ORAL DISEASES

Oral Diseases (2010) 16, 577–585. doi:10.1111/j.1601-0825.2010.01669.x

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INVITED MEDICAL REVIEW

Salivary glands – 'an unisex organ'?

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Usually no distinction is made between female and male salivary glands although cyclic changes of and/or differences in serum and salivary sex steroid concentrations characterize women and men. Moreover, sexual dimorphism is well recognized in salivary glands of rodents. Salivary glands contain estrogen and androgen receptors and are, according to modern high throughput technologies, subjected to gender differences not explainable by gene dose effects by the X chromosome alone. Because sex steroids are lipophilic, it is often thought that approximately 10% of them passively diffuse from plasma to saliva. Indeed, saliva can find use as sample material in sports medicine, pediatrics, veterinary medicine and behavioral sciences. Last but not least, humans and other primates are unique in that they have a reticular zone in their adrenal cortex, which produces dehydroepiandrosterone and androstendione pro-hormones. These are processed in peripheral tissues, not only in female breast and uterus and male prostate, but also in salivary glands by an intracrine enzymatic machinery to active 17β-estradiol, dihydrotestosterone and others, to satisfy and buffer against a constantly changing needs caused by circadian, menstrual, pregnancy and chronobiological hormonal changes in the systemic circulation. Female dominance of Sjögren's syndrome and certain forms of salivary gland cancer probably reflect these gender-based differences.

Oral Diseases (2010) **16**, 577–585

Keywords: salivary glands; sex steroids; DHEA; intracrinology; Sjögren's syndrome

Introduction

Human salivary glands comprise the large and paired parotid, submandibular (submaxillary) and sublingual glands and several hundred small submucosal glands. They are branching tubuloacinar glands composed of epithelial cells resting on basement membrane, embedded in a vascularised interstitial connective tissue stroma. Most of the watery saliva is produced as result of constitutive or stimulated transudation of plasma from peri-acinar capillary network as a result of basal or reflexive stimulation of the sympathetic and parasympathetic branches of the autonomic nervous system. These branches also regulate the exocrine secretion of mucins and proteins and the activity of ion channels responsible for the trans-acinar osmotic gradient, respectively. Mineral concentration, pH and protein composition of the primary saliva are modified as it passes through the salivary ducts, to finally reach the oral mucosal membranes, which in many ways are dependent on and regulated by the composition of the saliva. Little attention has been paid to the eventual role, if any, of sex steroids on salivary glands and in saliva.

Systemic sex steroids

Concentrations of sex steroids undergo major variations throughout life (Figure 1; Konttinen *et al*, 2009). In women in puberty 17β-estradiol levels rise dramatically to high values, but remain low in men, 0–0.13 nmol l^{-1}. From there on until menopause menstruation produces regular cyclic fluctuations of female estradiol levels, which are 0.11–0.44 nmol l^{-1} during the follicular phase, 0.55–1.29 nmol l^{-1} during the pre-ovulatory peak and 0.37–0.77 during the luteal phase. Correspondingly, progesterone levels in women are 0.3–2.5 nmol l^{-1} during the follicular phase and 7.80 nmol l^{-1} during the luteal phase of the menstrual cycle. In addition, due to placental production of human chorionic gonadotropin, progesterone and estrogens, pregnancy increases plasma levels of estrogen and progesterone even 30 and 10-fold, respectively. This not only helps to maintain pregnancy, but also induces a state of immune tolerance against the fetus, also reflected as remissions of rheumatoid arthritis during pregnancy. In postmenopausal women the major circulating estrogen is no more

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Received 29 September 2009; revised 12 November 2009; accepted 12 November 2009

