J. steroid Biochem. Vol. 35, No. 6, pp. 723–728, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

PROGESTERONE METABOLISM BY MAJOR SALIVARY GLANDS OF RAT—I. SUBMANDIBULAR AND SUBLINGUAL GLANDS

M. LAINE* and A. OJANOTKO-HARRI[1]
Institute of Dentistry and [1]Department of Physiology, University of Turku, SF-20520 Turku, Finland

(*Received* 9 *October* 1989)

Summary—The metabolism of progesterone by the submandibular and sublingual salivary glands of female (nonpregnant and pregnant) and male rats was studied.

The metabolism was in both sexes significantly greater in submandibular than in sublingual glands. Sex differences were not seen in sublingual glands but less metabolism was found in homogenates and microsomal fractions of female (nonpregnant and pregnant) submandibular glands compared to that of males. The metabolism did not differ between pregnant and nonpregnant female rats. The metabolites were mainly 5α-pregnane-compounds.

On the basis of the metabolites identified it can be concluded that rat submandibular and sublingual glands contain at least 3α-, 3β-, 20α- and 20β-hydroxysteroid dehydrogenase, 5α- and 5β-steroid hydrogenase and 17α-steroid hydroxylase activity. 5α-steroid hydrogenase activity was significantly higher in all preparations of male submandibular glands than in females. In sublingual glands some enzyme activities showed pregnancy-related decrease.

INTRODUCTION

The anatomy and biochemistry of the submandibular salivary glands of rodents have been extensively studied since Lacassagne[1] found that the submandibular glands of mice show androgen-dependent sexual dimorphism. In sexually mature mice the granular tubules are larger and more numerous in male than in female submandibular glands. The synthesis of some peptides and enzymes have been shown to be under the control of androgens [2–5]. Steroids perform many of their biological actions by binding to specific receptors. Mouse and rat submandibular glands are considered to be target organs for steroid hormones, especially testosterone, as there are specific receptor proteins [6–8]. Steroid hormones apparently have a specific role in the maintenance of the normal morphology and physiology of these glands. Testosterone is in this respect much more effective than estrogens or progestins [9, 10].

Many reports have been published concerning the steroid metabolism of submandibular glands. Progestins, estrogens and androgens are metabolized by the submandibular glands of various species [11–14]. Steroids have even been said to be synthesized in the submandibular glands of male rats [15].

In contrast to the relatively well explored metabolism of steroids in rodent submandibular glands, very little information is available about the sublingual glands. Only one study has been published concerning sterols in the sublingual gland [16], in which the authors found that the major sterol in male mouse sublingual gland but also in the submandibular and parotid glands is cholesterol, apparently synthesized in the glands.

Progesterone is the major precursor for androgen and estrogen biosynthesis. Rat submandibular glands, in both sexes, exhibit higher amounts of progesterone than of testosterone and estrogen [17]. The progesterone content in rat submandibular glands also rises significantly during pregnancy [17]. We have now examined in detail the metabolism of progesterone in female and male rats, and the possible effect of pregnancy on the metabolism.

EXPERIMENTAL

Preparation of tissues

Ten nonpregnant and nine pregnant female and ten male Long Evans rats (Turku strain), aged 6–9 months, were used. The animals were housed in a controlled environment (12 h light–12 h dark, temperature 23°C, humidity 50%) with free access to food and water. The stage of the estrus cycle of female rats was not checked. Female rats were placed overnight with males and the following day was called the first day of pregnancy, if pregnancy occurred. On the 19th to 21st day of pregnancy the rats

*Correspondence should be addressed to: Dr M. Laine, Department of Cariology, Institute of Dentistry, University of Turku, Lemminkäisenkatu 2, SF-20520 Turku, Finland.

were killed with ether. The submandibular and sublingual salivary glands were removed and weighed. The weights of the submandibular glands in nonpregnant, pregnant and male rats were 353 ± 27, 391 ± 42, and 490 ± 62 mg respectively, and the corresponding values for the sublingual glands were 66 ± 9, 76 ± 7, and 83 ± 5 mg respectively. 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 glands were homogenized in 10 ml of this sucrose buffer with a homogenizer of the Potter–Elvehjem type with a rotatory Teflon pestle using 15 strokes. After homogenization the 10 ml-samples were divided into two parts. The first 5 ml-portion was called the homogenate fraction. The other 5 ml-portion was first centrifuged for 15 min at 10,000 ***g*** to remove cell debris and large particles. The supernatant was then centrifuged for 60 min at 100,000 ***g***. The obtained pellet was resuspended in the phosphate buffer without sucrose and called the microsomal fraction. The supernatant from this centrifugation is referred to as the soluble fraction.

