Steroid Metabolism by Rabbit Olfactory-Specific P450 2G1

Xinxin Ding and Minor J. Coon
Department of Biological Chemistry, Medical School, University of Michigan, Ann Arbor, Michigan 48109-0606

Received June 7, 1994, and in revised form August 16, 1994

Cytochrome P450 2G1 (2G1), which is uniquely expressed in the olfactory mucosa in mammals, may have important physiological functions. In the present study, we have examined the catalytic activity of rabbit 2G1 toward a number of steroid sex hormones, including androstenedione, estradiol, progesterone, testosterone, and 5α-dihydrotestosterone; the purified cytochrome is active toward all of these compounds in a reconstituted enzyme system with turnover numbers of 1.84, 0.34, 1.46, 1.04, and 0.84, respectively, at a substrate concentration of 5 μM. In the presence of cytochrome b5, the turnover numbers are 1.58, 0.66, 1.66, 2.74, and 1.34, respectively. Estradiol is converted to the 2-hydroxy compound (major product) and 4-hydroxy compound (minor product) by 2G1, and progesterone is converted to the 16α-hydroxy derivative as well as the corresponding keto compound as a secondary product. The same products are formed in olfactory microsomal suspensions as major metabolites of progesterone, and the reactions are inhibited strongly by anti-2G1 IgG. In a reconstituted system, 2G1 has an apparent Km of 2.0 μM and a Vmax of 1.8 nmol/min/nmol P450 for the formation of the 16α-hydroxyprogesterone. Of particular interest, 2G1-catalyzed progesterone metabolism is effectively inhibited by the boar pheromones, 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol, and to a lesser extent by a variety of odorant compounds as well as by known P450 inhibitors, including ketoconazole and α-naphthoflavone. The broad substrate specificity and relatively high catalytic efficiency of 2G1 in sex steroid metabolism suggest a role for this unique P450 isozyme in the maintenance of steroid hormone homeostasis in the olfactory mucosa.

Key Words: nasal P450; P450 2G1; P450 NMB; cytochrome b5; sex steroids; steroid metabolism; olfactory mucosa.

The olfactory mucosa is the site of constant exchange of chemicals between the external environment in the nasal cavity and the rich blood supply in the submucosa. Lipophilic substances, such as endogenous steroid hormones or exogenous odorants, partition favorably into the membranous structure of the neuroepithelium. Thus, the efficient biotransformation system that converts these lipophilic compounds into metabolites with greater solubility is indispensable for maintaining the homeostasis of the chemosensory tissue. CYP2G1 is an abundant P450 cytochrome expressed specifically in the olfactory mucosa of mammalian species and is capable of catalyzing such transformations, as reviewed elsewhere (2). Rabbit 2G1, which is also called P450 NMB, was first purified to electrophoretic homogeneity from rabbit nasal microsomes and characterized in this laboratory (3), and its primary structure was subsequently determined by cDNA cloning (4). Rat 2G1, which is also called P450 olf1, was identified by cDNA cloning (5) and its gene structure has also been determined (6), but the enzyme has not been purified and its catalytic properties have therefore not been reported. The tissue-specific expression and early developmental expression of 2G1 have been demonstrated in both rat and rabbit, and the findings have led to speculation about the possible involvement of this P450 isozyme in physiological functions important for the olfactory chemosensory system (7-11).

1 This investigation was supported by Grants DC-01990 (to X.D.) and DK-10339 (to M.C.) from the National Institutes of Health.
2 Current address: Laboratory of Human Toxicology and Molecular Epidemiology, Division of Environmental Disease Prevention, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201-0509.
3 Abbreviations used: CYP2G1 or 2G1, cytochrome P450 isoform 2G1 (see Ref. 1 for updated P450 nomenclature); b5, cytochrome b5; and ANF, α-naphthoflavone.

Copyright © 1994 by Academic Press, Inc.
All rights of reproduction in any form reserved.
Our previous studies on the substrate specificity of rabbit 2G1 indicated that this isoform is active toward testosterone (3, 7) and arachidonic acid (12), as well as several xenobiotic compounds, including odorants, but not toward certain other endogenous substances, including prostaglandin E_2, retinoic acid, and retinol (cf. 2). In the present investigation, we have further examined the activity of 2G1 toward a number of sex steroids at physiologically relevant substrate concentrations and the effects of exogenous compounds, including odorants and the boar pheromones, 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol (13), on olfactory microsomal steroid metabolism. Our results indicate that 2G1 is highly active in the metabolism of all three classes of major sex steroid hormones, including androst-4-ene-3,17-dione, estradiol, 5α-dihydrotestosterone, testosterone, and progesterone, in a reconstituted enzyme system and apparently plays a major role in steroid hydroxylation in olfactory microsomes.

