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# PROGESTERONE METABOLISM BY MAJOR SALIVARY GLANDS OF RAT—II. PAROTID GLAND

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**Summary**—The metabolism of [4-$^{14}$C]progesterone in the parotid salivary glands of nonpregnant female, pregnant female and male rats was investigated *in vitro*. The metabolic activity of the male rats was significantly lower than in either of the female groups. The pregnant group was metabolically more active than the nonpregnant female group, but this difference was not statistically significant.

In homogenates and soluble fractions the main metabolite was 20α-hydroxy-4-pregnen-3-one in female rats. In male rats the main metabolites were 20α-hydroxy-4-pregnen-3-one and 3α-hydroxy-5α-pregnan-20-one in homogenates and 20α-hydroxy-4-pregnen-3-one in soluble fractions. In the microsomal fractions of both sexes polar compounds predominated.

The results indicated the presence of at least the following progesterone metabolizing enzymes in rat parotid salivary glands: 3α-, 3β-, 20α- and 20β-hydroxysteroid dehydrogenase, 5α- and 5β-steroid hydrogenase and 17α-steroid hydroxylase activities. In the homogenates and soluble fractions of female rats 20α-hydroxysteroid dehydrogenase activity was significantly higher than in males.

## INTRODUCTION

The concept that the digestive tract, like the reproductive tract, is sex steroid-dependent is not new. In a variety of tissues of the digestive tract hormonal receptors for androgen and estrogen have been demonstrated, e.g. in the endocrine and exocrine pancreas, liver and salivary glands [1–5].

The androgen dependence of mice submandibular glands was first reported by Lacassagne [6]. He found that androgens are essential for the maintenance of the structure of the granular tubules, which are more numerous and prominent in males than in females. Several ductal enzymes and growth factors are under the control of androgens [7–10]. The submandibular and parotid glands of female rats have features that are typical of an estrogen-dependent organ; a specific receptor for estrogen has been demonstrated and the peroxidase activity of the glands has been shown to be estrogen-dependent [4].

The metabolism of steroids, especially that of androgens, in rodent submandibular glands is relatively well characterized [11–14]. However, little is known about the steroid metabolism in parotid glands. In pigs, whose submandibular glands show sexual dimorphism, 16-androstenes were reduced by male parotid tissue, but to a lesser extent than by corresponding submandibular tissue [15]. Flood [16] showed histochemically in pigs that hydroxysteroid dehydrogenases were present mainly in the excretory and striated ducts of the submandibular glands, and only minimal activity was found in the parotid ducts. A similar metabolism exists in the parotid glands, and in species whose submandibular glands do not show sexual dimorphism, such as dogs and humans. The parotid and submandibular glands of male dogs are able to convert testosterone to androstenedione. The submandibular glands were, however, more active than the parotids in the conversion [17]. Djøseland *et al.* [18] demonstrated that in human parotid glands 17β-hydroxysteroid dehydrogenase was the main enzyme when testosterone, dihydrotestosterone or estradiol were used as substrates and that the metabolism was mainly oxidative. In another human study, Sirigu *et al.* [19] showed histologically that the submandibular and parotid glands show 3β- and 17β-hydroxysteroid dehydrogenase activity. The enzyme activities were

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mainly demonstrable in the duct area, and to a lesser degree in the acinar cells of female glands. The authors assumed that the androgen metabolism is mainly oxidative.

The parotid gland differs anatomically from the submandibular gland. Rat parotid glands do not have granular tubules [20]. The submandibular gland is a mixed gland with serous and mucous acini. The parotid gland is an exclusively serous gland, whose acinar cells anatomically resemble those of the exocrine pancreas. The parotid and pancreatic acini are digestive in function, secreting large amounts of α-amylase. These glandular tissues are also involved in glucose metabolism, by secreting insulin and glucagon. According to Kyle [21] there is a tendency for the development of diabetes during pregnancy; the pancreatic carbohydrate metabolism may be affected by the circulating sex hormones.

We now studied the metabolism of progesterone in rat parotid glands to determine if it resembles that in the other major salivary glands and if pregnancy has any effect on this metabolism. We have earlier studied the metabolism of progesterone in rat sublingual and submandibular glands [22].