Radioactive steroids

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

Incubations

The salivary gland homogenates and (microsomal and soluble) subcellular fractions were incubated in the buffer solution without sucrose with an NADPH-regenerating system consisting of 2.3 μmol NADP, 18.8 μmol glucose-6-phosphate and 3 units glucose-6-phosphate dehydrogenase (Boehringer Mannheim, Mannheim, F.R.G.). The reaction was started by adding 2–3 nmol of the radioactive progesterone dissolved in 1 ml of the buffer solution. [^{14}C]Progesterone was incubated without tissue (blank) in every incubation. The background activity was subtracted from the radioactivity values found after salivary gland incubations. 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 methyl acetate.

Extraction and chromatography of steroid metabolites

The reaction mixture was centrifuged and the methyl acetate phase removed. The procedure was then repeated three times with 4 ml of methyl acetate. The combined methyl phases (4 × 4 ml) were evaporated under a stream of nitrogen, at a temperature not exceeding 50°C.

5 ml ethanol was added to the lower aqueous phase. After evaporation at 100–105°C the radioactivity of the residuum was determined with liquid scintillation counting. Steroid conjugates (sulphates or glucuronidates), if present, remain in this fraction.

The residuum after combination and evaporation of the upper phases 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 the presence of lipoidal metabolites. The lower phase, containing unconjugated steroid metabolites, was used for bidimensional thin-layer chromatography. This fraction was the only one to be examined further, since most of the radioactivity was found there. About 10% of the added progesterone was metabolized, which confirmed the linearity of the reaction.

Non-radioactive steroid standards were added to this free steroid fraction, which was then applied to the corner of a silica gel plate. The plate was first developed in one direction with dichlormethane–methyl acetate (9:1, v/v) and, after a short drying period, in the second direction with hexane–1-hexanol (3:1, v/v). The running distance was about 10 cm in the first and 16 cm in the second direction.

In order to further characterize one metabolite found in the submandibular glands, tentatively identified as 3α, 17α-dihydroxy-5α-pregnan-3-one, additional thin-layer chromatography was performed after acetylation. The free steroid fraction was kept overnight at room temperature dissolved in a small amount of pyridine–acetic anhydride (1:1). After evaporation this material was run bidimensionally, together with acetylated steroid standards, with the following solvents: dichlormethane–methyl acetate (98:2, v/v) in the first direction and hexane–1-hexanol (92.5:7.5, v/v) in the second direction. The chromatographic mobilities of this metabolite were further characterized by additional thin-layer chromatography on magnesium silicate [18]. The area containing this metabolite on a silica gel plate was scraped off when the plate was still wet and extracted with ethanol. The extracted material was applied on a magnesium silicate plate and developed one-dimensionally with hexane–*t*-butanol (9:1, 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 after staining with ethanol-acetic anhydride-sulphuric acid. The radioactivity of different areas of the plates was determined by liquid scintillation counting.

Two metabolites, 20β-hydroxy-5α-pregnan-3-one and 3α-hydroxy-5β-pregnan-20-one, were quantified together as these compounds partially overlap on the chromatography plates. Several metabolites designated as nonpolar and most of the polar ones were quantified together, due to the unavailability of reference steroids. Most of the reference steroids were

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

Cellular preparation	Submandibular Nonpregnant female ($N = 10$)	Submandibular Pregnant female ($N = 9$)	Submandibular Male ($N = 9$)	Sublingual Nonpregnant female ($N = 10$)	Sublingual Pregnant female ($N = 9$)	Sublingual Male ($N = 10$)
Homogenate	1025 ± 108	894 ± 70	1569 ± 109	204 ± 31	167 ± 13	152 ± 19
Microsomal fraction	239 ± 56	178 ± 23	484 ± 80	67 ± 15	47 ± 10	55 ± 7
Soluble fraction	166 ± 19	124 ± 11	198 ± 28	29 ± 4 ($N = 9$)	26 ± 4	21 ± 4

Wilcoxon-test—level of significance: *$2\alpha \leq 0.1$; **$2\alpha \leq 0.05$; ***$2\alpha \leq 0.01$.

gifts from Professor D. N. Kirk, Steroid Reference Collection, Westfield College, London.

