HISTOCHEMICAL LOCALIZATION OF HYDROXYSTEROID DEHYDROGENASES IN THE MAXILLARY GLANDS OF PIGS

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The maxillary salivary gland of the boar is known (Patterson, 1968) to be rich in androstenol (3α -hydroxy- 5α -androst-16-ene) and to be capable of converting pregnenolone to 3β -hydroxyandrost-5,16-diene (Katkov & Gower, 1969). In addition, it has been suggested by Patterson (1968) that the maxillary gland may be a site of origin of androstenone (5α -androst-16-ene-3-one), but this compound is also present in testicular venous plasma (Gower, Harrison & Heap, 1970). Androstenone has pheromonal properties in the pig (Melrose, Reed & Patterson, 1971) and is apparently released during the profuse salivation which occurs in the male at the time of copulation. In an attempt to elucidate the site and nature of steroid metabolism in the maxillary gland, its hydroxysteroid dehydrogenases were studied histochemically. Hydroxysteroid dehydrogenases acting at the 11β position have already been reported in the ducts of porcine salivary glands (Ferguson, Glen & Mason, 1970): the present study is concerned with those acting at positions 3 and 17.

Maxillary and parotid glands were obtained from one male and one female fetus at 56, 73, 78 and 84 days' gestation, one male and one female piglet at 5 days of age, four gilts and four castrate males of about 80 kg live weight, six entire males with well-developed sex odour ranging from 80 to 100 kg live weight and three pregnant sows aged 4 to 5 years. In addition, maxillary glands alone were obtained from four 100-day-old gilts. The tissues were removed from the carcass at slaughter and frozen by vigorous agitation in liquid nitrogen.

The histochemical demonstration of hydroxysteroid dehydrogenases has been fully described by Baillie, Ferguson & Hart (1966). In the present study, cryostat sections were cut at $10\ \mu\text{m}$, mounted on coverslips and incubated in medium containing substrate (0.5 mmol/litre), nicotinamide-adenine dinucleotide (NAD—0.25 mg/ml) and nitro blue tetrazolium (0.1 mg/ml) in a phosphate buffer (0.5 mol/litre) adjusted to pH 7. The steroid solvent was dimethyl-formamide which proved satisfactory for all substrates other than androstenol which formed a heavy precipitate on addition to the aqueous medium. Control sections were incubated in medium lacking substrate and in medium lacking both substrate and NAD but containing NADH_2 (0.25 mg/ml). As an additional control, bovine and ovine maxillary gland tissue from adult females and castrate males was incubated in all media. The substrates used were testosterone and oestradiol- 17β (for 17β -hydroxysteroid dehydrogenases) dehydroepiandro-

sterone (Δ^5 - 3β), androsterone and androstenol (5α - 3α), 3α -hydroxy- 5β -androstan-17-one (5β - 3α), 3β -hydroxy- 5α -androstan-17-one (5α - 3β) and 3β -hydroxy- 5β -androstan-17-one (5β - 3β). An incubation time of 1 hr at 37°C was used with all substrates other than androstenol which was incubated for 2 hr. The substrate-free controls were also incubated for 2 hr.

Slight histochemical activity was present in a few ducts in the maxillary glands of the 78- and 84-day fetuses when 5α - 3α and 3β -hydroxy substrates were used (Pl. 1, Fig. 1); by 5 days *post partum*, activity was well developed in both the ducts and the acinar tissue (Pl. 1, Fig. 2). Similar strong reactions were seen in the 100-day-old gilts (Pl. 1, Fig. 3) and in all the older pigs. Activity was most intense in the excretory and striated ducts, less intense in the intercalated ducts and slight in the serous demilunes (Pl. 1, Figs 3 to 5). The mucous cells showed no activity. There was a clear difference between the distribution of pigment following incubation with 5α - 3α and 3β -hydroxy substrates. Activity in the ducts was particularly intense after the use of 3β substrates and that in the serous demilunes relatively slight (Pl. 1, Fig. 5) and the section appeared finely stippled to the naked eye; 5α - 3α substrates also gave maximal activity in the ducts but the contrast between these and the remainder of the gland was much less marked (Pl. 1, Fig. 6), and macroscopically the sections appeared uniform. Though activity was present in the parotid glands of pigs of 80 kg and over, the level of activity was very much less than that seen in the maxillary glands. However, as in the maxillary glands, the deposition of pigment was usually greatest in the ducts, though even here the rate of substrate oxidation was little above the minimum detectable by the method. Though the parotid and maxillary glands showed markedly different characteristics, there were no apparent differences in the enzyme distributions of boars, sows, gilts and hogs (Pl. 1, Figs 4 and 5). The intense Δ^5 - 3β hydroxysteroid dehydrogenase activity found in the serous cells of the mature boar by W. D. Booth, H. M. Dott and M. F. Hay (personal communication) was not seen: this is almost certainly attributable to the relative immaturity of the boars used in the present study.