## MATERIALS AND METHODS

### *Reagents*

Analytical grade reagents were used throughout and the solvents for chromatography and extractions were redistilled before use. [4-$^{14}$C]-Progesterone with a specific activity of 51 Ci/mol was purchased from New England Nuclear, Mass, U.S.A. It was purified before use with bidimensional thin-layer chromatography on silica gel using the solvent systems methyl acetate–dichloroethane (1:4, v/v) and 1-hexanol–hexane (7:13, v/v) respectively.

### *Tissues and their preparation*

Ten nonpregnant female, nine pregnant female and ten male Long–Evans rats (Turku strain), aged 6–9 months, were used. The group "pregnant rats" was established by placing female rats overnight with males. If pregnancy occurred, the day after insemination was designated as the first day of pregnancy. The animals were killed with ether when the pregnant rats were in the 19th–21st days of pregnancy. The tissues were cleaned of adjacent tissues, rinsed with cold buffer (0.067 M $KH_2PO_4$–$Na_2HPO_4$ containing 1.0 mM EDTA and 0.25 M sucrose, pH 7.4) and weighed. The samples varied from 60 to 220 mg. The preparation of the homogenates, 100,000 *g* microsomal and soluble fractions has been described in detail earlier [22].

### *Incubations*

The incubation mixture contained 2.3 μmol NADP, 18.8 μmol glucose-6-phosphate and 3 units glucose-6-phosphate dehydrogenase (Boehringer Mannheim, Mannheim, F.R.G.). To test the cofactor demands the parotid gland homogenate was divided into five parts. The incubation mixture contained phosphate buffer without sucrose, glucose-6-phosphate and glucose-6-phosphate dehydrogenase. The incubations were performed with or without cofactor supplementation. The cofactors were either NAD, NADH, NADP or NADH with an equal amount of NADP. The NADPH is formed by the NADPH-regenerating system (NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase). The incubation conditions were the same throughout all studies (tissue concentration 10 mg/ml).

The reaction was started by adding 2–3 nmol of the radioactive progesterone dissolved in 1 ml of the buffer solution to the different preparations of the parotid glands. The total volume of the incubation mixture was 10 ml. The incubations were carried out aerobically for 30 min at 37°C in a water bath. The reaction was stopped by placing the incubation tubes in an ice bath and adding, with shaking, 4 ml of methyl acetate.

### *Identification and quantitation of the metabolites*

The methyl acetate phase (upper phase) was removed after centrifugation. The extraction with methyl acetate was repeated three times. The combined methyl acetate phases were evaporated under a stream of nitrogen.

For steroid conjugate assay 5 ml ethanol was added to the lower aqueous phase. This phase was evaporated at 100–105°C. The radioactivity of the residuum was determined with liquid scintillation counting.

After the evaporation the upper or methyl acetate phase was redissolved in 1.7 ml methanol and 0.8 ml hexane. After shaking and centrifugation the upper hexane phase was removed and examined for lipoidal metabolites. The lower methanolic phase was used for bidimensional thin-layer chromatography. A small amount of lipoidal activity was found, but only traces of steroid conjugates were detected. The

radioactivity was found mainly in the free steroid fractions and therefore these were used for further examinations. About 90% of the radioactive progesterone remained unmetabolized, confirming the linearity of the reaction.

Nonradioactive steroid standards were added to these fractions and the silica gel plates were run with bidimensional thin-layer chromatography. The solvent systems were dichlormethane–methyl acetate (9:1, v/v) and 1-hexanol–hexane (1:3, v/v).

The thin-layer chromatography plates were autoradiographed with X-ray films with an exposure time of about 30 days. The radioactive metabolites were identified by comparing the spots on the film with steroid standards on the plate, visualized with staining with ethanol–acetic anhydride– sulphuric acid. The radioactivity was determined by liquid scintillation counting.

The activities of enzymes were obtained by summing all the relevant metabolites. The minor unidentified "nonpolar" and "polar" metabolites were omitted. In cases of incomplete separation of spots, the shares of the overlapping spots were estimated both visually from the X-ray films and from the calculated activity of the nonoverlapped spots.

Two minor metabolites, 20$\beta$-hydroxy-5$\alpha$-pregnan-3-one and 3$\alpha$-hydroxy-5$\beta$-pregnan-20-one, were quantified together, since they partially overlapped on the chromatography plates. All nonpolar and 2–3 derivates of the polar metabolites remained unidentified due to the unavailability of the reference steroids. Most of the reference steroids were generous gifts from Professor D. N. Kirk, Steroid Reference Collection, Westfield College, London.

### *Statistical analysis*

The statistical analysis of the results was performed with the Wilcoxon test.

