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PRODUCT-INHIBITION OF TESTICULAR STEROIDOGENIC ENZYMES IN THE MARMOSET *SAGUINUS OEDIPUS*

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SUMMARY

It has been reported that the marmoset testis converts pregnenolone to testosterone through the delta-4 pathway, with progesterone and 17-hydroxyprogesterone the major intermediate metabolites. The following studies were to determine the inhibition of specific testicular steroidogenic enzymes by the products of these enzymes, and to verify our initial delta-4 results with the marmoset testis. Marmoset testicular fragments (50 mg) were incubated with 10 μCi (200 nmol) of the following tritium-labelled substrates: pregnenolone, progesterone, 17-hydroxyprogesterone, androstenedione. Radiolabelled pregnenolone was incubated in the presence of unlabelled (200 nmol) progesterone or unlabelled 17-hydroxypregnenolone. Radiolabelled progesterone was incubated with 200 nmol of unlabelled 17-hydroxyprogesterone, while radiolabelled 17-hydroxyprogesterone was incubated with 200 nmol of unlabelled androstenedione. Radiolabelled androstenedione was incubated with 200 nmol of unlabelled testosterone. All radiolabelled incubates were compared to appropriate controls receiving no unlabelled product. Unlabelled progesterone decreased the metabolism of labelled pregnenolone by 19.6% compared to that of control incubates, while unlabelled 17-hydroxypregnenolone had no detectable effect upon the metabolism of pregnenolone. 17-Hydroxypregnenolone and dehydroepiandrosterone were not identified in any of the pregnenolone incubates. Unlabelled 17-hydroxyprogesterone substantially decreased the metabolism of progesterone, resulting in a 26.6% decline in the metabolism of progesterone compared to controls. Similarly, unlabelled androstenedione decreased the metabolism of 17-hydroxyprogesterone by 20.3% compared to controls receiving no unlabelled androstenedione. Unlabelled testosterone had no effect upon the metabolism of androstenedione by marmoset testicular fragments. These results suggest that the products of selected marmoset testicular steroidogenic enzymes can competitively inhibit the metabolism of their respective substrates, and that pregnenolone is converted to testosterone through the delta-4 pathway in the marmoset testis.

INTRODUCTION

We recently reported that the testis of the marmoset *Saguinus oedipus* converts pregnenolone to testosterone through the delta-4 pathway [1, 2]. Progesterone, 17-hydroxyprogesterone and androstenedione were the major metabolites identified in the incubates, with 17-hydroxyprogesterone most predominant. The delta-5 intermediates 17-hydroxypregnenolone and dehydroepiandrosterone were not identified in the pregnenolone incubates.

It is well documented that the metabolism of pregnenolone to testosterone is a consequence of the activities of specific sequential testicular enzymes [3, 4, 5]. The delta-4 conversion of pregnenolone to testosterone involves the sequential activities of the following enzymes acting upon their respective substrates: 3β-hydroxysteroid dehydrogenase and isomerase converts pregnenolone to progesterone; progesterone 17-hydroxylase converts progesterone to 17-hydroxyprogesterone; 17-hydroxypregnene-C_{17}-C_{20}-lyase converts 17-hydroxyprogesterone to androstenedione; 17β-hydroxysteroid dehydrogenase converts androstenedione to testosterone.

Although we have previously described testicular steroidogenesis in the marmoset, there have been no reports published with marmosets or with other subhuman primate species which determines the competitive inhibition of the activities of specific testicular steroidogenic enzymes by the products of these enzymes. The present studies were therefore undertaken to investigate product-inhibitions in the marmoset testis, and to further determine the delta-4 pathway as predominant for testosterone biosynthesis in this subhuman primate specie.

MATERIALS AND METHODS

Testicular tissues

Testes were removed from mature marmosets (*Saguinus oedipus*) and placed into ice-cold tris-sucrose buffer, pH 7.4. The testes were weighed, decapsulated, and cut into approximately equal 50 mg fragments. The fragments were teased apart and placed into flasks for incubation studies.

Materials

Solvents (nanograde) and silica-gel (silicar TLC-7Gf) were purchased from Mallinckrodt Chemical Works, St. Louis; nonradioactive steroid carriers were obtained from Steraloids, Wilton, New Hampshire. Radioactive substrates [^{3}H] and tracers [^{14}C] were purchased from Amersham Searle, Inc., Arlington Heights, Ill. Cofactors utilized for incubations

were obtained from Sigma Chemical Co., St. Louis. Paper (No. 1) for chromatographic separations was purchased from Whatmann Paper Company. Steroids were checked for purity by thin-layer chromatography, while paper and silica gel were washed with methanol prior to use in these studies.

