homogenate	2.03 ± 0.42 ($n = 7$)	2.81 ± 0.30 ($n = 4$)	5.71 ± 0.51 ($n = 10$)	4.80 ± 0.50 ($n = 6$)
Mitochondrial fraction	0.15 ± 0.05 ($n = 6$)	0.10 ± 0.01 ($n = 3$)	0.82 ± 0.27 ($n = 8$)	0.24 ± 0.07 ($n = 6$)
Microsomal fraction	1.16 ± 0.05 ($n = 6$)	0.07 ± 0.03 ($n = 3$)	0.26 ± 0.06 ($n = 7$)	0.29 ± 0.17 ($n = 6$)
Soluble fraction	1.72 ± 0.40 ($n = 6$)	1.42 ± 0.15 ($n = 3$)	5.73 ± 0.40 ($n = 7$)	3.43 ± 1.11 ($n = 5$)

(1:4, v/v) as the first and hexanol–hexane (7:13, v/v) as the second solvent system.

Incubations

The incubations were carried out in the phosphate buffer solution mentioned above but without sucrose. An NADPH-regenerating system consisting of 2.3 μmol of NADP, 18.8 μmol of glucose-6-phosphate and 3 units of glucose-6-phosphate dehydrogenase (Boehringer, Mannheim GmbH, Mannheim, West Germany) was used. The reaction was started by adding 2 to 4 nmol of substrate, dissolved in 1 ml of the buffer solution. The samples were incubated aerobically for 2 h at 37°C in a shaking water bath. The reaction was stopped with 50 ml of absolute ethanol.

Extraction and chromatography of steroids

The incubation mixtures were filtered and the precipitates washed with 100 ml of ethanol. A 0.8 ml vol of 1-octanol was added and the filtrate was evaporated until all the solvents except the octanol were evaporated. The filtrate was then fractionated into the main metabolite groups (lipids, free steroids, steroid conjugates) with Florisil columns [10].

The free steroid fractions were chromatographed bidimensionally on TLC silica gel plates [17]. After adding authentic non-radioactive standard steroids the first direction was run with methyl acetate–dichloroethane (1:4, v/v) and the second with hexanol–hexane (1:3, v/v). For the identification of the metabolites that did not separate sufficiently in this system, the areas containing these metabolites were scraped off the still wet plates and the metabolites rechromatographed with *t*-butanol–hexane (3:7, v/v). The TLC plates were radioautographed with X-ray films (exposure time about 30 days). The radioactive spots on the films were identified by comparing them with authentic standards on plates stained with ethanol–acetic anhydride-sulphuric acid. The radioactivity on the analyzed spots was determined by liquid scintillation counting. These methods have been described in detail earlier [18]. Most of the reference steroids were gifts from Professor D. N. Kirk, the M.R.C. Steroid Reference Collection, Westfield College, London.

Statistical analysis

The reliability and reproducibility of the steroid analysis method are good (*Hartiala* **19**, p. 35). The statistical analysis of the results was performed with Student's *t*-test.

RESULTS

The total metabolism of added progesterone by the various subcellular preparations of the human gingiva is presented in Table 1. The metabolic activity of the homogenates was highly significantly ($2P < 0.001$) greater with the inflamed than with the healthy samples; the difference was almost significant ($2P < 0.05$) in the soluble fraction incubations; there was only a trend ($2P < 0.1$) in the mitochondrial incubations. The difference in the metabolism in the microsomal fraction incubations was not statistically significant. The difference between the female healthy and inflamed samples was statistically greater than the corresponding difference be-

Table 2. Metabolites of progesterone identified in homogenate incubations of healthy and inflamed female and male gingiva (pmol/h/g of tissue ± SEM)