The activities of different enzymes were calculated by summing the metabolites having relevant molecular structures; e.g. the metabolites with a 5β-hydrogen were summed for the assessment of 5β-steroid hydrogenase activity.

Statistical analysis

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

RESULTS

The total metabolism of added progesterone by the homogenates and subcellular preparations of the rat submandibular and sublingual salivary glands is presented in Table 1. The total metabolic activity in the submandibular glands was about 5 times as high as in the corresponding female sublingual glands, and about 10 times as high as in the corresponding male sublingual glands. In both cases the soluble fractions were relatively inactive, contributing only about 13–16% to the metabolic activity present in the homogenates.

Pregnancy did not significantly affect the metabolism, although pregnant rats had lower metabolic activity in all preparations in both glands than nonpregnant females.

Sex differences were found in the total metabolism by homogenates and the microsomal fractions of submandibular glands. The metabolic activity in homogenates and microsomes of male rats was greater than in nonpregnant and pregnant females ($2\alpha < 0.01$).

The individual metabolites found after the incubation of [^{14}C]progesterone with homogenates and subcellular preparations are summarized in Tables 2–4. The metabolites were mainly 5α-pregnane-compounds. Small amounts were found of a compound which cochromatographed in several solvent systems with authentic 17α-hydroxy-4-pregnene-3, 20-dione (17α-hydroxyprogesterone), produced by both glands. Its identification was strengthened by the presence of similarly identified 3α,17α-dihydroxy-5α-pregnan-20-one after submandibular gland incubations. This compound is a logical metabolite of 17α-hydroxyprogesterone.

DISCUSSION

Our results indicate that progesterone is extensively metabolized by the rat submandibular glands and to a lesser extent by the sublingual glands. The reason for the lower metabolic activity in the sublingual

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

Metabolite	Submandibular Nonpregnant female ($N = 10$)	Submandibular Pregnant female ($N = 9$)	Submandibular Male ($N = 9$)	Sublingual Nonpregnant female ($N = 9$)	Sublingual Pregnant female ($N = 9$)	Sublingual Male ($N = 10$)
Polar compounds						
17α-hydroxy-4-pregnene-3,20-dione	9.6 ± 1.6	9.4 ± 2.2	12.9 ± 1.7	1.9 ± 0.2	1.1 ± 0.2	1.6 ± 0.2
3α,17α-dihydroxy-5-pregnan-20-one	5.7 ± 1.4	3.9 ± 1.3	5.2 ± 0.6	0.0	0.0	0.0
unidentified	70.1 ± 27.8	23.3 ± 7.8	78.1 ± 41.4	14.6 ± 2.4	9.7 ± 3.6	12.2 ± 1.9
20α-hydroxy-4-pregnen-3-one	51.9 ± 5.7	36.5 ± 5.6	28.6 ± 3.1	16.7 ± 2.5	19.0 ± 2.3	10.5 ± 1.5
5α-pregnane-3,20-dione	83.2 ± 15.0	101.5 ± 24.6	116.3 ± 13.0	45.4 ± 6.5	47.0 ± 5.9	37.5 ± 4.7
5β-pregnane-3,20-dione	4.0 ± 1.4	3.3 ± 2.6	4.8 ± 1.4	0.7 ± 0.2	0.6 ± 0.2	1.1 ± 0.3
3α-hydroxy-5α-pregnan-20-one	691.1 ± 94.5	633.3 ± 55.6	1119.4 ± 91.0	104.3 ± 23.7	72.5 ± 9.6	73.8 ± 14.9
3β-hydroxy-5α-pregnan-20-one	3.4 ± 1.3	4.9 ± 1.8	6.1 ± 0.2	4.8 ± 0.6	3.7 ± 0.5	3.5 ± 0.7
20α-hydroxy-5α-pregnan-3-one	2.9 ± 0.8	2.9 ± 1.3	5.5 ± 1.2	1.8 ± 0.4	3.0 ± 0.6	1.6 ± 0.2
20β-hydroxy-5α-pregnan-3-one / 3α-hydroxy-5β-pregnan-20-one	67.1 ± 8.8	45.4 ± 4.4	85.0 ± 5.7	7.7 ± 1.0	4.6 ± 0.7	5.0 ± 0.7
5α-pregnane-3α,20α-diol	34.2 ± 8.5	29.0 ± 6.0	25.1 ± 3.9	4.9 ± 1.3	6.2 ± 1.4	3.8 ± 1.1
5β-pregnane-3α,20β-diol	2.1 ± 0.8	0.9 ± 0.4	2.0 ± 1.0	0.4 ± 0.2	0.0	0.3 ± 0.2
Nonpolar compounds	0.0	0.0	5.6 ± 2.8	1.0 ± 0.6	0.0	0.6 ± 0.2