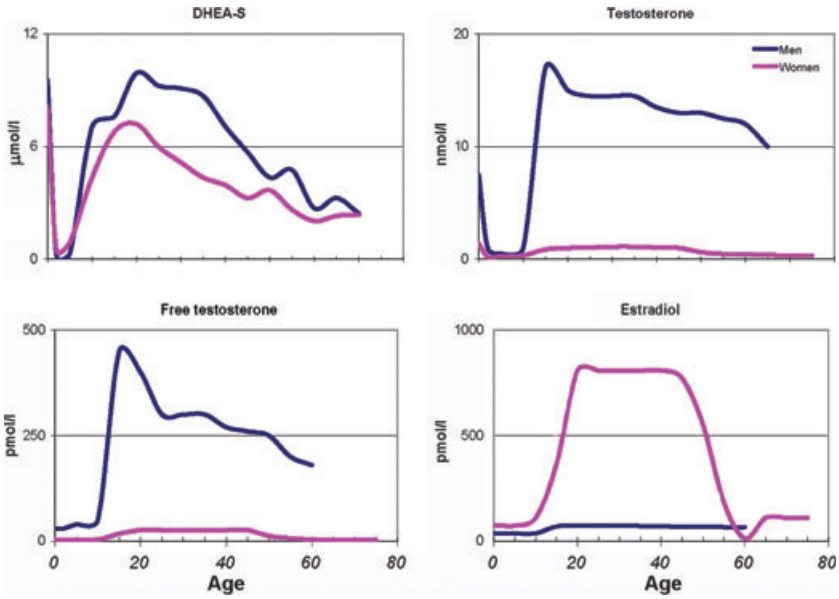

Figure 1 Some serum sex steroid concentrations in a life time scale in women and men. DHEA-S = dehydroepiandrosterone sulfate. Testosterone refers here to total testosterone, in contrast to free, unbound and biologically active testosterone. Age is expressed in years, from 0 to 80 years. (With kind permission of Transworld Research Network. Original Figure 5 'Serum dehydroepiandrosterone sulphate (DHEA-S), total testosterone, free testosterone and 17β-estradiol in men and women in a lifetime scale' from the book chapter 'Female dominance in Sjögren's syndrome – A paradox and a new paradigm' written by Konttinen YT, Spaan M, Stegaev V, Porola P, Lorés M, Vivó A, Koskenpato K, Przybyla BD in 'Sjögren's syndrome associated disorders', edited by Margit Zeher and Peter Szodoray, published in 2009, pp 59–77. ISBN: 978-81-7895-425-7)

ovarian-derived estradiol (E_2) but estrone (E_1) which is formed mainly in extraglandular tissues and lacks cyclic fluctuation.

The two most commonly used artificial sex steroid preparations among women are contraceptives and hormone replacement therapy (HRT). Oral contraceptives consist currently of combined estrogens and progestins or progestin alone and simulate the state of pregnancy in the body, thus preventing ovulation. HRT refers to administration of estrogens with or without progestins for perimenopausal, postmenopausal and surgically menopausal women.

In contrast, in men the serum total testosterone (Testo) levels rise in puberty to approximately 10–38 nmol l^{-1} but only 0.4–2 nmol l^{-1} in women. The lipophilic Testo is in serum mostly bound to sex hormone binding globulin (SHBG), androgen-binding protein and albumin and only approximately 2%–3 % is free, biologically active testosterone. Therefore, free testosterone levels measured in serum are circa 155–800 pmol l^{-1} in men and only 9–30 pmol l^{-1} in women. Finally, the serum levels of the most active androgen dihydrotestosterone (DHT) are approximately 1–10 nmol l^{-1} in adult men but only 0.3–1.2 nmol l^{-1} in women.

Salivary sex steroids

Conjugated steroids, such as dehydroepiandrosterone-sulfate (DHEA-S) and estrone-sulfate (E_1-S), are hydrophilic and negatively charged; their mode of uptake into the tubuloacinar cells and saliva is not quite clear, but may involve organic anion transporting polypeptides (OATP), in salivary glands perhaps OATP-2B1 (Pomari *et al*, 2009). DHEA-S is synthesized in the reticular zone of the adrenal cortex (Figure 2, Konttinen *et al*, in press) and its serum concentrations reach 200-fold higher than those of DHEA, but its salivary concentrations, although significant, may remain at levels lower than 1% of those measured in serum.

Unconjugated, lipophilic sex steroids pass from periductal and periacinar capillary plasma through the lipid bilayer of the cellular plasma membrane by passive diffusion along a concentration gradient and can further access saliva so that the free salivary hormone concentrations reach usually 10% of those of the plasma. Therefore, saliva forms a convenient source of samples, enabling stress-free and repeated sample collection (Gröschl, 2008). In this mini-review this aspect illustrates that sex steroids pass from plasma to salivary glands and saliva.

According to the above mentioned free hormone hypothesis, only free steroids are biologically relevant, whereas carrier bound steroids are inactive because they are blocked from entering target cells (Mendel, 1989; Willnow *et al*, 2008). However, megalin, an endocytic receptor for carrier bound vitamin A and D, also binds SHBG–sex steroid complexes (Hammes *et al*, 2005), followed by internalization of the receptor–ligand complex, degradation of the carrier and release of the sex steroid hormone.