## RESULTS

The total metabolism of added progesterone by homogenates and microsomal and soluble preparations from rat parotid salivary glands is presented in Table 1. The metabolic activity of soluble fractions of nonpregnant females ($2\alpha < 0.05$) and pregnant females ($2\alpha < 0.05$) was greater than in males. In homogenates the same trend was seen ($2\alpha < 0.1$ and $2\alpha < 0.05$, respectively). Neither statistically significant differences between the sexes, nor any pregnancy-related changes in the metabolism, were observed in the other preparations.

The individual metabolites of progesterone found after the incubations are presented in Tables 2 and 3, and the suggested metabolic pathways of progesterone in rat parotid glands are presented in Fig. 1.

In view of the large amount of unidentified polar metabolites in microsomal fractions, only homogenates and soluble fractions were used for the enzyme calculations. The results are summarized in Table 4.

The unsupplemented, NAD- and NADH-supplemented incubations showed low metabolic activity compared to NADP-supplemented incubations. Additional NAD- or NADH-supplementation did not change the metabolic activity in NADP-supplemented incubations or cause qualitative differences in metabolites.

## DISCUSSION

To our knowledge, the present paper is the first on the metabolism of progesterone by rat parotid glands. The results are quite similar to those obtained from rat submandibular and sublingual glands: the 5$\alpha$-pathway predominates [22].

The conversion of progesterone demonstrates that the rat parotid gland contains at least the following steroid metabolizing enzymes: 3$\alpha$-,

Table 1. Metabolism of progesterone (sum of all metabolites) by homogenates, microsomes and soluble fractions of parotid salivary glands of nonpregnant female, pregnant female and male rats (pmol of metabolized substrate/min/g of tissue ± SE)

| Cellular preparation | Nonpregnant female | Pregnant female | Male |
|---|---|---|---|
| Homogenate | 104 ± 17 ($N = 10$) | 121 ± 18 ($N = 9$) | 68 ± 7 ($N = 10$) |
| Microsomal fraction | 23 ± 4 ($N = 10$) | 33 ± 9 ($N = 9$) | 33 ± 4 ($N = 9$) |
| Soluble fraction | 67 ± 11 ($N = 9$) | 50 ± 4 ($N = 8$) | 29 ± 3 ($N = 10$) |

Level of significance (Wilcoxon test): *$2\alpha \leqslant 0.1$; **$2\alpha \leqslant 0.05$.

Table 2. Formation of metabolites of progesterone in homogenates of parotid glands of nonpregnant female, pregnant female and male rats (pmol/min/g of tissue ± SE)

| Metabolite | Nonpregnant female ($N = 10$) | Pregnant female ($N = 9$) | Male ($N = 10$) |
|---|---|---|---|
| Polar compounds | | | |
| 17α-Hydroxy-4-pregnene-3,20-dione | 1.2 ± 0.2 | 1.6 ± 0.5 | 2.4 ± 1.1 |
| Unidentified | 7.7 ± 2.4 | 7.7 ± 2.6 | 9.3 ± 2.8 |
| 20α-Hydroxy-4-pregnen-3-one | 58.6 ± 14.4 | 65.9 ± 15.0 | 20.1 ± 3.9 |
| 5α-Pregnane-3,20-dione | 6.1 ± 0.8 | 8.8 ± 0.8 | 9.2 ± 0.8 |
| 5β-Pregnane-3,20-dione | 0.7 ± 0.3 | 0.6 ± 0.2 | 1.5 ± 1.1 |
| 3α-Hydroxy-5α-pregnan-20-one | 16.0 ± 2.1 | 20.4 ± 3.0 | 17.4 ± 2.0 |
| 3β-Hydroxy-5α-pregnan-20-one | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.3 ± 0.1 |
| 20α-Hydroxy-5α-pregnan-3-one | 1.0 ± 0.2 | 2.0 ± 0.4 | 0.7 ± 0.2 |
| 20β-Hydroxy-5α-pregnan-3-one / 3α-Hydroxy-5β-pregnan-20-one | 7.2 ± 1.0 | 6.8 ± 0.5 | 5.5 ± 0.4 |
| 5α-Pregnane-3α,20α-diol | 4.1 ± 1.0 | 5.6 ± 0.8 | 1.6 ± 0.4 |
| 5β-Pregnane-3α,20β-diol | 0.8 ± 0.2 | 0.9 ± 0.2 | 0.1 ± 0.1 |
| Nonpolar compounds | 0.1 ± 0.1 | 0.0 | 0.4 ± 0.1 |

For the significance of the results, see Tables 1 and 4.