No histochemical activity was detected in the substrate-free controls though strong reactions always followed incubation with NADH_2 indicating adequate levels of the diaphorase linking the oxidation of NADH_2 to the reduction of nitro blue tetrazolium. No activity was found in any of the glands taken from 56- and 73-day fetuses or in parotid glands taken from any of the fetuses or the 5-day-old piglets. Likewise, no activity was found in the ruminant salivary glands examined and no reactions were obtained from any of the tissues following incubation with 17β -hydroxy substrates and 3α -hydroxy- 5β -androstan-17-one.

These results suggest that the maxillary gland of the pig can be an important site of steroid metabolism and they are therefore in agreement with those of Patterson (1968) and Katkov & Gower (1969). The fact that only minimal activity was observed in parotid tissue is consistent with the finding that androstenone levels in the parotid gland differ little from those in adipose tissue (Claus, Hoffman & Karg, 1971) and with the absence of sex odour in the parotid glands of all but the most heavily tainted boar carcasses (Patterson, 1968). The results show that steroid metabolism is most likely to occur in the

FIG. 1. The maxillary gland of an 84-day pig fetus showing that $5\beta-3\beta$ -hydroxysteroid dehydrogenase activity is confined to a few cells of the excretory duct epithelium. $\times 95$.

FIG. 2. The ducts and acinar tissue of the maxillary gland of a 5-day-old gilt showing the distribution of $5\beta-3\beta$ -hydroxysteroid dehydrogenase activity. $\times 95$.

FIG. 3. The maxillary gland of a 100-day-old gilt showing that moderate $5\beta-3\beta$ -hydroxysteroid dehydrogenase activity is present in the serous demilunes. There is intense activity in the striated ducts on the right. $\times 95$.

FIG. 4. The maxillary gland of a castrate male showing $5\beta-3\beta$ -hydroxysteroid dehydrogenase activity. $\times 95$.

FIG. 5. The maxillary gland of a boar showing $\Delta^5-3\beta$ -hydroxysteroid dehydrogenase activity. $\times 95$.

FIG. 6. The maxillary gland of a boar showing $5\alpha-3\alpha$ -hydroxysteroid dehydrogenase activity. Deposition of pigment in the striated ducts is significantly less than that seen in Fig. 5 which shows an adjacent section. $\times 95$.

ducts but may also occur to a limited extent in the serous demilunes. Further, they indicate the possibility of hydroxysteroid dehydrogenase secretion in the saliva. The fact that 3α and 3β substrates gave different pigment distributions suggests that at least two enzymes are involved, one utilizing 3β -hydroxy substrates having 5α , 5β or Δ^5 configurations and one utilizing 3α -hydroxy substrates with the 5α configuration but not those with the 5β form. The ability of the 3α enzyme to utilize androstenol suggests that it may be responsible for the oxidation of androstenol to androstenone and it could account for the fact that, while the boar maxillary gland smells most strongly of the alcohol, the saliva, breath and fat smell most strongly of the ketone (Patterson, 1968). The function of hydroxysteroid dehydrogenases in the maxillary glands of females, castrates and young pigs is unexplained though it seems possible they form part of a mechanism whereby internal changes in sex hormone status are expressed as an external olfactory signal.

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