For fertility studies, it would be possible to demonstrate the above mentioned follicular, pre-ovulatory and luteal phases of the menstrual cycle by daily measurement

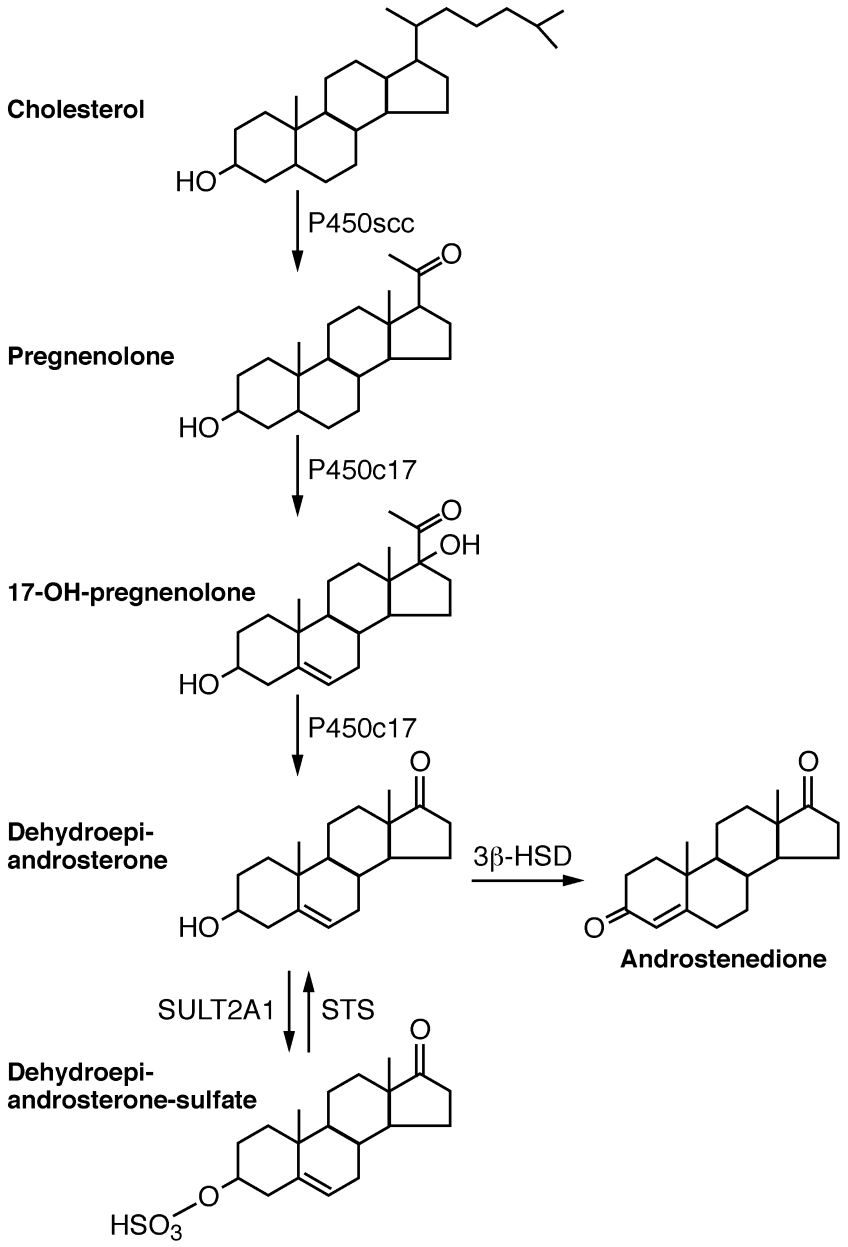

Figure 2 Synthesis of dehydroepiandrosterone-sulfate and androstenedione from cholesterol by steroidogenic enzymes in the reticular zone of the adrenal cortex. P450scc = cholesterol side-chain cleavage enzyme, P450c17 = 17α-hydroxylase, 3β-HSD = 3β-hydroxysteroid dehydrogenase, SULT2A1 = steroid sulfotransferase, STS = steroid sulfatase. (With kind permission of Springer Science and Business Media. Original Figure 4 'Conversion of cholesterol to dehydroepiandrosterone-sulfate in the reticular zone of the adrenal cortex' from the book chapter 'Neurobiology and hormonal control of lacrimal and salivary gland function' written by Konttinen YT, Vivó A, Porola P, Koskenpato K, Lorés M, Pöllänen R, Stegaev V, Virkki L, Spaan M, Przyzbyla BD in 'Sjögren's Syndrome: Pathogenesis and Therapy', edited by Robert I. Fox and Carla Fox, to be published in July 2010. ISBN: 978-1-60327-956-7)

of salivary estradiol and progesterone and, similarly, serum free testosterone correlates so well with the salivary testosterone that also the diagnosis of male hypogonadism should be possible although, at least this far, the more conventional serum measurements are preferred. Salivary measurements in general might offer advantages in sports medicine, pediatrics, veterinary medicine and behavioral research, due to easy and painless sampling (Lewis, 2006). In any case, ample evidence from sialochemistry of sex steroids suggests that salivary glands are exposed to such hormones.