3β-, 20α- and 20β-hydroxysteroid dehydrogenases, 5α- and 5β-steroid hydrogenases and 17α-steroid hydroxylase activities. The presence of other hydroxylases could not be confirmed [22]. As in submandibular glands [22], the enzyme activities seemed to be NADPH-dependent and they are favored by a reductive environment.

The enzyme activities were higher in females (pregnant and nonpregnant) than in males (Table 4). The formation of 20α-hydroxy-4-pregnen-3-one as the major metabolite of progesterone demonstrates high 20α-hydroxysteroid dehydrogenase activity. 20α-Hydroxy-4-pregnen-3-one retains progestational activity [23]. It is interesting to note that the homogenates and the soluble fractions of the female glands produce much larger amounts of this compound than those of the male glands.

The main metabolite in the homogenates from the parotid glands was 20α-hydroxy-4-pregnen-3-one, as is also the case in rat gingiva [24]. Furthermore, the metabolism in rat gingiva and parotid glands is qualitatively quite similar, and females are more active than males in converting progesterone. In contrast to the parotid glands, the gingiva undergoes metabolic changes during pregnancy, when the metabolism decreases significantly [24]. In the submandibular and sublingual glands progesterone is reduced mainly to 3α-hydroxy-5α-pregnan-

Table 3. Formation of metabolites of progesterone in subcellular preparations of parotid glands of nonpregnant female, pregnant female and male rats (pmol/min/g of tissue ± SE)

| Metabolite | Nonpregnant female | Pregnant female | Male |
|---|---|---|---|
| *Microsomes* | ($N = 10$) | ($N = 9$) | ($N = 9$) |
| Polar compounds | | | |
| 17α-Hydroxy-4-pregnene-3,20-dione | 0.8 ± 0.1 | 0.9 ± 0.2 | 0.7 ± 0.1 |
| Unidentified | 12.1 ± 3.2 | 21.9 ± 9.0 | 14.5 ± 3.1 |
| 20α-Hydroxy-4-pregnen-3-one | 2.4 ± 0.6 | 1.9 ± 0.4 | 2.1 ± 0.9 |
| 5α-Pregnane-3,20-dione | 5.6 ± 0.8 | 5.9 ± 1.4 | 10.1 ± 1.4 |
| 5β-Pregnane-3,20-dione | 0.9 ± 0.2 | 0.7 ± 0.2 | 1.0 ± 0.3 |
| 3α-Hydroxy-5α-pregnan-20-one | 0.8 ± 0.2 | 0.9 ± 0.2 | 2.1 ± 0.8 |
| 3β-Hydroxy-5α-pregnan-20-one | 0.0 | 0.0 | 0.4 ± 0.2 |
| 20α-Hydroxy-5α-pregnan-3-one | 0.0 | 0.0 | 0.1 ± 0.1 |
| 20β-Hydroxy-5α-pregnan-3-one / 3α-Hydroxy-5β-pregnan-20-one | 0.7 ± 0.1 | 0.4 ± 0.1 | 1.0 ± 0.3 |
| 5α-Pregnane-3α,20α-diol | 0.0 | 0.0 | 0.1 ± 0.1 |
| Nonpolar compounds | 0.1 ± 0.1 | 0.0 | 0.9 ± 0.3 |
| *Soluble fraction* | ($N = 9$) | ($N = 8$) | ($N = 10$) |
| Polar compounds | | | |
| 17α-Hydroxy-4-pregnene-3,20-dione | 0.7 ± 0.1 | 0.7 ± 0.1 | 0.6 ± 0.2 |
| Unidentified | 5.0 ± 1.9 | 2.4 ± 1.1 | 3.9 ± 1.0 |
| 20α-Hydroxy-4-pregnen-3-one | 51.2 ± 10.2 | 37.4 ± 4.0 | 16.1 ± 2.8 |
| 5α-Pregnane-3,20-dione | 0.6 ± 0.1 | 0.5 ± 0.2 | 0.7 ± 0.2 |
| 5β-Pregnane-3,20-dione | 0.6 ± 0.2 | 0.5 ± 0.2 | 0.4 ± 0.1 |
| 3α-Hydroxy-5α-pregnan-20-one | 0.2 ± 0.1 | 0.7 ± 0.2 | 0.3 ± 0.1 |
| 20β-Hydroxy-5α-pregnan-3-one / 3α-Hydroxy-5β-pregnan-20-one | 8.2 ± 1.0 | 7.4 ± 0.6 | 6.9 ± 0.5 |
| 5β-Pregnane-3α,20β-diol | 0.6 ± 0.1 | 0.3 ± 0.1 | 0.1 ± 0.1 |
| Nonpolar compounds | 0.0 | 0.0 | 0.3 ± 0.1 |

For the significance of the results see Tables 1 and 4.