MATERIALS AND METHODS

Steroid hydroxylation assays. All enzymatic reactions were initiated by the final addition of NADPH. For reactions in which bH was included, this cytochrome was added to the reaction mixture after the addition of the reconstituted P450 system, as described previously (14). The contents of the various reaction mixtures are described in detail in the table and figure legends. For quantitation of steroid metabolites, the reactions were stopped by the addition of an ice-cold solution of perchloric acid and methanol at final concentrations of 2 and 45%, respectively. Each mixture was centrifuged, and an aliquot of the supernatant layer was analyzed by HPLC as described below. The recovery of labeled steroids in the supernatant fraction was nearly complete.

HPLC analysis of steroid metabolites was performed essentially as previously described by van der Hoeven (15) and Wood et al. (16) for metabolites of testosterone, with use of a Novapak C18 reverse-phase column from Waters (150 x 3.9 mm i.d.) and a liquid chromatographic system as described previously (17). The metabolites were eluted isocratically with a methanol/water/acetonitrile (47.5/42.5/10) mobile phase for androst-4-ene-3,17-dione, 5α-dihydrotestosterone, estradiol, and testosterone metabolites, and a methanol/water/acetonitrile (52.5/37.5/10) mobile phase was used for progesterone metabolites at a flow rate of 1.0 ml/min. Elocite(+1) liquid scintillation solution was added to the flow detector at a level of 2.0 ml/min.

The steroid metabolites were quantitated by determination of the radioactivity of the individual peaks as determined by an on-line flow detector, and the amounts were calculated based on the recovery of total radioactivity. For studies on the effects of odorants and pheromones on olfactory microsomal progesterone metabolism, unlabeled progesterone was used and the levels of the 16α-hydroxy product and starting material in the reaction mixtures were quantitated by determination of the absorbance of the individual peaks at 240 nm. The amounts were calculated by comparison with the absorbance of authentic standards.

TLC separation of estradiol and its metabolites was performed according to Schaub and Johnson (18). The reactions were stopped by the addition of ice-cold chloroform, and the organic extracts were evaporated to dryness under nitrogen gas. The dried residues were resuspended in 0.05 ml of ethyl acetate and applied with a micropipet to channeled Silica Gel (250-μm) GF TLC plates (Analtech, Newark, DE) previously treated with ascorbic acid. The chromatograms were developed with ethyl acetate/cyclohexane (1:1). 14C-labeled estradiol and its metabolites were visualized by autoradiography, and the metabolites were identified by direct comparison of the relative mobilities with those of authentic compounds as visualized under UV light. The TLC system used permitted simultaneous resolution of estrone, estradiol, and estradiol hydroxylated in the 2, 4, 6α, or 16α position.

Other methods. Microsomes prepared from the olfactory mucosa of adult male or female New Zealand White rabbits as described (7) had 0.4 to 0.7 nmol of P450 per mg of protein. Protein was determined by the method of Lowry et al. (19), and the concentration of total P450 in microsomal suspensions was determined according to Omura and Sato (20). Electrophoretically homogenous 2G1 was prepared from rabbit olfactory microsomes as described previously (3, 21) and had a content of 16.4 nmol/mg of protein. The method of Strittmatter et al. (22) was used for the purification of bH from rabbit liver microsomes. The preparations had specific contents of 40 to 50 nmol of bH/mg of protein. NADPH-P450 reductase was purified from microsomes obtained from phenobarbital-treated rabbits (23) and had a specific activity of 45 to 60 nmol of cytochrome c reduced per minute per milligram of protein at 30°C. The preparation and characterization of polyclonal antibodies to rabbit 2G1 have been described (7).