Salivary sex steroid receptors

Sex steroids affect cellular functions by binding to sex steroid receptors, which are ligand-regulated gene transcription factors. A simplified summary of sex steroid receptors in salivary glands is given in Table 1.

Estrogen receptors (ER) were quite early tentatively localized in human salivary glands using ligand binding studies (Dimery *et al*, 1987). However, using immunohistochemical staining of ERs, two groups reported the lack of ERs in human salivary glands (Shick *et al*, 1995;

Table 1 A simplified scheme of androgen receptors (AR), estrogen receptors (ER-α and ER-*β*) and progesterone receptors (PR) in human salivary glands

	AR	*ER-α*	*ER-β*	*Not specified ER*	*PR*
Acini	+[1,2]	−[3,4]	+[4,5]		+/−[7]
Intercalleted ducts	+[2]	−[3,4]	+[4,5]	+[6,7]	+[7]
Striated ducts	+[1,2]	−[3,4]	+[4,5]	+[7]	+[7]
Excretory ducts	+[2]	−[3,4]	+[4,5]		+[7]

[1]Morrell *et al*, 1987; [2]Laine *et al*, 1993; [3]Vadlamudi *et al*, 2005; [4]Ohshiro *et al*, 2006; [5]Williams *et al*, 2007; [6]Weinreb *et al*, 2009; [7]Ozono *et al*, 1992. The '+' indicates presence, the '–' indicates absence, and the '+/– ' indicates an equivocal result.

Leimola-Virtanen *et al*, 2000). These two studies, however, used ER-ICA antibody, which has been later shown to recognize only ERα. A more recent and well controlled immunohistochemical study confirmed the lack of ERα in acinar and ductal cells, using a monoclonal mouse anti-human ERα antibody (ID5, diluted 1:200), but showed the presence of ER*β* in acinar and ductal epithelial cells in human parotid, submandibular and minor salivary glands, in both women and men, using a specific ER*β* 503 antibody (Saji *et al*, 2000) subjected to rigorous antigen blocking control with purified human ER*β* protein (Välimaa *et al*, 2004). This latter work seems to resolve some of the earlier controversies and suggests that the more recently discovered novel ER*β*, rather then the classical gonadal ERα typical for breast and uterine tissues, mediates estrogen effects on salivary glands.

Progesterone receptors were not found in acinar cells studied using a radio labeled progesterone binding assay, although cytoplasmic binding was seen in intercalated, striated and excretory ducts. Immunohistochemical staining suggested the occasional presence of progesterone receptors on acinar cells and in nuclei of some ductal epithelial cells (Ozono *et al*, 1992; Shick *et al*, 1995). On the other hand, in a more recent study, all 26 benign salivary gland tumours studied were negative for progesterone receptors, although 5 out of 52 malignant tumours contained immunoreactive progesterone receptors (Nasser *et al*, 2003).

Androgen receptors (AR) have been shown by immunohistochemistry in parotid, submandibular and minor salivary glands in both sexes in the nuclei of almost all acinar cells and in the majority of nuclei in ductal cells, whereas very few of the nuclei of connective tissue and endothelial cells stained positively (Laine *et al*, 1993). Accordingly, all 26 benign salivary gland tumors studied were immunoreactive for ARs (Nasser *et al*, 2003).

Apart from these classical ligand-regulated intracellular ARs/transcription factors, androgens exert a wide range of rapid, non-genomic actions of potential relevance in salivary gland biology and diseases, such as increase of ornithine decarboxylase and polyamines. These actions may be mediated via classical AR modulating second messenger cascades, a non-classical G-protein coupled AR (e.g. one binding the above mentioned SHBG-androgen complex), direct binding to an ion channel or other targets or membrane fluidity effects (Michels and Hoppe, 2008). Such effects might explain observations, which have suggested that an AR may exist for, e.g. DHEA, separate from that known to exert the effects of Testo and DHT.

It thus seems that ER*β* and AR are the main sex steroid receptors in human salivary glands, found in both acinar and ductal cells, whereas the presence of ERα in tubuloacinar structures is in doubt and the presence and distribution of progesterone receptors requires confirmation. These findings suggest that sex steroids passing from serum to salivary glands can affect the tubuloacinar epithelial cells of these glands.