Incubations

Testicular fragments were placed into incubation flasks containing Krebs–Ringer bicarbonate buffer, pH 7.4, fortified with NADH, and an NADPH generating system which contained NADP, nicotinamide, glucose-6-phosphate, pyruvate, glucose-6-phosphate dehydrogenase, and lactic dehydrogenase. Incubations were in a Dubnoff metabolic shaking incubator at 37°C under an atmosphere of 95% O_2/5% CO_2. Upon completion of the incubations the reactions were terminated with 0.5 ml 1N HCl and the incubates immediately frozen [2, 6].

Extractions

Incubates were thawed and the testicular fragments were homogenized in Krebs–Ringer bicarbonate buffer, pH 7.4. The homogenates were pooled with their respective incubation media, radiolabelled tracers [^{14}C] and unlabelled carriers were added to each pool, and the pools were extracted ten times with cold diethyl ether–chloroform (4:1, v/v). The solvents were evaporated under an atmosphere of nitrogen, and the residues were concentrated. Methanol (5 ml) was added to each tube and aliquots were removed for estimates of recoveries. The remaining solvent was evaporated under nitrogen, with the residues placed onto paper strips for chromatographic separations.

Chromatographic procedures

The residues were placed onto formamide-impregnated Whatmann No. 1 paper strips (2.5 × 50 cm) and the strips chromatographed in hexane, followed by a second chromatographic separation in hexane–benzene. Upon completion of the chromatographic separations the strips were dried in a warm air oven. Radioactive peaks were located with a Packard Model 385 Recording Ratemeter, the peaks eluted from the paper with 80 ml of methanol, and the metabolites isolated by thin-layer chromatography in selected solvent systems. The systems utilized in these studies were benzene–ethyl acetate (80:20; 60:40, v/v), benzene–methanol (98:2; 95:5, v/v), and chloroform–acetone (90:10; 80:20, v/v). Testosterone was acetylated [7] and isolated by thin-layer chromatography. Aliquots for recovery determinations were obtained prior to each thin-layer separation, and prior to crystallization of each metabolite.

Crystallization procedures

Following isolation and tentative identity by thin-layer chromatography, final identity of the metabolites was established by crystallization to constant specific activities and $^3H/^{14}C$ ratios through three successive solvent combinations (acetone–hexane; acetone–cyclohexane; acetone–hexane). Conversion of substrates to metabolites was calculated by determining the total [^{3}H]-DPM for each metabolite from crystallization data, correcting the total [^{3}H]-DPM for procedural losses, and dividing the corrected total [^{3}H]-DPM for each metabolite by the [^{3}H]-DPM of the total incubate. Conversions were expressed as a percentage of the [^{3}H]-DPM of the total incubate.

Experimental procedures

In the experiments described below, the inhibition of each specific enzyme was determined by incubating teased marmoset testicular fragments (50 mg) with the tritium-labelled substrate (10 μCi; 200 nmol) for that enzyme in both the presence and absence of 200 nmol of unlabelled product. The specific inhibition of each enzymic reaction was obtained by determining the activity of each enzyme in the presence of unlabelled product, and comparing this conversion to that obtained when unlabelled product was not present in the incubates. Each substrate was run in triplicate in both the presence and absence of unlabelled product.

For determination of inhibition of 3β-hydroxysteroid dehydrogenase and isomerase activities, testicular fragments were incubated with pregnenolone-7-[^{3}H] (10 μCi; 200 nmol) in the presence of 200 nmol of unlabelled progesterone. For pregnenolone 17-hydroxylase activity, testicular fragments were incubated with pregnenolone-7-[^{3}H] (10 μCi; 200 nmol) with 200 nmol of unlabelled 17-hydroxypregnenolone present in the incubates. Testicular fragments incubated with radiolabelled pregnenolone in the absence of any unlabelled product was utilized as controls in these experiments.