Materials. [4-14C]Androst-4-ene-3,17-dione, [1,2,4,5,6,7-3H(N)]5α-dihydrotestosterone, [6,7-3H(N)]estradiol, [4-14C]estradiol, [1,2,6,7,21-3H(N)]progesterone, and [1,2,6,7-3H(N)]testosterone were from Du Pont—New England Nuclear. Unlabeled steroids, including 5α-dihydrotestosterone, 19-nortestosterone, testosterone, progesterone, 11α-, 11β-, 16α-, 17α-, 20α-, and 20β-hydroxyprogesterone, estradiol, and estrone were from Sigma; 2α-, 6α-, 6β-, and 21-hydroxyprogesterone, 4-, 6α-, and 16α-hydroxyestradiol, 5α-androst-16-en-3-one, and 5α-androst-16-en-3α-ol were from Steroloids; and odorants, ketaconazole, and ANF were from Aldrich. The sources of standards for testosterone derivatives have been described earlier (9), and other materials were obtained as described previously (3, 6, 17).

RESULTS AND DISCUSSION

Activity of P450 2G1 toward a variety of sex steroids. The turnover numbers of androst-4-ene-3,17-dione, estradiol, progesterone, 5α-dihydrotestosterone, and testosterone metabolism in a reconstituted enzyme system containing 2G1, P450 reductase, and phospholipid are shown in Table I. At a substrate concentration of 5 μM, these steroids were metabolized by 2G1 at relatively high rates, with turnover numbers ranging from 0.34 to 1.84 nmol of total metabolites formed per minute per nanomole of the cytochrome at 37°C. In experiments not presented, 2G1 was also shown to be active toward other steroids, including 19-nortestosterone, 17-hydroxyprogesterone, and 16α-hydroxyprogesterone.

Multiple metabolites were formed from 5α-dihydrotestosterone (retention time, 17.3 min), with the two major products having retention times of 3.8 and 4.6 min. Several metabolites were also formed from androst-4-ene-3,17-dione (retention time, 10.2 min), with the major metabolite having a retention time of 4.7 min. The structures of the metabolites of 5α-dihydrotestosterone and androst-4-ene-3,17-dione have not been determined. The identification of the testosterone metabolites, including the 15α-, 16α-, 11α-, and 16β-hydroxy compounds, has been reported previously (3, 7), and one additional metabolite that did not comigrate with available standards of testosterone derivatives has been found by direct probe mass spectrometry to correspond to a keto derivative, most likely 15-ketotestosterone (unpublished data). Two metabolites that were formed from estradiol comigrated with
authentic 2- and 4-hydroxy standards upon TLC analysis (Fig. 1).

The HPLC profile of progesterone metabolites generated by purified 2G1 is shown in Fig. 2. The identification of the 16α-hydroxy compound was made by a direct comparison of its retention time with that of the authentic standards, which included the 2α-, 6α-, 6β-, 11α-, 11β-, 16α-, 17α-, 20α-, 20β-, and 21-hydroxy compounds. The other metabolite, which did not comigrate with any of the available standards, was tentatively identified as the 16-keto compound. This conclusion was based on the observation that the retention time, which suggested a monohydroxy or monoketo derivative of the parent compound, was identical to that of a metabolite of authentic 16α-hydroxyprogesterone in reactions with purified 2G1 in a reconstituted system containing b5 (data not shown).

Furthermore, this metabolite had absorbance at 240 nm, indicating that reduction of the conjugated double bonds in the A ring had not occurred. Interestingly, the conversion of the recovered 16α-hydroxyprogesterone to the 16-keto compound was much slower than the rate of formation of the keto steroid from progesterone, suggesting that the newly formed hydroxy compound is further oxidized without leaving the active site.

**Effects of b5 on steroid oxidation.** The level of b5 in olfactory mucosa is quite high, about 0.7 nmol per milli-

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Turnover No. (nmol products/min/nmol 2G1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No b5</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>1.04</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>1.84</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.84</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.34</td>
</tr>
<tr>
<td>2-Hydroxyestradiol formation</td>
<td>0.24</td>
</tr>
<tr>
<td>4-Hydroxyestradiol formation</td>
<td>0.10</td>
</tr>
<tr>
<td>Progesterone</td>
<td>1.46</td>
</tr>
<tr>
<td>16α-Hydroxyprogesterone formation</td>
<td>1.20</td>
</tr>
<tr>
<td>16-Ketohydroxyprogesterone formation</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* Incubation mixtures contained 25 pmol of 2G1, 100 pmol of NADPH-P450 reductase, 15 μg of dilauroylglyceryl-3-phosphorylcholine, 50 mM phosphate buffer, pH 7.4, 1 mM ascorbic acid, substrate added in 10 μl of methanol, 1 mM NADPH, and, when applicable, 100 pmol of b5 in a final volume of 1.0 ml. The substrates used (5 μM final concentration) were [4,14C]androst-4-ene-3,17-dione (53.9 mCi/mmol), [1,2,4,5,6,7,11H(N)]dihydrotestosterone (2.0 Ci/mmol), [6,7,11H(N)]estradiol (2.0 Ci/mmol), [1,2,6,7,12H(N)]progesterone (2.1 Ci/mmol), and [1,2,6,7,12H(N)]testosterone (6.0 Ci/mmol). The reactions were carried out in duplicate at 37°C for 10 min. Procedures for HPLC analysis and quantification of metabolites are described under Materials and Methods.