Salivary glands as sex steroid targets

Although the sexual dimorphism of the granular duct of the submandibular gland in mice has been amply studied, recently a possible sexual dimorphism of human salivary glands has raised interest. Maybe the most convincing evidence comes from DNA-microarray studies, confirmed by real time-polymerase chain reaction. Such a study disclosed two major findings of interest and possibly related to sex steroids. First, 787 genes differed at least 1.5-fold between men and women, with 59% of them showing higher expression in females. These included genes like vesicle-associated membrane protein 3 VAMP3, synaptosomal-associated protein SNAP23, RAS oncogene family member RAB1A and the syntaxin binding protein STXBP1 with potential relevance to salivary gland function and diseases. Second, comparison of the youngest and oldest women revealed 228 gene alterations during aging, including 22 of the 30 probes (73%) associated with potential down-regulation of immune responses (Srivastava *et al*, 2008). This is in line with the earlier observations suggesting that HRT changes total protein and peroxidise and immunoglobulin composition of saliva (Leimola-Virtanen *et al*, 1997b). These gender and age dependent differences may in part relate to the gene copy number or dose differences between XX and XY positive healthy or 47,XXY and 45,XO positive diseased individuals (Scofield, 2009) as well as to hormonal effects. Therefore, studies on aging and HRT could shed more light on this dilemma (Table 2).

Decreased salivary flow rate upon aging has been, although not proven (Ship *et al*, 1991; Evio *et al*, 2006), at least suggested (Percival, *et al*, 1994). On the other hand, salivary flow rate improved, salivary pH increased and the buffering capacity of the saliva was enhanced upon HRT (Laine and Leimola-Virtanen, 1996), which suggests a direct effect of estrogens on salivary gland function. With the recognition of the oral mucosal membrane as direct ER*β*-provided estrogen target (Välimaa *et al*, 2004), and without considering how important the serum and salivary sources of estrogens are, the diminishing feeling of oral dryness and discomfort in postmenopausal women on HRT also provides another potential mechanism of action (Leimola-Virtanen *et al*, 1997a; Eliasson *et al*, 2003). Due to wide

Table 2 Effects of hormone replacement therapy on salivary glands and oral microbes. References with non-confirming results are shown below the table as footnotes

Increased salivation[1]	Hietala *et al*, 1993; Laine and Leimola-Virtanen, 1996; Eliasson *et al*, 2003; Yalçin *et al*, 2005
Ease of post-menopausal oral discomfort[2]	Leimola-Virtanen *et al*, 1997b; Eliasson *et al*, 2003; Yalçin *et al*, 2005
Improvement of salivary buffer capacity[3]	Hietala *et al*, 1993; Laine and Leimola-Virtanen, 1996
Increase in the output of salivary peroxidase	Leimola-Virtanen *et al*, 1997b
Decrease in the concentration of salivary calcium	Sewón *et al*, 2000
Lower scores of lactobacilli[4]	Hietala *et al*, 1993

[1] Ship *et al*, 1991; Eviö *et al*, 2006; [2] Eviö *et al*, 2006; [3] Eviö *et al*, 2006; [4] Leimola-Virtanen *et al*, 1997b.

inter-individual variation, many of these clinical studies or series suffer from the lack of power and results are partly inconclusive, but it seems that gender and in part sex steroids affect salivary glands and mucosal membranes.

Salivary glands as intracrine organs

One of the major reasons for the slow advancement of ideas of salivary glands as sexually dimorphic organs relates to the fact that primates are unique since they possess an endocrine organ producing large quantities of DHEA and androstenedione pro-hormones and apparently multiple intracrine peripheral organs, which tailor-make their steroids from these pro-hormones for their local intracrine needs (Figure 3). In fact, this incracrine enzyme apparatus can be considered as a buffer against circadian, menstrual, pregnancy and chronobiological changes, which the human body encounters during its hopefully long and happy life. This compensatory mechanism in the human body may obscure the sexual dimorphism of the human salivary glands. On the other hand, rodents, such as mice, rats and rabbits, often used for experimental endocrine work, are not *per se* suitable for intracrine studies, and therefore aspects important for the human salivary glands would be easily missed.

Serum DHEA-S can be desulfated by steroid sulfatase, but also resulfated by sulfontransferase back to DHEA-S, and then further metabolized to DHT or E_2 via several additional enzymes. Notice that considering an eventual DHT demand in female salivary glands, several enzymes have to act in concert and the reaction has to proceed along the right metabolic pathway, without going astray, to be able to produce DHT from DHEA-S.

Intracrine enzyme architecture (Figure 4) is such that those enzymes, which participate in the proximal metabolic pathways, seem to locate close the serum DHEA-S and DHEA source, near the basolateral aspects of the epithelial cell. Interestingly, 5α-reductase isotype 1, the major isotype found in the healthy human labial salivary glands, was largely localized in the acinar cell nuclei, which at first glance seems astonishing thinking of the regular idea of cytoplasmic AR binding its Testo or DHT ligand, followed by translocation of the complex to the nucleus (Spaan *et al*, 2009). Recent studies have disclosed a much more complex regulated nuclear import, nuclear export and nuclear mobility between subnuclear compartments, i.e. AR shuttling, than was earlier envisioned (Black and Paschal, 2004). Localized synthesis close to AR and its co-activators and the classical androgen-responsive elements might add to the site-specific regulated androgen action. Another interesting finding was that aromatase seemed to be located in the apical acinar cell membrane, as if E_1 and E_2 would be produced at a site physically separate from DHT and suitable for export to saliva and further to the oral mucosa.