	Healthy		Inflamed	
Metabolite	Female ($n = 7$)	Male ($n = 4$)	Female ($n = 10$)	Male ($n = 6$)
20α-Hydroxy-4-pregnen-3-one	1076 ± 316	1904 ± 237	2828 ± 434	2757 ± 533
5α-Pregnane-3,20-dione	510 ± 92	439 ± 151	880 ± 161	979 ± 237
5β-Pregnane-3,20-dione	20 ± 16	52 ± 46	125 ± 73	78 ± 40
3α-Hydroxy-5α-pregnan-20-one	52 ± 24	33 ± 9	291 ± 177	294 ± 180
3β-Hydroxy-5α-pregnan-20-one	80 ± 18	140 ± 45	459 ± 131	426 ± 115
20α-Hydroxy-5α-pregnan-3-one	96 ± 33	115 ± 52	444 ± 86	229 ± 58
20β-Hydroxy-5α-pregnan-3-one / 3α-Hydroxy-5β-pregnan-20-one	29 ± 10	21 ± 16	115 ± 57	104 ± 62
5α-Pregnane-3α,20α-diol	16 ± 10	17 ± 2	86 ± 29	74 ± 46
5α-Pregnane-3β,20α-diol	3 ± 3	3 ± 3	71 ± 29	17 ± 8
5β-Pregnane-3α,20α-diol / 5β-Pregnane-3α,20β-diol	2 ± 2	8 ± 4	41 ± 17	23 ± 18
"More polar" metabolites	92 ± 19	72 ± 20	321 ± 68	211 ± 34
"Less polar" metabolites	40 ± 13	3 ± 3	54 ± 22	121 ± 60

Table 3. Metabolites of progesterone identified in mitochondrial fraction incubations of healthy and inflamed female and male gingiva (pmol/h/g of tissue ± SEM)

Metabolite	Healthy		Inflamed	
	Female ($n = 6$)	Male ($n = 3$)	Female ($n = 8$)	Male ($n = 6$)
20α-Hydroxy-4-pregnen-3-one	21 ± 5	45 ± 3	65 ± 3	97 ± 16
5α-Pregnane-3,20-dione	38 ± 12	15 ± 8	493 ± 198	52 ± 16
5β-Pregnane-3,20-dione	14 ± 11	0.0	4 ± 4	7 ± 7
3α-Hydroxy-5α-pregnan-20-one	0.0	0.0	20 ± 13	0.0
3β-Hydroxy-5α-pregnan-20-one	3 ± 3	0.0	46 ± 16	6 ± 4
20β-Hydroxy-5α-pregnan-3-one + 3α-Hydroxy-5β-pregnan-20-one	0.0	0.0	27 ± 27	0.0
"More polar" compounds	66 ± 37	12 ± 4	93 ± 42	80 ± 54
"Less polar" compounds	12 ± 9	12 ± 6	61 ± 31	6 ± 6

tween the male samples. However, statistically significant differences between the sexes could not be detected (see Table 1).

The individual metabolites of the progesterone found after the homogenate, mitochondrial, microsomal and soluble fraction incubations are presented in Tables 2–5. Some metabolites, viz. 20β-hydroxy-5α-pregnan-3-one and 3α-hydroxy-5β-pregnan-20-one, and also 5β-pregnane-3α,20α-diol and 5β-pregnane-3α,20β-diol, are quantified together because of partial overlapping in the chromatography plates. In the homogenate incubations more of 20β-hydroxy-5α-pregnan-3-one was usually found than of 3α-hydroxy-5β-pregnan-20-one. After the soluble fraction incubations more of the latter was present. Several metabolites designed as "more polar" were quantified together. Their area on the silica gel plates contains, according to earlier studies [17], the steroid metabolites with at least three keto and/or hydroxy groups, e.g. monohydroxylated diketopregnanes. One spot was cochromatographed with authentic 3α,6α-dihydroxy-5β-pregnan-20-one in several TLC systems. The others have not been identified because of the unavailability of reference steroids. The group of "less polar" metabolites usually consisted of two unidentified derivates. They were found after bidimensional TLC in the general area of steroid acetates.