Table 3. Formation of metabolites of progesterone in microsomal fractions of submandibular and sublingual salivary glands of nonpregnant female, pregnant female and male rats (pmol/min/g of tissue ± SE)

Metabolite	Submandibular			Sublingual		
	Nonpregnant female ($N = 10$)	Pregnant female ($N = 9$)	Male ($N = 9$)	Nonpregnant female ($N = 9$)	Pregnant female ($N = 9$)	Male ($N = 10$)
Polar compounds						
17α-hydroxy-4-pregnene-3,20-dione	6.7 ± 1.5	3.1 ± 1.0	9.5 ± 1.8	0.9 ± 0.1	1.1 ± 0.3	1.0 ± 0.1
unidentified	36.2 ± 9.8	62.7 ± 21.3	123.9 ± 31.0	21.6 ± 6.1	25.9 ± 10.0	21.8 ± 3.8
20α-hydroxy-4-pregnen-3-one	3.9 ± 1.0	3.5 ± 0.8	6.1 ± 1.1	1.2 ± 0.2	0.9 ± 0.1	0.9 ± 0.1
5α-pregnane-3,20-dione	154.3 ± 47.4	79.3 ± 15.8	246.1 ± 37.4	35.1 ± 15.8	15.6 ± 3.6	23.1 ± 4.2
5β-pregnane-3,20-dione	4.5 ± 0.9	3.1 ± 0.8	8.7 ± 2.6	1.2 ± 0.3	0.8 ± 0.3	1.2 ± 0.3
3α-hydroxy-5α-pregnan-20-one	26.8 ± 10.1	20.8 ± 6.4	62.2 ± 16.6	5.2 ± 2.5	2.2 ± 0.6	5.0 ± 1.5
3β-hydroxy-5α-pregnan-20-one	0.0	0.0	4.1 ± 2.3	0.0	0.0	0.4 ± 0.2
20β-hydroxy-5α-pregnan-3-one / 3α-hydroxy-5β-pregnan-20-one	4.8 ± 1.0	2.9 ± 0.9	7.5 ± 1.4	0.8 ± 0.2	0.6 ± 0.1	0.4 ± 0.1
5α-pregnane-3α,20α-diol	0.0	0.0	1.3 ± 0.9	0.0	0.0	0.0
5β-pregnane-3α,20β-diol	0.0	0.0	1.4 ± 0.9	0.2 ± 0.1	0.0	0.0
Nonpolar compounds	0.0	1.3 ± 1.0	13.6 ± 4.5	1.0 ± 0.5	0.1 ± 0.1	1.5 ± 0.5

glands may be their anatomy. In rat sublingual glands, striated ducts are few and granular tubules are absent [19]. During puberty the striated ducts of rodent submandibular glands are transformed to granular tubules, which are sexually dimorphic [20]. The steroid metabolizing enzymes are mainly found in the duct area both in humans and animals [21, 22]. The time of the estrus cycle was not checked, which may explain the slightly larger variations found in the total metabolism in nonpregnant females. The weights of both glands of pregnant females were slightly higher than in nonpregnant females. This agrees with the findings of Travill[23], who found that the weight of mice submandibular glands was greater immediately after pregnancy than in virgin females.