Sex steroids in salivary gland diseases and aging

Normal salivary gland function in the light of sex steroids seems complicated enough, yet medicine looks for some additional evidence related to their eventual role in human diseases.

Maybe the hallmark disease of the salivary glands in this respect is Sjögren's syndrome (SS), characterized by dryness of the eyes and mouth and diminished exocrine secretory function of the lacrimal and salivary glands, which occurs in an autoimmune context, in the presence of focal sialadenitis and/or autoantibodies (SS-A/Ro and/or SS-B/La). One unexplained but characteristic feature of this syndrome is its skewed gender distribution, with nine out of ten patients being women, who in addition often contract their disease at the time of menopause, when they are 40–50 years old. It was first described by Sigridur Valtysdottir and her coworkers that patients with primary SS are characterized by low serum DHEA-S values (Valtysdóttir *et al*, 2001), as if the reticular zone of their adrenal glands would be failing. In the light of current data it seems that such diminution in the feeding of the salivary intracrine enzyme machinery with DHEA substrate may act as a stress factor, unmasking a subclinical failure to locally to produce enough DHT from DHEA-S to maintain glandular remodeling.

This was first suggested by low salivary DHEA concentrations in SS coupled with low glandular and salivary levels of an androgen-regulated biomarker, cysteine-rich secretory protein-3 (Laine *et al*, 2007) and supported by a salivary sex steroid profile in primary SS (Porola *et al*, 2008), also during DHEA substitution treatment (Virkki *et al*, in press; Porola *et al*, unpublished data). Although not quite clear yet, the underlying mechanism may include deficient uptake of DHEA-S, its ineffective processing to DHT and/or its diversion into other sex steroids and sex steroid metabolites (Spaan *et al*, 2009).

Only recently the eventual mechanism of the effect of the intracrine deficiency started to become clear,