Inhibition of progesterone 17-hydroxylase activity was determined by incubating marmoset testicular fragments with progesterone-7-[^{3}H] (10 μCi; 200 nmol) in the presence and absence of unlabelled (200 nmol) 17-hydroxyprogesterone in the incubates. For determination of 17-hydroxypregnene-C_{17}-C_{20}-lyase inhibition, fragments were incubated with 17-hydroxyprogesterone-7-[^{3}H] (10 μCi; 200 nmol) with unlabelled androstenedione (200 nmol) both present and absent from the incubates. Inhibition of 17β-hydroxysteroid dehydrogenase was determined by incubating testicular fragments with androstenedione-7-[^{3}H] (10 μCi; 200 nmol) in the presence of 200 nmol of testosterone, and comparing this conversion to that obtained from incubation of radiolabelled androstenedione in the absence of unlabelled testosterone.

RESULTS

3β-Hydroxysteroid dehydrogenase and isomerase, and pregnenolone 17-hydroxylase

Marmoset testicular 3β-hydroxysteroid dehydrogenase and isomerase converted 86.4% (172.8 nmol)

of the radiolabelled pregnenolone into delta-4-3-keto steroids when no unlabelled progesterone was present in the incubates. 17-Hydroxyprogesterone was the major metabolite of pregnenolone with 32.5% (65.0 nmol) of the total radioactivity, while progesterone was a lesser metabolite with 21.3% (42.6 nmol) of the radioactivity in the incubates. Androstenedione was 16.0% (33.8 nmol) and testosterone was 12.5% (25.2 nmol) of the total radioactivity. Non-metabolized pregnenolone substrate was identified as 13.6% (27.2 nmol), while 3.1% (6.2 nmol) was present as minor unidentified metabolites of pregnenolone. As in previous reports we have published with *Saguinus*, [1, 2] there was no radioactivity identified as 17-hydroxypregnenolone or dehydroepiandrosterone present in these control incubates.

Incubation of marmoset testicular fragments with radiolabelled pregnenolone in the presence of 200 nmol of unlabelled progesterone substantially decreased the activity of 3β-hydroxysteroid dehydrogenase and isomerase as evidenced by the decline in the amount of radiolabelled pregnenolone metabolized by the fragments. In these incubates, 66.8% (133.6 nmol) of pregnenolone substrate was metabolized into delta-4-3-keto steroids, a 19.6% decline from that of control incubates. When unlabelled progesterone was present in the pregnenolone incubates, radiolabelled progesterone was the major metabolite of radiolabelled pregnenolone with 22.4% (44.8 nmol) of the radioactivity, while 17-hydroxyprogesterone was 9.0% (18.1 nmol) of the total radioactivity. Both androstenedione (15.6%; 31.2 nmol) and testosterone (16.2%; 33.4 nmol) were formed in amounts similar to that of control incubates receiving no unlabelled progesterone. Non-metabolized pregnenolone substrate was identified as 33.2% (66.3 nmol) of the total radioactivity. As in control pregnenolone incubates,

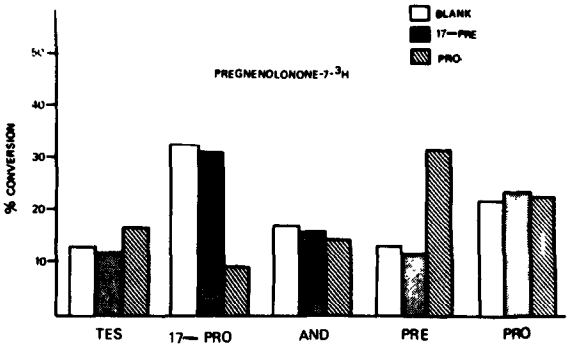

Fig. 1. Metabolism of radiolabelled pregnenolone (10 μCi; 200 nmol) by marmoset (*Saguinus oedipus*) testicular fragments in the presence of 200 nmol of unlabelled 17-hydroxypregnenolone or in the presence 200 nmol of unlabelled progesterone. Control incubates received no unlabelled steroids. The following abbreviations are used in the figure: TES, testosterone as testosterone acetate; 17-PRO, 17-hydroxyprogesterone; PRO, progesterone; 17-PRE, 17-hydroxypregnenolone.