The proposed metabolic pathways of progesterone in the rat submandibular and sublingual salivary glands according to our results are presented in Fig. 1. The reductive 5α-pathway predominates over the 5β-pathway. The metabolites formed suggest that at least the following steroid metabolizing enzymes are present: 5α- and 5β-steroid hydrogenases, 3α-, 3β-, 20α- and 20β-hydroxysteroid dehydrogenases and 17α-steroid hydroxylase. The presence of other hydroxylases could not be confirmed because of the unavailability of reference steroids. Several metabolites, which in two-dimensional chromatography moved to the general area of steroids with more than two functional groups (hydroxyl and/or keto) were found. These polar metabolites are not, however, necessarily hydroxylated [24]. Sex differences were also found in some of the progesterone metabolizing enzyme activities in the submandibular glands. The homogenate and microsomal 3α-hydroxysteroid dehydrogenase and the soluble fraction 20β-hydroxysteroid dehydrogenase activity were greater in males than in females ($2\alpha < 0.01$). In all preparations from the submandibular glands 5α-steroid hydrogenase activity was significantly greater in males than in females ($2\alpha < 0.01$). Similar results concerning androgen metabolism and 5α-steroid hydrogenase have been published by Katsukawa *et al.*[25], who found that in the submandibular glands of male mice 5α-steroid hydrogenase activity is greater than in females. In addition this enzyme activity was found to be androgen-dependent.

In the sublingual glands no sex-related, but pregnancy-related changes were found in some enzyme activities. In nonpregnant rats the 5β-steroid hydrogenase, 20β-hydroxysteroid dehydrogenase and 17α-

Table 4. Formation of metabolites of progesterone in soluble fractions of submandibular and sublingual salivary glands of nonpregnant female, pregnant female and male rats (pmol/min/g of tissue ± SE)

Metabolite	Submandibular			Sublingual		
	Nonpregnant female ($N = 10$)	Pregnant female ($N = 9$)	Male ($N = 9$)	Nonpregnant female ($N = 9$)	Pregnant female ($N = 9$)	Male ($N = 10$)
Polar compounds						
17α-hydroxy-4-pregnene-3,20-dione	5.3 ± 1.9	4.1 ± 1.3	3.3 ± 1.1	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.1
unidentified	17.8 ± 8.6	10.2 ± 3.0	12.1 ± 3.4	3.2 ± 2.0	2.7 ± 0.9	3.2 ± 0.9
20α-hydroxy-4-pregnen-3-one	43.9 ± 10.5	41.2 ± 8.8	30.5 ± 5.0	11.0 ± 2.0	14.3 ± 2.9	8.6 ± 1.1
5α-pregnane-3,20-dione	6.6 ± 1.6	3.0 ± 0.9	8.7 ± 2.1	1.2 ± 0.2	1.1 ± 0.4	0.9 ± 0.2
5β-pregnane-3,20-dione	3.2 ± 1.1	2.7 ± 1.4	7.3 ± 2.5	1.1 ± 0.2	0.4 ± 0.1	0.7 ± 0.2
3α-hydroxy-5α-pregnan-20-one	16.6 ± 3.5	9.2 ± 1.9	26.4 ± 5.2	1.2 ± 1.1	0.9 ± 0.2	0.8 ± 0.2
3β-hydroxy-5α-pregnan-20-one	0.0	0.0	2.8 ± 1.6	0.0	0.0	0.1 ± 0.1
20α-hydroxy-5α-pregnan-3-one	1.2 ± 0.8	0.0	1.4 ± 0.9	0.0	0.0	0.0
20β-hydroxy-5α-pregnan-3-one / 3α-hydroxy-5β-pregnan-20-one	65.7 ± 10.2	51.2 ± 2.7	102.2 ± 20.7	9.5 ± 1.6	5.7 ± 2.0	5.6 ± 1.3
5α-pregnane-3α,20α-diol	1.1 ± 0.7	0.0	0.7 ± 0.7	0.2 ± 0.2	0.0	0.0
5β-pregnane-3α,20β-diol	3.5 ± 2.4	2.4 ± 1.0	0.7 ± 0.7	0.3 ± 0.2	0.0	0.2 ± 0.2
Nonpolar compounds	1.4 ± 1.0	0.0	2.0 ± 1.0	0.4 ± 0.4	0.0	0.3 ± 0.2