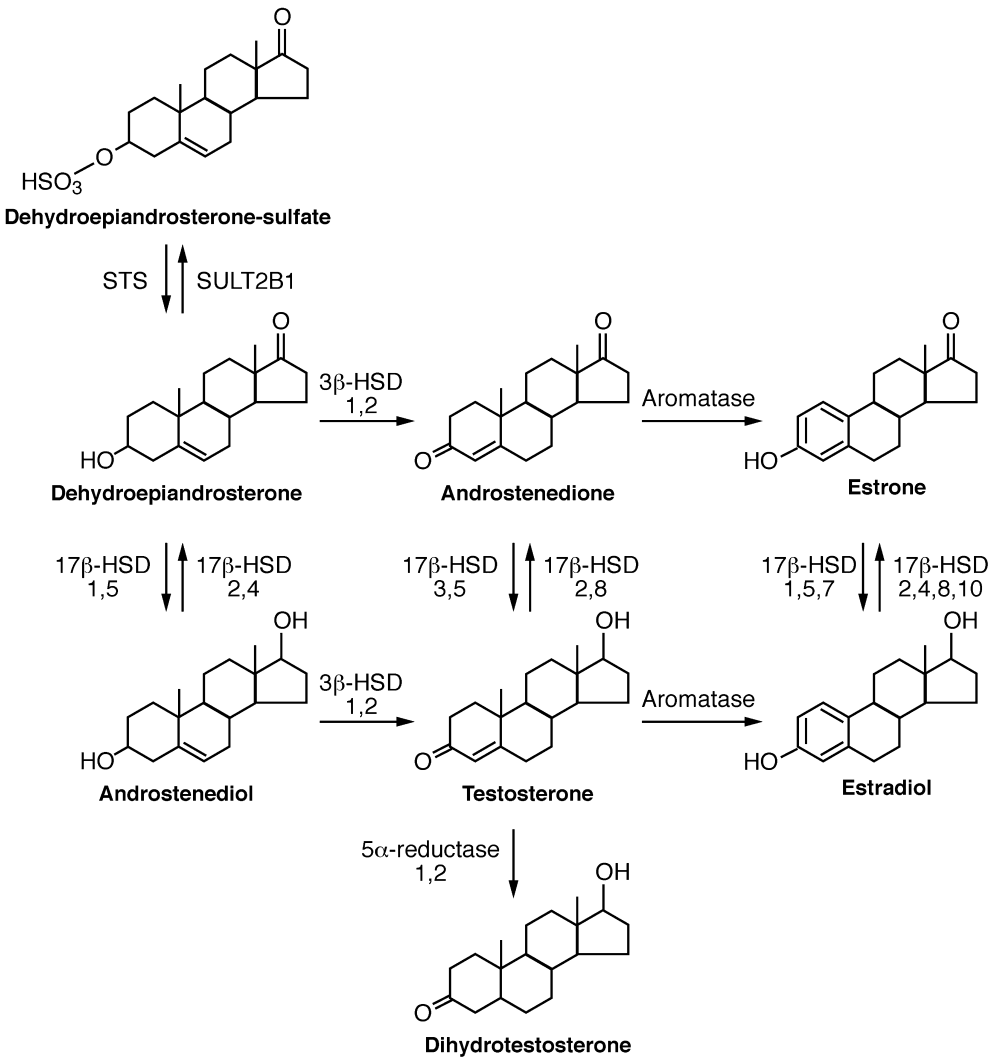

Figure 3 Intracrine processing of dehydroepiandrosterone-sulfate to 17β-estradiol and dihydrotestoserone in peripheral human tissues. STS = steroid sulfatase, SULT2B1 = steroid sulfotransferase, 3β-HSD = 3β-hydroxysteroid dehydrogenase, 17β-HSD = 17β-hydroxysteroid dehydrogenase. Various isoforms are indicated by a number after the name or abbreviation of the enzyme. (With kind permission of Springer Science and Business Media. Original Figure 6 'Intracrine metabolism of DHEA-S in peripheral tissues' from the book chapter 'Neurobiology and hormonal control of lacrimal and salivary gland function' written by Konttinen YT, Vivó A, Porola P, Koskenpato K, Lorés M, Pöllänen R, Stegaev V, Virkki L, Spaan M, Przyzbyla BD in 'Sjögren's Syndrome: Pathogenesis and Therapy', edited by Robert I. Fox and Carla Fox, to be published in July 2010. ISBN: 978-1-60327-956-7)

indicating that it affects the maintenance of salivary acini. Intercalated duct cells have earlier been suggested to represent acinar cell progenitors based on labeling studies (Man *et al*, 2001), cloning of interacted duct cells (Sato *et al*, 1987) and studies on SS, which is characterized by acinar cell atrophy and loss, i.e. failure of acinar maintenance (Laine *et al*, 2007). It seems that laminin-111 (LM-111) both in *in vivo* and *in vitro* experiments is necessary for the intercalated duct-to-acinar cell trans-differentiation. Such LM-111 is restricted to the basement membrane of the acinar compartment of the salivary glands, but not found in the basement membrane around the salivary ducts. The growth factor-depleted Matrigel contains the very same LM-111, which was used in the above mentioned cloning and trans-differentiation experiments (Sato *et al*, 1987); growth factors are depleted to get rid of an eventual confounding factor.

Intercalated duct cells express and seem to use integrin $\alpha_1\beta_1$ and integrin $\alpha_2\beta_1$ receptors for two different but related functions (Laine *et al*, 2008). The intercalated duct progenitor cells probably undergo asymmetric divisions, one of the daughter cells maintaining the progenitor cell pool (unipotent stemness), while the other terminally differentiates to an acinar cell. It migrates from the intercalated duct to the acinus and once there, uses LM-111-to-Int $\alpha_1\beta_1$ and $\alpha_2\beta_1$ outside-in signaling for trans-differentiation. Ongoing work suggests that DHEA, upon conversion to DHT, up-regulates these integrin $\alpha_1\beta_1$ and $\alpha_2\beta_1$ receptors ten-fold, thus facilitating their migration and trans-differentiation (Porola *et al*, unpublished data). Such a