* Unless otherwise indicated, the combined rates of formation of all metabolites are reported.

---

gram of microsomal protein from adult rabbits (unpublished data). Addition of b5 to the incubation mixtures resulted in significant changes in the rates of steroid metabolism, as shown in Table I. Thus, whereas b5 stimulated 5α-dihydrotestosterone metabolism by almost threefold, it caused a slight inhibition of androst-4-ene-3,17-dione metabolism. With estradiol, the formation of both the 2- and the 4-hydroxy derivatives was increased by b5 (Table I and Fig. 1), but with progesterone (5 μM) the formation of the 16-keto but not the 16α-hydroxy compound was increased by b5. Interestingly, addition of b5 to the reaction mixture with 15 nM progesterone (a known physiological concentration) resulted in stimulation of formation of both products in the first few minutes (data not shown). At longer times, much of the hydroxy product was converted to the keto steroid (Fig 2). In experiments not presented, alteration of product stoichiometry by b5 was also observed with testosterone.

**Kinetic analysis and inhibition of 2G1-catalyzed progesterone metabolism.** The kinetic parameters of 16α-hydroxyprogesterone formation in the reconstituted system containing 2G1 are shown in Table II. The K_m and V_max values were determined from Lineweaver–Burk and Eadie–Hofstee plot analyses (not shown) with substrate concentrations in the range of 0.15 to 7.5 μM. The amounts

---

**FIG. 1.** Autoradiogram of TLC separation of estradiol and metabolites generated in incubations with purified P450 2G1. Complete incubation mixtures contained 50 mM potassium phosphate buffer, pH 7.4, 1 mM L-ascorbic acid, 5 μM [1,2,6,7,12C]estradiol (56.4 mCi/mmol) added in 10 μl of methanol, and a reconstituted system containing 0.1 μM 2G1, 0.3 μM NADPH-P450 reductase, and 30 μg of phospholipid in a final volume of 1.0 ml. When applicable, 0.4 mM b5 was also included. The reaction was carried out at 37°C for 20 min. Control reactions were performed without addition of NADPH. The origin of chromatography and the position of authentic estradiol (E2), 4-hydroxyestradiol (4-OHE2), and 2-hydroxyestradiol (2-OHE2) are indicated. The chromatographic procedures are described under Materials and Methods.
of P450 reductase and phospholipid used in the reconstituted system as well as the pH of the reaction mixture were optimal. The apparent catalytic efficiency, indicated by the $V_{\text{max}}/K_m$ ratio, is relatively high, being close to 1.0. For comparison, this is close to the value reported for P450 2C3v, the high-efficiency hepatic progesterone 16α-hydroxylase from inbred III/J rabbits (24, 25).

Addition of ANF, which is known to serve as an allosteric activator for some P450 isoforms and a competitive inhibitor for others (18, 26), caused a slight increase in $V_{\text{max}}$ and a large increase in $K_m$, resulting in a decreased catalytic efficiency. The effects of ANF are further complicated by the observation that, with progesterone at 1 μM, the inhibition of 2G1-catalyzed hydroxylation plateaued at ANF concentrations between 5 and 15 μM (Fig. 3). The incomplete inhibition by ANF was also observed with 5 μM progesterone (data not shown). In contrast, a plateau was not observed with ketoconazole as an inhibitor. Thus, it is possible that ANF may act both as an inhibitor and an effector for 2G1-catalyzed progesterone hydroxylation in a concentration-dependent fashion.