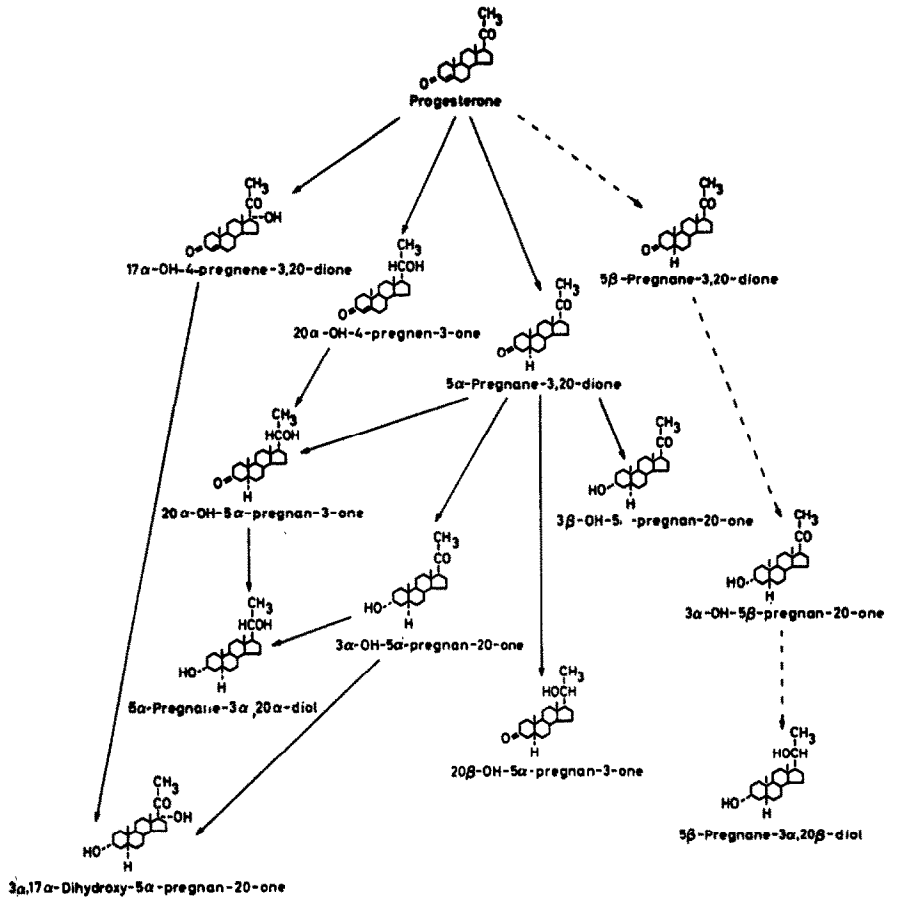

Fig. 1. Proposed metabolic pathways of progesterone in the submandibular and sublingual salivary glands of the rat. (3α,17α-Dihydroxy-5α-pregnan-20-one was not detected in sublingual glands.) 5α-pathway →; 5β-pathway - - →.

hydroxylase activities were greater than in pregnant rats.

The relationship between the submandibular glands and the endocrine system is complex. The submandibular glands of rats have been shown to be target organs for male hormones. The morphology and excretion of some enzymes and growth factors are under the control of androgens and thyroxine [26, 27]. The submandibular glands are mainly exocrine in nature, but they have endocrine features, although specialized endocrine cells like those in the pancreas do not exist. Endocrine functions have, however, been reported. Banerjee *et al.*[28] found that removal of the submandibular glands of immature female rats is followed by a 3-fold increase in weight of the uterus. On the basis of increased plasma estradiol and uterine peroxidase levels, they suggested that estrogen was responsible for the changes. They also suggested that rat submandibular glands have a factor which acts either on the ovaries or through the pituitary gland. Boyer *et al.*[29] found that removal of the submandibular glands from male rats decreased the weight of both the testes and the total body. Since no changes in plasma testosterone were found, they suggested that factors of salivary gland origin, probably Epidermal Growth Factor (EGF) and Nerve Growth Factor (NGF), act directly through the pituitary gland.