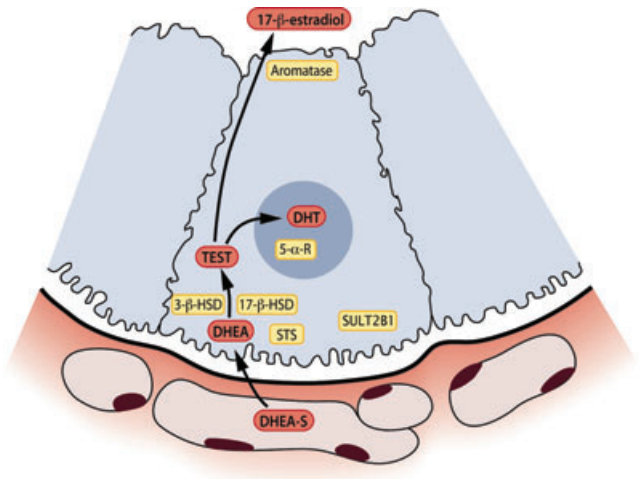

Figure 4 Intracrine enzymes and their tissue architecture in the acinar cells of the human salivary glands as assessed based on immunohistochemical staining of human labial salivary glands. STS = steroid sulfatase, SULT2B1 = steroid sulfotransferase, 3β-HSD = 3β-hydroxysteroid dehydrogenase, 17β-HSD = 17β-hydroxysteroid dehydrogenase, 5-α-R = 5α-reductase, TEST = testosterone, DHT = dihydrotestosterone, DHEA = dehydroepiandrosterone, DHEA-S = dehydroepiandrosterone sulfate. (With kind permission of John Wiley & Sons Ltd. Original Figure 7 'The architecture of the steroidogenic enzymes in a healthy salivary gland acinar cell' in original article 'Healthy human salivary glands contain a DHEA-sulphate processing intracrine machinery, which is deranged in primary Sjögren's syndrome' written by Spaan M, Porola P, Laine M, Rozman B, Azuma M, Konttinen YT and published in Journal of Cellular and Molecular Medicine, 2009 Jul;13(7):1261–70)

link would nicely tie together the normal maintenance of acinar cells and its androgen support.

Much more attention has been paid to the eventual role of sex steroids for lymphoid tissue and cell function than to their effects on tubuloalveolar epithelial cells. Both central and peripheral lymphoid organs co-express ER-α and ER-β (Brandenberger *et al*, 1997; Enmark *et al*, 1997; Kuiper *et al*, 1997; Shim *et al*, 2006). Estrogens affect postnatal thymocyte development (Screpanti *et al*, 1991; Okuyama *et al*, 1992; Rijhsinghani *et al*, 1996; Okasha *et al*, 2001; Staples *et al*, 1999) and reduce B cell precursors in the bone marrow (Smithson *et al*, 1994).

ER-α and ER-β have also been identified on purified CD4+ T cells (Phiel *et al*, 2005), predominantly express ER-α, whereas B cells exhibit the opposite pattern showing a predominant ER-β expression, while CD8+ T-lymphocytes express low and equal levels of both receptors (Phiel *et al*, 2005). Estrogens regulate T cell migration by affecting their chemokine-receptor expression and cytoskeletal reorganization (Mo *et al*, 2005; Acconcia *et al*, 2006; Simoncini *et al*, 2006; Lengi *et al*, 2007). The expression of co-stimulatory molecules, pivotal in T cell activation (e.g. CD40L), is altered by estrogens (Rider *et al*, 2001; Quezada *et al*, 2004). Low doses of estrogen seem directly and indirectly to induce expression of interferon-γ in T cells, while high estrogen levels associate with the induction of T-helper-2 responses (Pernis, 2007). Estrogens expand TNF-α producing T cells by multiple mechanisms (Weitzmann and Pacifici, 2006).

Pregnancy doses of estradiol slow down maturation of pro-B cells to early pre-B cell stage (Medina *et al*, 1994, 2001) and also other stages of B cell development are in part regulated by estrogens and estrogen derivates (Grimaldi *et al*, 2001). Estrogen induces a genetic program that alters survival and activation of B cells and thus skews the naive immune system toward autoreactivity (Grimaldi *et al*, 2002). It is concluded that sex steroids may affect SS and focal adenitis also via their effects on immune cells.

Apart from the above mentioned SS, examples of run-away diseases caused by pathological effects of sex steroids on salivary gland cells are seen in many different types of salivary gland cancers that have been shown to carry androgen or estrogen receptors and/or to be regulated by sex steroids or sex steroid depletion (Nasser *et al*, 2003; Williams *et al*, 2007; Sygut *et al*, 2008). ARs can be used for the classification of some tumors and in the treatment of these diseases in particular androgen deprivation treatments appear promising so that partial or complete remissions of parotid gland carcinoma have been reported (van der Hulst *et al*, 1994; Locati *et al*, 2003). There are also reports of successful usage of the ER antagonist tamoxifen in the treatment of salivary gland adenoid cystic carcinoma (Shadaba *et al*, 1997; Elkin and Jacobs, 2008) although contradictory reports exist indicating no or only minor expression of estrogen and progesterone receptors in salivary gland cancers (Miller *et al*, 1994; Dori *et al*, 2000).

Effects of aging are quite dramatic on human parotid, submandibular and labial salivary glands so that their acinar volume decreased by 32% (Scott *et al*, 1987), 37% (Scott, 1977) and 45% (Scott, 1980), respectively, between adult maturity and old age. If this is in part due to failure of androgen support and failure of maintenance of acini is not known at present.

Acknowledgments

This study was supported by the Academy of Finland, HUCS evo grant, ORTON Orthopaedic Hospital of the Invalid Foundation, Finska Läkaresällskapet, CIMO, Biomedicum Foundation, Orion-Farmos Foundation, Emil Aaltonen Foundation, Maire Lisko Foundation and the Finnish PhD Graduate School in Musculoskeletal Diseases and Biomaterials.

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