J. Steroid Biochem. Molec. Biol. Vol. 43, No. 8, pp. 1095–1098, 1992
Printed in Great Britain.

0960-0760/92 $5.00 + 0.00

EXTRAGLANDULAR HORMONAL STEROIDOGENESIS IN AGED RATS

LUISA DALLA VALLE, PAOLA BELVEDERE, CLAUDIA SIMONTACCHI and LORENZO COLOMBO*
Department of Biology, University of Padua, Via Trieste 75, 35121 Padua, Italy

Summary—We have examined the metabolism *in vitro* of [4-^{14}C]pregnenolone by the following organs of 2.4-year-old rats: submandibular gland, stomach, duodenum, liver, lung, heart, spleen, kidney, skin, prostate, testis and adrenal. All tissues converted pregnenolone to progesterone, the highest yields being observed with adrenal, testis and skin. Androgen formation was intense in the testis and absent in the adrenal. Moreover, 17α-hydroxylation of pregnenolone occurred moderately in kidney, skin and submandibular gland and markedly in duodenum and stomach, which also produced high amounts of dehydroepiandrosterone and/or 5-androstene-3β,17β-diol. Extratesticular synthesis of androstenedione and testosterone was very low. A significant formation of 20α-dihydropregnenolone was observed in all tissues but stomach, duodenum and steroidogenic endocrines. Corticosteroids were not synthesized extraadrenally, except a small amount of 11-deoxycorticosterone in the testis. These results indicate that key steroid-biosynthetic enzymes, such as 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase, 17β- and 20α-hydroxysteroid dehydrogenases and steroid 17α-monooxygenase/17,20-lyase are also expressed extraglandularly in the rat.

INTRODUCTION

An increasing number of studies are indicating that peripheral organs are involved not only in steroid hormone transformations (e.g. testosterone 5α-reduction and aromatization), prohormone activation (e.g. 17-keto reduction of androstenedione and estrone) and steroid catabolism but also in more extensive biosynthetic performances.

In the rat, submandibular glands were shown to convert acetate into cholesterol, which was further transformed into pregnenolone and dehydroepiandrosterone (DHEA) [1]. Steroid 17α-monooxygenase activity was reported in both rat submandibular and sublingual glands [2] and small intestine [3]. Rat brain oligodendrocytes were found to utilize mevalonolactone to synthesize cholesterol and pregnenolone with subsequent production of progesterone and 5α-pregnane neurosteroids acting on GABAergic neurons [4].

The present work on the metabolism of [4-^{14}C]pregnenolone by several organs of 2.4-year-old rats indicates that extraglandular steroidogenesis may be widespread in this species.

Proceedings of the First International Symposium on A Molecular View of Steroid Biosynthesis and Metabolism, Jerusalem, Israel, 14–17 October 1991.
*To whom correspondence should be addressed.

EXPERIMENTAL

Chemicals

[4-^{14}C]Pregnenolone (sp. act. 2.07 GBq/mmol) was obtained from Amersham (Bucks., England) and ^{3}H-labeled steroids were purchased from New England Nuclear Corp. (Boston, MA, U.S.A.) and purified by two-dimensional TLC prior to use. Reference steroids were obtained from Steraloids, Inc. (Pawling, NY, U.S.A.). Plastic sheets precoated with silica gel 60 F_{254} (20 × 20 cm × 0.2 mm thickness) were from Merck (Darmstadt, Germany). No-screen medical X-ray safety films, type S, from 3M (Milan, Italy) were utilized for autoradiography.

Animals and tissue preparation

Six male Wistar rats, weighing approx. 800 g and 2.4 years old, were obtained from Fidia Research (Abano Terme, Padua, Italy). Animals were beheaded and the following organs dissected out: submandibular glands, stomach, duodenum, liver, lungs, heart, spleen, kidneys, skin, prostate, testes and adrenals. Tissues were rinsed in ice-cold 0.15 M KCl, minced, pooled from all donors and incubated separately.

Incubations

Samples of 1 g of each tissue (80 mg for adrenals) were incubated in 10 ml of medium

(153 mM NaCl, 2.0 mM KCl, 0.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.8 mM $MgSO_4$, 2.5 mM Na_2HPO_4, 0.6 mM KH_2PO_4, 26 mM $NaHCO_3$ and 5.5 mM glucose) containing 18.5 kBq of [4-^{14}C]pregnenolone (0.9 μM) in 100 μl of propylene glycol. Incubations were carried out in a Dubnoff shaker for 4 h at 37°C under carbogen (95% O_2–5% CO_2). Metabolism was terminated with 2 vol of ethanol–ethyl acetate (3:1) and incubates were stored at −20°C until processed. An incubate with rat minced boiled liver was done as control.