Baldi and Charreau[30] found that testosterone is metabolized in male submandibular glands slower than in female glands. In our study 20α-hydroxy-4-pregnen-3-one, which is a biologically active progestin [31], was metabolized more rapidly by male submandibular glands. The greater formation of less active metabolites of progesterone and the retention of the active form of androgen may provide a control mechanism for progesterone and testosterone action. Small amounts of 17α-hydroxyprogesterone were formed. This compound, which is an intermediate for androgen and estrogen biosynthesis, is not normally formed by other than steroid-synthesizing tissues.

To summarize, the pattern of metabolites in the salivary glands studied appears to be similar, although the submandibular gland was much more active in converting progesterone. In the submandibular glands of males, progesterone is rapidly metabolized to several metabolites with less progestational activity.

In contrast to some other parts of the mouth and the alimentary tract, e.g. gingiva and small intes-

tine [32, 33], salivary glands did not show marked pregnancy-related metabolic changes in the total steroid metabolism. It should be noted that *in vivo* the supply of progesterone to the tissues is increased. In the present study, the effect of substrate concentration on the metabolism was not studied. The major metabolites were the same as those formed *in vitro* by the uterus of nonpregnant and pregnant rats [34]. In submandibular glands sex-related and in sublingual glands pregnancy-related changes were found in some enzyme activities. In conclusion, it seems that the role of the salivary glands in steroid metabolism is more extensive than thought earlier; although they are probably not organs where only peripheral steroid catabolism occurs.

Acknowledgements—The authors wish to thank Ms P. Jäkälä and Ms T. Laiho for their skilful technical assistance, and Dr W. Nienstedt for valuable advice during the preparation of this report. This study was supported by a grant from the N.I.H. (ES-91684-21) and the Finnish Dental Society.