Table 1. Crystallization data for metabolism of pregnenolone-[^{3}H] (10 μCi; 200 nmol) by marmoset (*Saguinus oedipus*) testicular fragments in the presence and absence of unlabelled progesterone (200 nmol) or unlabelled 17-hydroxypregnenolone (200 nmol) at 3 h of incubation

Metabolite*†	^{3}H d.p.m. (I)§	^{3}H d.p.m./mg‖¶	^{14}C d.p.m./mg‖¶	^{3}H/^{14}C	^{3}H d.p.m. (T)**	% Purity††
Control incubates						
TES	1638.2	74.7	2.8	26.5	1497.3	91.4
AND	2069.5	93.8	1.7	52.3	1879.1	90.8
17-PRO	4504.7	199.3	2.0	96.3	3991.2	88.6
PRO	2671.8	123.1	2.5	48.6	2466.1	92.3
PRE‡	1817.3	81.7	2.6	30.6	1637.4	90.1
Progesterone incubates						
TES	2889.8	129.0	2.8	44.5	2583.5	89.4
AND	2579.4	117.2	1.9	61.3	2347.3	91.0
17-PRO	1391.8	64.5	1.9	33.7	1291.6	92.8
PRO	3811.4	172.3	2.6	64.5	3449.4	89.9
PRE‡	5420.6	245.0	2.6	94.2	4905.7	90.5
Pregnenolone incubates						
TES	1696.5	79.1	2.6	29.3	1584.5	93.4
AND	2320.4	104.4	1.9	52.9	2090.6	90.1
17-PRO	4772.1	212.8	1.9	108.0	4261.4	89.3
PRO	3577.6	162.9	2.5	63.0	3262.8	91.2
PRE‡	1809.1	83.4	2.6	31.8	1669.8	92.3

* The following abbreviations are used in the table: TES, Testosterone as testosterone acetate; AND, androstenedione; 17-PRO, 17-hydroxyprogesterone; PRO, progesterone; PRE, pregnenolone.

† Approximately 20 mg of crystalline steroid was added to each sample prior to crystallization.

‡ Non-metabolized substrate.

§ Initial ^{3}H d.p.m. ($\times 10^3$) before crystallization.

‖ Data as mean d.p.m. ($\times 10^3$) of three successive crystallizations of triplicate samples.

¶ Solvent systems for crystallizations were: I. acetone–hexane; II. acetone–cyclohexane; III. acetone–hexane.

** Total ^{3}H d.p.m. ($\times 10^3$) following crystallization.

‡‡ % Purity = ^{3}H d.p.m. (T)/^{3}H d.p.m. (I).

there was no 17-hydroxypregnenolone or dehydroepiandrosterone identified in incubations of testicular fragments with radiolabelled pregnenolone in the presence of unlabelled progesterone.

When marmoset testicular fragments were incubated with radiolabelled pregnenolone and unlabelled 17-hydroxypregnenolone, the unlabelled product had no effect upon the metabolism of radiolabelled substrate. In these incubations, 87.2% (174.4 nmol) of radiolabelled pregnenolone was metabolized into delta-4-3-keto steroid metabolites. 17-Hydroxyprogesterone was the major metabolite with 31.1% (62.2 nmol) of the radioactivity, while progesterone was 23.0% (46.0 nmol) of the total radioactivity in the incubates. Androstenedione (16.2%; 32.4 nmol) and testosterone (11.9%; 23.8 nmol) were also formed similar to that of control incubates. Unmetabolized pregnenolone substrate was 12.8% (25.6 nmol) of the radioactivity, while radiolabelled 17-hydroxypregnenolone and dehydroepiandrosterone were not identified in these incubates. Figure 1 and Table 1 present data for the metabolism of radiolabelled pregnenolone by marmoset testicular fragments.

Progesterone 17-hydroxylase

Marmoset testicular progesterone 17-hydroxylase metabolized 55.4% (110.8 nmol) of radiolabelled progesterone substrate into metabolites when no unlabelled 17-hydroxyprogesterone was present in the incubates. As in control pregnenolone incubates, 17-hydroxyprogesterone (28.2%, 56.3 nmol) was the major metabolite in these control progesterone incubates. Androstenedione (14.6%; 29.1 nmol) and testosterone (12.6%; 25.3 nmol) were lesser metabolites and were formed in amounts similar to control pregnenolone incubates.