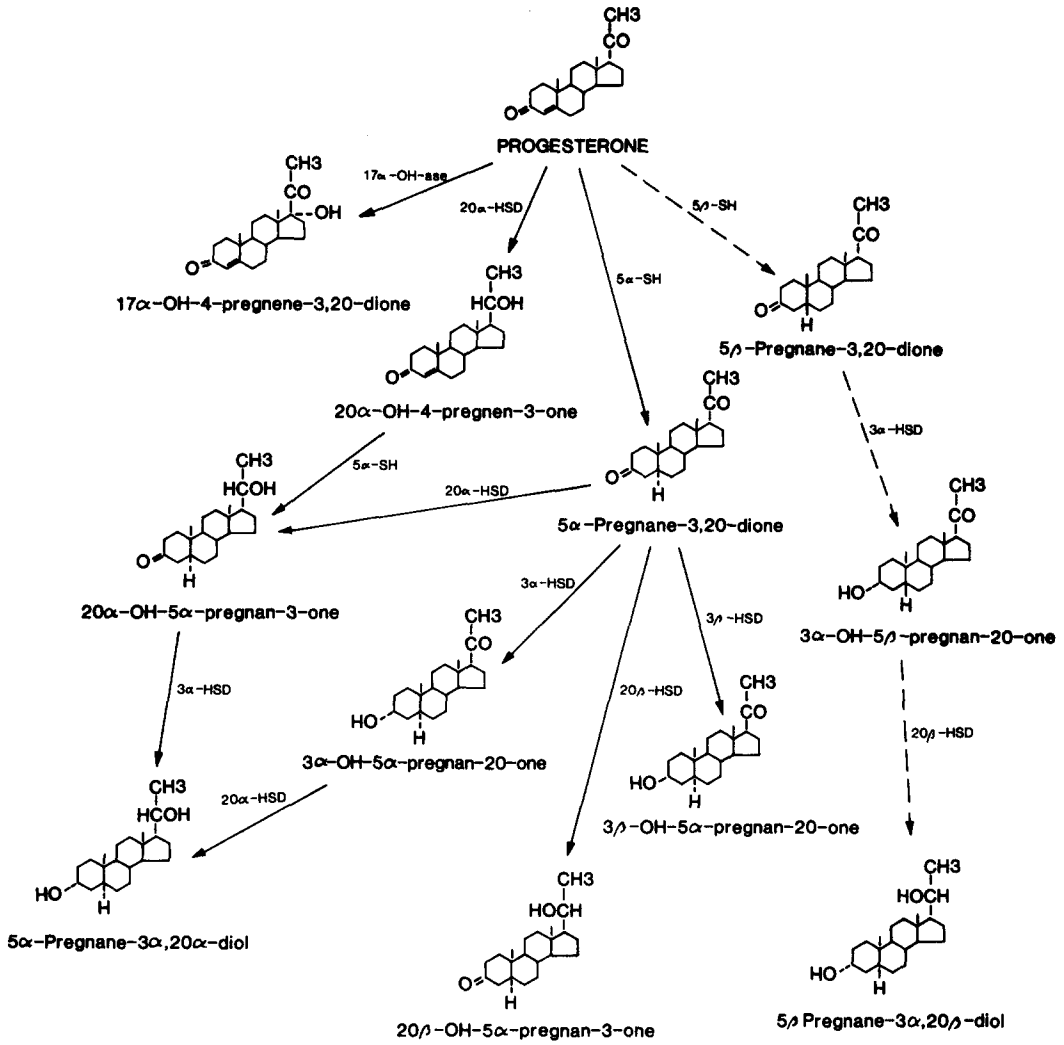


Fig. 1. Suggested metabolic pathways of progesterone in parotid salivary glands of the rat. ⟶ 5α-pathway; - - - ⟶ 5β-pathway. SH = steroid hydrogenase; HSD = hydroxysteroid dehydrogenase; OH-ase = steroid hydroxylase.