REFERENCES

1. Lacassagne A.: Dimorphisme sexuel de la glande sous-maxillaire chez la souris. *C.R. Seanc. Soc. Biol.* **133** (1940) 180–181.
2. Levi-Montalcini R. and Angeletti P. U.: Hormonal control of the nerve growth factor content in the submaxillary glands of mice. In *Salivary Glands and Their Secretions* (Edited by L. M. Sreebny and J. Meyer). Pergamon Press, Oxford (1964) pp. 129–141.
3. Shear M., Barbakow F. and King C.: Histochemically demonstrable changes in amylase activity in submaxillary glands of puberal male mice following castration. *S. Afr. J. Med. Sci.* **40** (1975) 133–144.
4. Nakamura T., Fujii M., Kaiho M. and Kumegawa M.: Sex difference in glucose-6-phosphate dehydrogenase activity in the submandibular gland of mice. *Biochim. Biophys. Acta* **362** (1974) 110–120.
5. Hosoi K., Kobayashi S. and Ueha T.: Influence of androgens on β-glucuronidase activity in liver, kidney and submandibular salivary gland of the mouse. *Archs Oral Biol.* **23** (1978) 905–909.
6. Verhoeven G. and Wilson J. D.: Cytosol androgen binding in submandibular gland and kidney of the normal mouse and the mouse with testicular feminization. *Endocrinology* **99** (1976) 79–92.
7. Laine M. and Tenovuo J.: Effect of peroxidase activity and specific binding of the hormone 17β-oestradiol and rat salivary glands. *Archs Oral Biol.* **28** (1983) 847–852.
8. Sakabe K., Seiki K., Fujii-Hanamoto H. and Ogawa H.: Progestin receptor and its localization in submandibular gland of male rats. *IRCS Med. Sci.* **14** (1986) 928–929.
9. Dunn J. F. and Wilson J. D.: Developmental study of androgen responsiveness in the submandibular gland of the mouse. *Endocrinology* **96** (1975) 1571–1578.
10. Flynn E. A., Yelland K. T. and Shklar G.: Effect of estradiol on ultrastructure of granular ducts in submandibular glands of female rats. *Anat. Rec.* **206** (1983) 23–30.
11. Coffey J. C.: *In vitro* progesterone metabolism in rat submaxillary gland: the formation of 20α-hydroxy-4-pregnen-3-one and other substances. *Steroids* **22** (1973) 561–566.
12. ElAttar T. M. A.: *In vitro* metabolism of estrone 2,4,6,7-^{3}H and 4-androstene-3,17-dione-1,2-^{3}H in submandibular gland and submandibular gland cancer tumor. *Steroids* **24** (1974) 519–526.
13. Kadis B. and Chyn L. C.: Steroid transformations in porcine salivary glands. *J. Steroid Biochem.* **6** (1975) 1543–1548.
14. Djøseland O., de Besche A., Høglo S. and Rennie P. S. Steroid metabolism by normal and neoplastic parotid tissue. *J. Steroid Biochem.* **16** (1982) 397–402.
15. Rosner J. M., Macome J. C. and Cardinali D. P.: *In vitro* biosynthesis of sterols and steroids by rat submaxillary glands. *Endocrinology* **85** (1969) 1000–1003.
16. Hindy A. M., Yoshiga K., Takada K. and Okuda K.: Sterol composition and biosynthesis in mouse salivary glands. *Archs Oral Biol.* **31** (1986) 87–93.
17. Paulo E. and Szoltys M.: Steroid hormone content in the submandibular gland of normal and pregnant rats. *Archs Oral Biol.* **27** (1982) 887–890.
18. Nienstedt W.: "Atypical" thin-layer chromatography in the characterization of C_{21}-steroids I. *Eur. J. Steroids* **2** (1967) 473–481.
19. Young J. A. and van Lennep E. W.: *The Morphology of Salivary Glands*. Academic Press, London (1978) p. 28.
20. Navia J. M. *Animal Models in Dental Research*. The University of Alabama Press, Alabama (1977) p. 230.
21. Booth W. D., Hay M. F. and Dott H. M.: Sexual dimorphism in the submandibular gland of the pig. *J. Reprod. Fert.* **33** (1973) 163–166.
22. Sirigu P., Cossu M., Perra M. T. and Puxeddu P.: Histochemistry of the 3β-hydroxysteroid, 17β-hydroxysteroid and 3α-hydroxysteroid dehydrogenases in human salivary glands. *Archs Oral Biol.* **27** (1982) 547–551.
23. Travill A.: The effect of pregnancy on the submandibular glands of mice. *Anat. Rec.* (1966) 217–222.
24. Nienstedt W.: Characterization of C_{19}-steroids by two-dimensional thin-layer chromatography. *J. Chromat.* **329** (1985) 171–177.
25. Katsukawa H., Tanabe Y. and Funakoshi M.: Effects of androgens on the activity of steroid-metabolizing enzymes in murine submaxillary gland. *J. Dent. Res.* **62** (1983) 725–727.
26. Leblond C. P. and Grad B.: The necessity of testis and thyroid hormones for the maintenance of the serous tubules of the submaxillary gland in the male rat. *Endocrinology* **45** (1949) 250–266.
27. Mori M., Hamada K., Naito R., Tsukitani K. and Asano K.: Immunohistochemical localization of epidermal growth factor in rodent submandibular glands. *Acta Histochem. Cytochem.* **16** (1983) 536–548.
28. Banerjee P. N., Bhattacharyya J., Datta A. and Datta A. G.: The effect of submaxillariectomy on the uterine peroxidase activity and [^{14}C]phenylalanine incorporation into uterine protein in rats. *Acta Endocr. (Copenh.)* **114** (1987) 208–213.
29. Boyer R., Escola R. and Arancibia S.: Effect of submandibular salivary gland removal on body weight, plasmatic testosterone levels, testicular weight and spermatogenesis in rats. *J. Biol. Buccale* **14** (1986) 187–193.
30. Baldi A. and Charreau E. H.: 17β-Hydroxysteroid dehydrogenase activity in rat submaxillary glands. Its relation to sex and age. *Endocrinology* **90** (1972) 1643–1646.
31 Goldman B. C. and Zarrow M. X.: *Handbook of Physiology: Section 7: Endocrinology*, Vol. II (Edited by R. O. Greep and E. B. Astwood). American Physiological Society, Washington D.C. (1973) p. 562.
32. Ojanotko A. and Harri M.-P.: Progesterone metabolism by rat oral mucosa. II. The effect of pregnancy. *J. Periodont. Res.* **17** (1982) 196–201.
33. Savolainen T., Ojanotko A. and Harri M.-P.: Metabolism of progesterone in small intestine of normal and pregnant rat. *J. Steroid Biochem.* **17** (1982) 285.
34. Howard P. D. and Wiest W. G.: Progesterone metabolism by uterine tissue of pregnant rats. *Steroids* **19** (1972) 35–45.