Extraction, chromatography and identification of steroid metabolites

Incubates were extracted 4 times with 10 ml of 95% ethanol. The extracts were centrifuged to precipitate the tissue and the supernatants were pooled. One fifth of every extract was analyzed first to establish the content of metabolites. Appropriate carriers (3 μg) and ^{3}H-tracer steroids for recovery control were then added to the remainder. These were evaporated under air at 40°C and the aqueous residues (brought down to 2.5 ml and then diluted to 5 ml with distilled water) were extracted 4 times with 10 ml of ethyl acetate to obtain the free steroid fraction.

Metabolites were separated by two-dimensional TLC with the solvent systems cyclohexane–ethyl acetate (95:5, v/v; 5–7 runs) in the first direction for defatting, toluene–acetone (8:2, v/v; 2 runs) in the second direction and cyclo-hexane–ethyl acetate (1:1, v/v; 2 runs) again in the first direction. Chromatoplates were then autoradiographed for 5 days and the zones containing labeled carriers were removed, eluted in 20 ml of acetone and counted in Emulsifier scintillant (Packard, Milan) by means of a liquid scintillation spectrometer (Packard 1500 Tri-Carb).

For each incubate, conclusive identification of labeled metabolites was based on recrystallization in acetone–water (or pyridine–acetone–water for 17α-hydroxypregnenolone, 17α-hydroxyprogesterone and 5-androstene-3β,17β-diol) to constant ^{3}H/^{14}C ratio (values within ±5% from the mean) of the unchanged compounds or their acetylated derivatives in the case of 17α-hydroxypregnenolone, DHEA and 5-androstene-3β,17β-diol. When radioactivity was low (<1% conversion), pooled samples were recrystallized. Percent conversions were calculated from the last ^{3}H/^{14}C ratio in the purification procedure of individual steroids and corrected for losses. Yields of 20α-dihydropregnenolone were not corrected for losses for lack of ^{3}H-tracer and this compound was recrystallized as its diacetate to constant ^{14}C dpm/mg carrier ratio.

RESULTS

After correction for losses, an average of 98% (range 87–102%) of precursor radioactivity was recovered in the free steroid fraction of all incubates, except that with liver in which 37% of label was associated with water-soluble compounds.

Testis, adrenal and liver metabolized 87, 82 and 66% of [4-^{14}C]pregnenolone, respectively, whereas metabolism in the remaining organs ranged from 9 to 23% (Tables 1 and 2).

All tissues examined converted pregnenolone to progesterone. The highest yields were observed with adrenal, testis and skin, while accumulation in the other incubates was within 0.1–0.6% (Tables 1 and 2).

As expected, androgen formation through 17α-hydroxylated C_{21} intermediates was intense in the testicular tissue and absent in the adrenal incubate (Table 1). Moreover, 17α-hydroxylation of pregnenolone occurred moderately in kidney, skin and submandibular gland and markedly in duodenum and stomach which also produced high amounts of DHEA. In addition, there was a conspicuous accumulation of 5-androstene-3β,17β-diol in the stomach incubate. Percent conversions of these 5-ene-3β-hydroxysteroids in the other tissues were below 0.2% and extratesticular synthesis of androstenedione and testosterone was below 0.1% (Table 2).

A significant formation of 20α-dihydropregnenolone was observed in all tissues except stomach, duodenum and steroidogenic endocrines (Table 2). Finally, among the corticosteroids, only 11-deoxycorticosterone was found

Table 1. Metabolites of [^{14}C]pregnenolone after incubation with rat testis and adrenal[a]

Steroid	Testis	Adrenal
Pregnenolone (unmetabolized)	12.8	18.1
17α-Hydroxypregnenolone	8.8	—
Progesterone	2.8	33.2
17α-Hydroxyprogesterone	1.6	—
20α-Dihydroprogesterone	0.2	—
Dehydroepiandrosterone	0.2	—
Androstenedione	4.6	—
Testosterone	29.8	—
5-Androstene-3β,17β-diol	3.3	—
11-Deoxycorticosterone	0.2	16.6
11-Dehydrocorticosterone[b]	—	8.5
Corticosterone[b]	—	5.0

[a] Results are expressed as percentage of the substrate original radioactivity.
[b] Yields not corrected for procedural losses.