DISCUSSION

The results indicate that the metabolism of pro-

Table 4. Metabolites of progesterone identified in microsomal fraction incubations of healthy and inflamed female and male gingiva (pmol/h/g of tissue ± SEM)

Metabolite	Healthy		Inflamed	
	Female ($n = 6$)	Male ($n = 3$)	Female ($n = 7$)	Male ($n = 6$)
20α-Hydroxy-4-pregnen-3-one	18 ± 6	27 ± 19	37 ± 9	35 ± 12
5α-Pregnane-3,20-dione	44 ± 17	35 ± 4	100 ± 35	56 ± 24
5β-Pregnane-3,20-dione	22 ± 17	0.0	18 ± 14	24 ± 24
3α-Hydroxy-5α-pregnan-20-one	3 ± 3	0.0	3 ± 3	3 ± 2
3β-Hydroxy-5α-pregnan-20-one	0.0	0.0	6 ± 6	5 ± 5
20α-Hydroxy-5α-pregnan-3-one	3 ± 3	0.0	0.0	0.0
20β-Hydroxy-5α-pregnan-3-one + 3α-Hydroxy-5β-pregnan-20-one	0.0	0.0	28 ± 19	0.0
"More polar" compounds	61 ± 38	13 ± 7	72 ± 19	182 ± 162
"Less polar" compounds	3 ± 3	0.0	7 ∓ 4	0.0

Table 5. Metabolites of progesterone identified in soluble fraction incubations of healthy and inflamed female and male gingiva (pmol/h/g of tissue ± SEM)

Metabolite	Healthy		Inflamed	
	Female ($n = 6$)	Male ($n = 3$)	Female ($n = 7$)	Male ($n = 5$)
20α-Hydroxy-4-pregnen-3-one	1137 ± 316	1365 ± 181	3740 ± 920	3166 ± 1086
5α-Pregnane-3,20-dione	6 ± 4	19 ± 12	19 ± 8	10 ± 5
5β-Pregnane-3,20-dione	60 ± 26	0.0	62 ± 24	39 ± 15
3α-Hydroxy-5α-pregnan-20-one	3 ± 3	0.0	7 ± 7	3 ± 3
3β-Hydroxy-5α-pregnan-20-one	3 ± 3	0.0	14 ± 8	9 ± 6
20β-Hydroxy-5α-pregnan-3-one + 3α-Hydroxy-5β-pregnan-20-one	363 ± 246	19 ± 12	409 ± 218	91 ± 16
5α-Pregnane-3α,20α-diol	0.0	0.0	9 ± 5	5 ± 5
5β-Pregnane-3α,20α-diol + 5β-Pregnane-3α,20α-diol	93 ± 68	5 ± 5	116 ± 63	27 ± 23
"More polar" compounds	40 ± 13	12 ± 6	90 ± 61	77 ± 39
"Less polar" compounds	15 ± 19	0.0	71 ± 69	0.0
5β-Pregnane-3α,20α-diol + 5β-Pregnane-3α,20β-diol	0.0	0.0	9 ± 5	5 ± 5
"More polar" compounds	93 ± 68	5 ± 5	116 ± 63	27 ± 23
"Less polar" compounds	15 ± 19	0.0	71 ± 69	0.0

gesterone in human gingival preparations is increased by inflammation. In homogenate and soluble fraction incubations the increase of the metabolism is about 2 to 3-fold. The increase is larger in female than in male gingiva. With other subcellular preparations the trend was also the same. The progesterone metabolism by human mitochondria, microsomes and soluble fraction has not been reported earlier. The influence of an inflammation is even more pronounced with testosterone, as the healthy gingiva of females does not metabolize testosterone appreciably [10].

The results indicate that the human gingiva contains at least 3α-, 3β-, 20α- and 20β-hydroxysteroid dehydrogenases, Δ^4-5α- and Δ^4-5β steroid hydrogenases and probably also steroid hydroxylases. The gingival tissue is thus capable of reductive metabolism that leads to inactivation of steroid hormones. No statistically significant metabolic difference in the overall metabolism could be detected between the sexes, as could be done with the oral mucosa of rats [20]. Neither were statistically significant differences found between the sexes in the activities of the enzymes in human gingiva.