The presence of unlabelled 17-hydroxyprogesterone in the progesterone incubates substantially decreased the activity of progesterone 17-hydroxylase, as only 28.8% (57.6 nmol) of progesterone substrate was converted into radiolabelled metabolites, a decline of 26.6% from control incubates. 17-Hydroxyprogesterone (8.9%; 17.7 nmol), androstenedione (10.2%; 20.4 nmol) and testosterone (9.8%; 19.8 nmol) were identified as the major metabolites of progesterone. In these incubates, unmetabolized progesterone was 71.2% (142.1 nmol) of the total radioactivity. Figure 2 and Table 2 demonstrate percent conversion and crystallization data for the progesterone incubations.

17-Hydroxypregnene-C_{17}-C_{20}-lyase

Marmoset testicular 17-hydroxypregnene-C_{17}-C_{20}-lyase controls converted 58.8% (116.2 nmol) of radiolabelled 17-hydroxyprogesterone into metabolites (Fig. 3; Table 3). The metabolites of 17-hydroxyprogesterone were androstenedione (25.3%; 50.6 nmol) and testosterone (32.7%; 65.5 nmol) while unmetabolized 17-hydroxyprogesterone was 41.2% (82.4 nmol) of the total radioactivity.

When 200 nmol of unlabelled androstenedione was added to the 17-hydroxyprogesterone incubates there was a substantial decline in the metabolism of radiolabelled 17-hydroxyprogesterone substrate by marmoset testicular 17-hydroxypregnene-C_{17}-C_{20}-lyase.

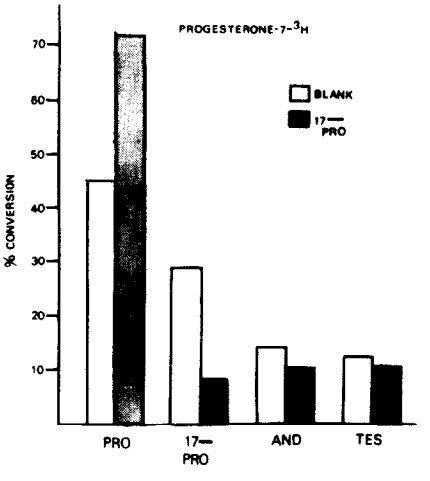

Fig. 2. Metabolism of radiolabelled progesterone (10 μCi; 200 nmol) by marmoset (*Saguinus oedipus*) testicular fragments in the presence or absence of 200 nmol of unlabelled 17-hydroxyprogesterone. The following abbreviations are used in the figure: PRO, progesterone; 17-PRO, 17-hydroxyprogesterone; AND, androstenedione; TES, testosterone as testosterone acetate.

Table 2. Crystallization data for metabolism of progesterone-7-[^{3}H] (10 μCi; 200 nmol) by marmoset (*Saguinus oedipus*) testicular fragments in the presence and absence of unlabelled 17-hydroxyprogesterone (200 nmol) at 3 h of incubation

Metabolite*†	^{3}H d.p.m. (I)§	^{3}H d.p.m./mg‖¶	^{14}C d.p.m./mg‖¶	^{3}H/^{14}C	^{3}H d.p.m. (T)**	% Purity††
Control incubates						
TES	1106.1	49.4	2.7	17.9	989.9	89.5
AND	1278.8	58.6	1.9	30.3	1173.9	91.8
17-PRO	2473.1	114.3	2.0	56.0	2290.1	92.6
PRO‡	4251.3	187.7	2.7	68.3	3758.1	88.4
17-Hydroxyprogesterone incubates						
TES	1252.6	57.1	2.8	19.9	1144.9	91.4
AND	1348.4	58.8	1.9	30.2	1177.1	87.3
17-PRO	1179.6	53.3	2.1	25.1	1067.6	90.5
PRO‡	8739.4	407.2	2.6	155.3	8153.9	93.3

* Legend as in Table 1.

Fragments metabolized only 38.5% (77.0 nmol) of 17-hydroxyprogesterone substrate, a decline of 20.3% from that of control incubates. Androstenedione (22.9%; 45.9 nmol) and testosterone (16.5%; 32.9 nmol) were the only metabolites of 17-hydroxyprogesterone identified in the incubates.

17β-Hydroxysteroid dehydrogenase

17β-Hydroxysteroid dehydrogenase efficiently converted radiolabelled androstenedione into testosterone when no unlabelled testosterone was present in the incubates. Fragments converted 89.3% (178.6 nmol) of androstenedione to testosterone, with the remaining radioactivity (10.7%; 21.3 nmol) identified as unmetabolized androstenedione substrate. When 200 nmol of unlabelled testosterone was present in the incubates there was no detectable decline in the metabolism of androstenedione to testosterone by 17β-hydroxysteroid dehydrogenase. There were no estrogens or 5α-reduced compounds identified in the androstenedione incubates. Figure 4 and Table 4 demonstrate conversion data for the androstenedione incubates.