20-one, as also happens in the rat uterus, a known target tissue for progesterone [25]. This observation differs from that by Coffey [13], who found that reduction of progesterone occurred in rat submandibular glands, resulting in the formation of 20α-hydroxy-4-pregnen-3-one as the main metabolite.

The 5α-steroid hydrogenase activity was higher in all preparations of male submandibular glands than in corresponding female glands.

Table 4. Activities of some enzymes involved in progesterone metabolism in homogenates and subcellular preparation of the rat parotid gland. Sum of corresponding metabolites formed, pmol/min/g of tissue ± SE[a]

| | Homogenate | | | Soluble fraction | | |
|---|---|---|---|---|---|---|
| | Nonpregnant female | Pregnant female | Male | Nonpregnant female | Pregnant female | Male |
| 5α-Steroid hydrogenase | 34.8 ± 3.9 | 44.0 ± 4.3 | 34.4 ± 2.9 | 9.2 ± 1.0 | 8.6 ± 0.6 | 7.9 ± 0.6 |
| 5β-Steroid hydrogenase | 8.7 ± 1.0 | 8.2 ± 0.6 | 5.6 ± 1.6 | 9.6 ± 0.9 | 8.1 ± 0.6 | 5.6 ± 1.1 |
| | | * | | | | |
| 3α-Hydroxysteroid dehydrogenase | 28.1 ± 3.3 | 33.6 ± 3.7 | 23.1 ± 2.7 | 9.2 ± 1.0 | 8.4 ± 0.6 | 5.5 ± 1.2 |
| 3β-Hydroxysteroid dehydrogenase | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.2 ± 0.1 | 0 | 0 | 0 |
| | | ** | | | *** | |
| 20α-Hydroxysteroid dehydrogenase | 63.8 ± 15.5 | 72.7 ± 16.0 | ***-23.5 ± 3.9 | 51.2 ± 10.2 | 37.7 ± 3.8 | ***-14.1 ± 3.2 |
| 20β-Hydroxysteroid dehydrogenase | 8.0 ± 1.1 | 7.6 ± 0.7 | **- 5.5 ± 0.4 | 9.0 ± 1.0 | 7.7 ± 0.6 | 6.4 ± 0.9 |
| 17α-Steroid hydroxylase | 1.2 ± 0.2 | 2.2 ± 0.8 | 1.4 ± 0.1 | 0.7 ± 0.6 | 0.7 ± 0.1 | 0.6 ± 0.1 |

Level of significance (Wilcoxon test): *$2\alpha \leqslant 0.1$; **$2\alpha \leqslant 0.05$; ***$2\alpha \leqslant 0.01$.
[a]Excluding unidentified nonpolar and polar metabolites.

This is in contrast to progesterone metabolism in the liver, where homogenates progesterone is reduced about 3–10 times faster in females than in males [26]. In the parotid gland microsomal fractions unidentified polar metabolites predominated. In the soluble fractions of the parotid glands, 20α-hydroxy-4-pregnen-3-one was the main metabolite.

The 5α-pathway dominated over the 5β-pathway in all salivary glands. It is known from previous studies [27, 28] that in androgen-dependent tissues testosterone undergoes 5α-reduction; testosterone is converted to a more potent androgenic hormone, dihydrotestosterone. In progesterone metabolism the main end product of 5α-reduced metabolites of progesterone in our experimental conditions after prolonged incubations of salivary glands (data not shown) was 5α-pregnane-3α, 20α-diol (5α-pregnanediol), as is found in the rat myometrium [29]. Although the metabolism seems mainly to consist of peripheral catabolism, some interesting differences in response to different steroids are seen between the glands.

In submandibular glands of male rats testosterone is the main regulatory factor of the structure and metabolic activity of the gland [6–9], as is also the case in the exorbital lacrimal of the rat [30]. Testosterone receptors are found [5, 31] in both glands, suggesting that these glands are target tissues for androgen action. In addition the active forms of testosterone are retained in the submandibular gland [32] when the active form of progesterone is rapidly metabolized [22].

As in the pancreas, estrogens also play a major role in metabolism in the parotid glands. Both these glands have estrogen receptors [1, 2, 4]. In the parotid glands the peroxidase activity seems to be much more estrogen-dependent than in submandibular and sublingual glands [4] and the female parotid glands have much more progestational activity than those of males.

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