Compared with the results of El Attar *et al.*[15] with frozen human gingival slices interesting differences can be seen. Methodological differences might account for most of them; for instance, the incubation time in their study was 3 times longer than in this study. Their main metabolite was 20α-hydroxy-4-pregnen-3-one, the amount of which increased 4-fold in their experiments (in this study the increase was 2-fold). In addition they found 5α-pregnane-3,20-dione only in healthy and 5β-pregnane-3,20-dione only in inflamed samples, while in the present study both of these metabolites were found after incubations with both healthy and inflamed gingivae. In addition El Attar *et al.* found three unknown metabolites. In this study, all metabolites except some of the "more polar" or "less polar" groups, were identified in both healthy and inflamed gingiva, with larger amounts in inflamed gingiva. The main metabolites are 20α-hydroxy-4-pregnen-3-one, 5α-pregnane-3,20-dione, 3β-hydroxy-5α-pregnan-20-one, 20α-hydroxy-5α-pregnan-3-one, and some of the "more polar" metabolites. At least one of the main metabolites, viz. 20α-hydroxy-4-pregnen-3-one, is still an active progestational agent. In soluble fraction incubations there are more 5β-derivates than in homogenate incubations. This is also the case elsewhere in the alimentary canal [18].

Vittek *et al.*[22] have demonstrated a positive correlation between the level of plasma progesterone, the degree of gingival inflammation and the Δ^4-5α-steroid hydrogenase of human gingiva. This phenomenon might explain why the difference between a healthy and an inflamed gingiva is greater in the female than in the male. The fact that the amount of all metabolites increases, could possibly be explained by assuming the enhancement of some enzyme or enzymes at the start of the metabolic pathway. One likely enzyme could be the Δ^4-5α-steroid hydrogenase. Lindhe *et al.*[22] have noticed that there is a peak in the gingival exudation in all females studied on the day of ovulation and they also found that the fluctuation was most pronounced with pre-existing gingivitis.

Steroid hormones have significance in gingival physiology and it seems that they have different effects on different kinds of gingival cells. Vittek *et al.* [9] have demonstrated in dry autoradiographic experiments with gingiva a specific nuclear localization of [^{3}H]estradiol predominantly in the basal and the spinous cell layer of the gingival epithelium, and also in connective tissue cells (fibroblasts) and endothelial cells and pericytes of small blood vessels of the lamina propria. A similar distribution of progesterone has been found by Mohamed[13] in rabbit gingiva. Later Vittek *et al.*[23] also demonstrated specific progesterone receptors in rabbit gingiva. The existence of steroid hormone receptors is thought to mean some specific function of the steroid in the target tissue, e.g. synthesis of a specific protein. Many histological and morphological experiments with gingiva have shown that estradiol and progesterone have special effects for instance on the gingival vessels.

No direct experiments with the different cell types of the gingiva have been done in order to find out their roles in gingival steroid metabolism. It has been shown [24] that human leukocytes metabolize progesterone to 5α-pregnane-3,20-dione, 20α-hydroxy-4-pregnen-3-one and to pregnanolone-like derivatives (presumably 3α-hydroxy-5α-pregnan-20-one and 3β-hydroxy-5α-pregnan-20-one). Both the mononuclear and the polymorphonuclear leukocytes produced each of the found metabolites, though the polymorphonuclear leukocytes produced more of the pregnanolone-like derivates. All these metabolites were produced by gingival tissue in the present study. It is thus conceivable that some of the metabolism observed here is due to the leukocytes especially in the inflamed gingiva. An acutely inflamed gingiva contains more polymorphonuclear leukocytes, while in a chronically inflamed gingiva monocytes predominate [25].

The metabolism possibly due to gingival microorganisms must be very low for several reasons. Certain oral bacteria can metabolize steroids, but the conversion rate per mg of bacterial material is small [26–28]. With the small tissue sample sizes used in the present study, the amount of contaminating bacteria is negligible. In addition, the mouth rinse with chlorhexidine is known to reduce the number of oral bacteria by 95% [29]. The metabolism that might result from bacterial contamination of the incubation vessels is not included in the results, as the very small background metabolism obtained with no added tissue was always subtracted from the results.

The results seem to indicate that the metabolism of progesterone, as that of other steroids, is different in

healthy and inflamed gingiva. The exact mechanism of this difference remains to be elucidated.

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