Table 2. Metabolites of [^{14}C]pregnenolone after incubation with rat peripheral organs[a]

Organ	Steroids						
	PRE[b]	17α-PRE	20α-PRE[c]	PRO	DHEA	DIOL	AND
Duodenum	78.5	6.4	—	0.1	4.9	—	0.03
Stomach	80.0	5.3	—	0.08	8.5	4.4	0.02
Submandibular gland	77.4	1.4	8.5	0.3	0.1	—	—
Liver	33.9	0.2	1.5	0.4	0.08	0.1	0.01
Lung	79.3	0.05	7.6	0.2	0.2	0.2	—
Kidney	81.1	1.7	2.0	0.6	0.2	—	0.01
Spleen	82.8	0.02	1.1	0.3	0.02	0.05	—
Heart	90.0	0.03	0.9	0.1	0.08	0.06	—
Skin	90.6	1.4	1.7	2.5	—	—	—
Prostate	87.5	0.08	6.6	0.2	0.04	—	—

Steroid abbreviations: PRE = pregnenolone; 17α-PRE = 17α-hydroxypregnenolone; 20α-PRE = 20α-dihydropregnenolone; PRO = progesterone; DHEA = dehydroepiandrosterone; AND = androstenedione; DIOL = 5-androstene-3β,17β-diol.
[a] Results are expressed as percentage of the substrate original radioactivity. Yields below 0.01% (100 dpm) are indicated with a hyphen.
[b] Unmetabolized precursor.
[c] Yields not corrected for procedural losses.

extraadrenally in the testicular incubate in a low amount (Table 1). None of the above metabolites was detected in the incubate with boiled tissue.

Although chemical identity of metabolites was mostly checked by recrystallization separately for each incubate, only representative recrystallization data for each steroid are reported in Table 3. Several unidentified metabolites of low and high polarity were revealed in the TLC autoradiographs of liver, testis and adrenal, fewer of them being present in the other incubates.

DISCUSSION

Apart from the liver, peripheral organs displayed lower pregnenolone-transforming capacity and less complex metabolite profiles than testis and adrenal. Steroid conjugation, as evidenced by water-soluble products, was detectable only in the liver whereas steroid esterification with fatty acids, as evidenced by low polarity compounds, appeared to be ubiquitous.

Table 3. Recrystallization data of some of the steroid metabolites formed from [^{14}C]pregnenolone by rat testis, adrenal and peripheral organs

Steroid	Organ	^{3}H/^{14}C			
		C 1	C 2	C 3	ML
17α-PRE[a]	Stomach	4.30	4.22	4.23	4.25
20α-PRE[b]	Lung	46.76	46.38	45.15	49.22
PRO	Skin	8.94	8.89	8.87	8.94
17α-PRO	Testis	16.27	16.02	15.95	16.02
20α-PRO	Testis	0.80	0.75	0.74	0.74
DHEA	Duodenum	4.53	4.45	4.56	4.73
AND	Pool[c]	1.85	1.85	1.85	1.85
TST	Testis	0.11	0.11	0.11	0.12
DIOL[a]	Stomach	1.56	1.54	1.55	1.55
DOC	Adrenal	1.93	1.90	1.92	1.93

Symbols: C 1, C 2, C 3 = 1st, 2nd, 3rd crystal crops; ML = mother liquor.
[a] Recrystallized after acetylation.
[b] Recrystallized after acetylation to constant ^{14}C dpm/mg carrier ratio.
[c] Metabolite pooled from the incubates with duodenum, stomach, liver and kidney.

All tissues contained 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) activity, higher levels occurring in adrenal, testis and skin, in agreement with a recent report on the conversion of labeled pregnenolone to progesterone by homogenates of a similar series of rat organs [5]. Two types of 3β-HSD cDNAs with differential tissue distribution have been characterized in the rat [5], a third type being expressed in male liver only [6]. Type I is more active than type II but neither type discriminates between C_{21} and C_{19} substrates [7].

Although peripheral 3β-HSD in man appears to be involved mainly in the transformation of circulating adrenal DHEA and DHEA sulfate into hormonally active or aromatazible androgens [8], in rat this role should be less important, given the lack of adrenal androgens and the low level (below 0.3 ng/ml) of plasma DHEA in the intact animal [9]. Rather, our results indicate that this enzymatic complex could be implicated in the production of active steroids from 5-ene-3β-hydroxypregnenes and/or 5-ene-3β-hydroxyandrostenes synthesized locally.