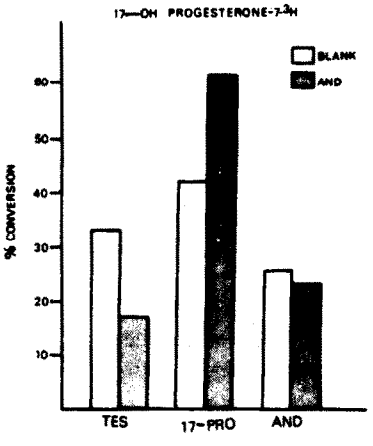

Fig. 3. Metabolism of radiolabelled 17-hydroxyprogesterone (10 μCi; 200 nmol) by marmoset (*Saguinus oedipus*) testicular fragments in the presence or absence of 200 nmol of unlabelled androstenedione. The following abbreviations are used in the figure: TES, testosterone as testosterone acetate; 17-PRO, 17-hydroxyprogesterone; AND, androstenedione.

DISCUSSION

The results of these studies demonstrate that exogenous products of specific steroidogenic enzymes can, by product-inhibition, decrease the amount of substrate which is metabolized by testicular tissue. Exogenous unlabelled progesterone substantially decreased the conversion of radiolabelled pregnenolone into radiolabelled progesterone by 3β-hydroxysteroid dehydrogenase and isomerase, while exogenous 17-hydroxyprogesterone inhibited the activity of progesterone 17-hydroxylase. Furthermore, 17-hydroxypregnene-C_{17}-C_{20}-lyase activity was inhibited by the presence of exogenous unlabelled androstenedione in the medium. These inhibitions occurred when a concentration of 200 nmol for both labelled substrates and unlabelled products, which is saturating, were used in the incubates.

It is significant that 17-hydroxypregnenolone had no effect upon the metabolism of pregnenolone, and thereby was similar to pregnenolone controls in which no unlabelled products were present in the incubates. These results, along with the observations in past studies with marmoset testes [1, 2], further suggests that the delta-5 pathway is not involved in the metabolism of pregnenolone to testosterone in marmoset testicular fragments.

We have recently reported [6] that the testis of the marmoset *Saguinus oedipus* efficiently converts the delta-5 intermediates 17-hydroxypregnenolone and dehydroepiandrosterone into testosterone, while the delta-4 intermediates progesterone and 17-hydroxyprogesterone are less efficiently converted into testosterone. These results suggest that the enzymes of the delta-5 pathway are active in the marmoset testis, and will efficiently convert their respective substrates into products once the substrates become available. However, the lack of any detectable 17-hydroxylation of pregnenolone to 17-hydroxypregnenolone by pregnenolone 17-hydroxylase appears to be the regulatory step for the predominant delta-4 pathway in the mar-

Table 3. Crystallization data for metabolism of 17-hydroxyprogesterone-7-[^{3}H] (10 μCi; 200 nmol) by marmoset (*Saguinus oedipus*) testicular fragments in the presence and absence of unlabelled androstenedione (200 nmol) at 3 h of incubation

Metabolite*†	^{3}H d.p.m. (I)§	^{3}H d.p.m./mg‖¶	^{14}C d.p.m./mg‖¶	^{3}H/^{14}C	^{3}H d.p.m. (T)**	% Purity††
Control incubates						
TES	6965.3	318.0	2.7	116.4	6366.3	91.4
AND	2098.1	97.1	1.9	50.1	1944.9	92.7
17-PRO	3571.4	159.8	2.0	79.1	3199.9	89.6
Androstenedione incubates						
TES	9532.9	419.5	2.8	148.7	8398.5	88.1
AND	2523.3	113.9	2.0	56.3	2281.0	90.4
17-PRO	6276.6	288.4	2.0	144.2	5774.5	92.0

* Legend as in Table 1.

moset testis. Since no detectable 17-hydroxylation of pregnenolone occurs in the testis of this subhuman primate, pregnenolone is metabolized to progesterone by 3β-hydroxysteroid dehydrogenase and isomerase, with a consequent predominant delta-4 pathway for testosterone synthesis.