**Role of 2G1 in olfactory microsomal steroid metabolism.** The effect of anti-2G1 antibodies on olfactory microsomal progesterone metabolism is shown in Fig. 4, with progesterone at 15 nM, which is within the range of plasma levels of this hormone in vivo (27). Addition of anti-2G1 at 10 μg of IgG per nanomole of microsomal P450 almost completely inhibited formation of the 16α-hydroxy and

---

**TABLE II**

<table>
<thead>
<tr>
<th>Addition</th>
<th>$K_m$ (μM)</th>
<th>$V_{\text{max}}$ (nmol/min/nmol P450)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.0 ± 0.5</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>ANF</td>
<td>13.2 ± 4.7</td>
<td>3.2 ± 0.4</td>
</tr>
</tbody>
</table>

*The contents of the incubation mixtures were the same as described in Table I. The concentration of [1,2,6,7,21-3H(N)]progesterone (1.3-40.1 Ci/nmol) ranged from 0.15 to 7.5 μM, and $b_0$ was not included. When present, ANF was at a concentration of 5 μM. The reaction was carried out in triplicate at 37°C for 4 min, and the rates of 16α-hydroxy product formation were determined. The values reported are the means of two separate experiments, with the standard deviations indicated.

---

**FIG. 3.** Inhibition of 2G1-catalyzed progesterone metabolism by ketoconazole and α-naphthoflavone. The contents of the incubation mixtures were the same as described in the legend to Table II, except that ketoconazole (●) or ANF (○) was included at the specified concentrations. [1,2,6,7,21-3H(N)]progesterone (10 Ci/nmol) concentration was 1 μM. The reactions were carried out in duplicate at 37°C for 3 min, and the rates of 16α-hydroxyprogesterone formation were determined. Similar results were obtained in two separate experiments.
16-keto compounds. Formation of a small amount of 20β-hydroxyprogesterone, apparently catalyzed by P450 enzymes other than 2G1, is detectable when substrate consumption by 2G1 is blocked. The relatively high level of 16-ketopregosterone formation (Fig. 4A) is probably a result of stimulation by β3 and the depletion of progesterone in the incubation mixture. The 16α-hydroxy compound apparently competes with progesterone as a substrate. The effects of anti-2G1 antibody on microsomal estradiol and testosterone metabolism are more complex, due to the presence of steroid oxidoreductases that convert testosterone to androst-5-ene-3,17-dione and estradiol to estrone. However, 2G1-dependent metabolites account for the major portion of total products generated in microsomes at substrate concentrations lower than 10 μM, and their formation is effectively suppressed by anti-2G1 (data not shown). Thus, 2G1 is the major P450 enzyme involved in the microsomal metabolism of these steroid hormones at substrate concentrations close to the physiological levels.

**Effects of pheromones and odorants on microsomal progesterone metabolism.** The effects of the boar pheromones and a number of odorant compounds on 2G1-catalyzed progesterone metabolism were examined in olfactory microsomes (Table III). At the concentration of progesterone used, 16α-hydroxyprogesterone was the major metabolite. The 16α-hydroxylation reaction as well as total progesterone metabolism, as measured by substrate disappearance, were inhibited by more than 90% by anti-2G1 IgG (added at 5 μg of IgG per nmol of microsomal P450, data not shown). As indicated in Table III, both 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol inhibited progesterone metabolism at concentrations identical to that of the substrate (2 μM), and 100 μM androstenedione was completely inhibitory. In experiments not presented, the apparent $K_m$ of progesterone for 16α-hydroxyprogesterone formation in olfactory microsomes was approximately 2 μM, with an apparent $V_{max}$ of 0.7 nmol/min/nmol microsomal P450 at 30°C. Kinetic analysis suggests that 5α-androstenedione is a competitive inhibitor of microsomal 16α-hydroxyprogesterone formation. The odorant compounds inhibited progesterone metabolism at much higher concentrations than those of the pheromones, with octanal and 3,7-dimethyloctanol being the most effective and benzyl benzoate the least. The extent of inhibition of the 16α-hydroxylation generally agrees with that of total progesterone metabolism with all inhibitors tested, consistent with 2G1 being the major catalyst. At substrate concentrations higher than 10 μM, the rate of formation of additional metabolites not formed by purified 2G1 becomes significant (not shown), indicating the presence of additional steroid-metabolizing enzymes with higher $K_m$ values in olfactory microsomes.