As a matter of fact, the C_{21}-steroid side-chain cleavage system, comprising steroid 17α-monooxygenase/17,20-lyase activities, is more widespread peripherally in the rat than previously claimed [1–3] and is prominent in stomach and intestine. Pregnenolone 17α-hydroxylation was also observed in porcine salivary glands [10].

Our data suggest that extraglandular androgen synthesis in rat follows predominantly the 5-ene-3β-hydroxysteroid route, since 17α-hydroxyprogesterone was not detected. Though formation of androstenedione and testosterone was always low, except in the testis, the

high yield of 5-androstene-3β,17β-diol in the stomach is of interest, since this compound has been shown to exert direct estrogenic effects in the rat [11]. Stomach and duodenum were the only peripheral organs devoid of 20α-HSD activity. Formation of 20α-dihydroprogesterone, a biologically active progestin, from progesterone was previously described in rat salivary glands [2].

Furthermore, we have established that rat duodenum can not only transform DHEA into aromatazible androgens and the latter into estrogens [12] but also converts cholesterol to pregnenolone (in preparation). Rat salivary glands and rat brain oligodendrocytes were also found to contain cytochrome *P*450scc [1, 13].

Thus, a number of peripheral organs in the rat may act not just as terminal activators of glandular precursors but rather as autonomous centres of hormonal steroidogenesis. Interestingly, among the organs examined, those with the highest steroid-transforming potential, namely the gastro-duodenal tract and skin, are also characterized by the most intense epithelial cell renewal.

Acknowledgements—Research aided by the Ministry of the University and Scientific and Technological Research, grant MURST 40%, and the National Research Council, Special Project on Evolutionary Biology and Differentiation, Italy. We are grateful to Fidia Research for providing the aged rats.

REFERENCES

1. 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.
2. Laine M. and Ojanotko-Harri A.: Progesterone metabolism by major salivary glands of rat—I. Submandibular and sublingual glands. *J. Steroid Biochem.* **35** (1990) 723–728.
3. 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.
4. Jung-Testas I., Hu Z. Y., Baulieu E. E. and Robel P.: Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology* **125** (1989) 2083–2091.
5. Zhao H.-F., Labrie C., Simard J., de Launoit Y., Trudel C., Martel C., Rhéaume E., Dupont E., Luu-The V., Pelletier G. and Labrie F.: Characterization of rat 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase cDNAs and differential tissue-specific expression of the corresponding mRNAs in steroidogenic and peripheral tissues. *J. Biol. Chem.* **266** (1991) 583–593.
6. Zhao H.-F., Rhéaume E., Trudel C., Couët J., Labrie F. and Simard J.: Structure and sexual dimorphic expression of a liver-specific rat 3β-hydroxysteroid dehydrogenase/isomerase. *Endocrinology* **127** (1990) 3237–3239.
7. Simard J., de Launoit Y. and Labrie F.: Characterization of the structure–activity relationships of rat types I and II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase by site-directed mutagenesis and expression in HeLa cells. *J. Biol. Chem.* **266** (1991) 14842–14845.
8. Adams J. B.: Control of secretion and the function of C_{19} Δ^5-steroids of the human adrenal gland. *Molec. Cell. Endocr.* **41** (1985) 1–17.
9. Bélanger B., Bélanger A., Labrie B., Dupont A., Cusan L. and Monfette G.: Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in man. *J. Steroid Biochem.* **32** (1989) 695–698.
10. Kadis B. and Ching Chyn L.: Steroid transformations in porcine salivary glands. *J. Steroid Biochem.* **6** (1975) 1543–1548.
11. Simard J., Vincent A., Duchesne R. and Labrie F.: Full oestrogenic activity of C_{19}-^{5}adrenal steroids in rat pituitary lactotrophs and somatotrophs. *Molec. Cell. Endocr.* **55** (1988) 233–242.
12. Colombo L., Dalla Valle L., Belvedere P., Simontacchi C. and Marcon P.: Hormonal steroidogenesis by duodenal tissue of rat. *II Course on Molecular Biology of Hormone Action in Endocrinology and Pharmacology*, Milan, October (1990) Abstr. p. 85.
13. Le Goascogne C., Robel P., Gouézou M., Sananes N., Baulieu E. E. and Waterman M.: Neurosteroids: cytochrome P-450_{scc} in rat brain. *Science* **237** (1987) 1212–1215.