**Biological significance of olfactory steroid oxidation.** Extensive metabolism of estradiol, testosterone, and progesterone in rodent olfactory mucosa was demonstrated previously by Brittebo and Rafter (28, 29) with nasal mucosa tissue slices and in vivo by autoradiography following injection of 14C-labeled steroids. Our earlier study demonstrated that rabbit olfactory microsomes are

### TABLE III

Inhibition of Olfactory Microsomal Progesterone Metabolism by Pheromones and Odorant Compounds

<table>
<thead>
<tr>
<th>Inhibitor addition</th>
<th>Concentration</th>
<th>16α-Hydroxyprogesterone formation (%)</th>
<th>Progesterone disappearance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5α-Androst-16-en-3-one</td>
<td>2 μM</td>
<td>63</td>
<td>54</td>
</tr>
<tr>
<td>5α-Androst-16-en-3-one</td>
<td>10 μM</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>5α-Androst-16-en-3α-one</td>
<td>100 μM</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5α-Androst-16-en-3α-ol</td>
<td>2 μM</td>
<td>67</td>
<td>NDa</td>
</tr>
<tr>
<td>5α-Androst-16-en-3α-ol</td>
<td>10 μM</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>Octanal</td>
<td>1 mm</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3,7-Dimethyl-1-octanol</td>
<td>10 μM</td>
<td>42</td>
<td>50</td>
</tr>
<tr>
<td>3,7-Dimethyl-1-octanol</td>
<td>1 mm</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Anisole</td>
<td>1 mm</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>Benzyl benzoate</td>
<td>1 mm</td>
<td>33</td>
<td>13</td>
</tr>
</tbody>
</table>

*The rates of 16α-hydroxyprogesterone formation and progesterone disappearance in olfactory microsomal incubations in the presence of an inhibitor were determined by comparing the absorbance or absence of an inhibitor were determined by comparing the absorbance peak area at 240 nm with that of the standards. The reaction mixtures contained 50 mM phosphate buffer, pH 7.4, 2.0 μM progesterone, 0.1 nmol of olfactory microsomal P450, an inhibitor added in 10 μl of methanol or methanol alone, and 1 mM NADPH in a final volume of 1.0 ml. The reactions were carried out at 30°C for 10 min. The values reported are the average of triplicate determinations. The procedures for HPLC analysis and product quantification are described under Materials and Methods.

*ND, not determined.
much more active than respiratory nasal mucosa microsomes and liver microsomes toward testoste- rone (7). The present results as well as those obtained previously indicate that rabbit P450 2G1 is highly active in the metabolism of a number of steroid sex hormones, including androst-4-ene-3,17-dione, estradiol, 5α-dihydrotestosterone, testosterone, and progesterone, and plays a major role in steroid hydroxylations activities in olfactory microsomes. Kinetic analysis of 2G1-catalyzed progesterone metabolism indicates that the enzyme is highly efficient in hydroxylation of this substrate. In addition, consistent with the lack of gender difference in 2G1 expression (5, 7), no significant sex difference in olfactory microsomal progesterone, testosterone, or estradiol metabolism was found with regard to either the metabolite profile or the turnover numbers (data not shown).

Much remains to be learned about the role of 2G1 in olfactory function. However, the relatively high efficiency and broad substrate specificity toward sex steroids and the early expression of the gene during prenatal development (10, 11) suggest that it may be important for metabolic clearance of systemically derived endogenous compounds that accumulate in olfactory tissue. In this connection, Rosenblum et al. (30) recently reported that receptor binding of a known steroidophore, 17α,20β-dihydroxy-4-pregnen-3-one, to goldfish olfactory epithelium is competitively inhibited by progesterone, 17α-hydroxyprogesterone, androst-4-ene-3,17-dione, and testosterone, which suggests a potential for regulation of pheromone signal transduction by endogenous compounds in this tissue. Furthermore, the olfactory mucosa is a known target tissue for steroid hormone action (31–33). For instance, in the male rat, the morphology of the olfactory epithelium is altered by castration, and testosterone replacement counteracts these changes (31). Thus, it may be speculated that, by inhibiting steroid metabolism, foreign compounds may alter the homeostasis of such bioactive substances in the olfactory mucosa and consequently induce biological responses such as stimulation of progenitor cell differentiation and accelerated regeneration of the olfactory neurons. Further studies designed to link knowledge of the biochemistry and biology of olfactory P450 are warranted.

ACKNOWLEDGMENTS

We are grateful to Drs. Steven J. Pernecky and Alfin D. N. Vaz for helpful discussions.

REFERENCES