It is interesting that exogenous testosterone did not decrease the conversion of radiolabelled androstenedione into radiolabelled testosterone by 17β-hydroxysteroid dehydrogenase. We recently reported [6] that the marmoset testis efficiently converts androstenedione into testosterone, but does not efficiently convert testosterone into androstenedione. It is possible that in the present studies the concentration of unlabelled testosterone added to the radiolabelled androstenedione incubates may have not been sufficient to inhibit the metabolism of androstenedione by 17β-hydroxysteroid dehydrogenase, whereas higher concentrations may have been inhibitory.

There have been previous reports published which describe the inhibition of the conversion of progesterone to androgens by selected steroids. Nayfeh and Baggett[8] and Matsumoto *et al.*[9] reported that 17-hydroxyprogesterone produced a small but significant reduction of the conversion of progesterone to 17-hydroxyprogesterone by 17-hydroxylase. In mouse [9] and in rat [10] testicular homogenates, exogenous progesterone suppressed the conversion of 17-hydroxyprogesterone to androstenedione by C_{17}-C_{20}-lyase, while exogenous 17-hydroxyprogesterone had little effect upon the activity of C_{17}-C_{20}-lyase. Matsumoto *et al.*[9] speculated that the active sites for the 17-hydroxylation of progesterone and for the side chain cleavage of 17-hydroxyprogesterone may be so closely associated that the progesterone molecule may remain attached at the common interacting site as it undergoes consecutive reactions. They further speculated that progesterone and 17-hydroxyprogesterone compete for the same active site, so the competition of 17-hydroxyprogesterone with progesterone would be upon the 17-hydroxylation of progesterone. This close association of the interacting sites would also explain the inhibition of C_{17}-C_{20}-lyase by progesterone.

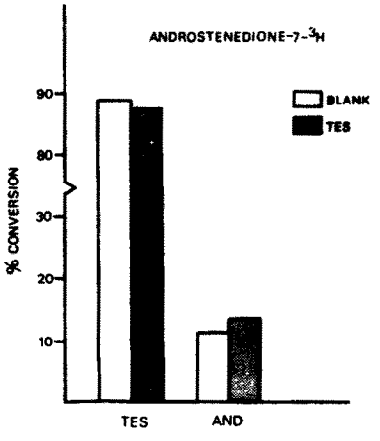

Fig. 4. Metabolism of radiolabelled androstenedione (10 μCi; 200 nmol) by marmoset (*Saguinus oedipus*) testicular fragments in the presence or absence of 200 nmol of unlabelled testosterone. The following abbreviations are used in the figure: TES, testosterone as testosterone acetate; AND, androstenedione.

Results from the present and from previous studies [1, 2] suggests that progesterone is the preferred substrate for marmoset testicular 17-hydroxylase. Progesterone was efficiently 17-hydroxylated into 17-hydroxyprogesterone. However, pregnenolone was not a suitable substrate for 17-hydroxylase since 17-hydroxypregnenolone was not identified in the pregnenolone incubates. These results are consistent with those of Tamaoki[11] in which progesterone was reported as the preferred substrate for 17-hydroxylase in the rat testis. However, Fevold[12], and Fevold and Drummond[13], and more recently, Kremers[14] reported in the rat that pregnenolone is the preferred substrate for 17-hydroxylation.

In the incubates of radiolabelled pregnenolone substrate with unlabelled progesterone, the lack of any decrease in the percentage of substrate converted into androstenedione and testosterone compared to control incubates may be explained on the basis of the activity of 17-hydroxypregnene-C_{17}-C_{20}-lyase. The accumulation of 17-hydroxyprogesterone in the control pregnenolone incubates indicates that the C_{17}-C_{20}-lyase may not be capable of converting a substantial portion of the 17-hydroxyprogesterone formed from pregnenolone to androstenedione within the 3 h period of incubation, resulting in an apparent accumulation of 17-hydroxyprogesterone in the incubates. However, in the pregnenolone incubates receiv-

Table 4. Crystallization data for metabolism of androstenedione-7-[^{3}H] (10 μCi; 200 nmol) by marmoset (*Saguinus oedipus*) testicular fragments in the presence and absence of testosterone (200 nmol) at 3 h of incubation

Metabolite*†	^{3}H d.p.m. (I)§	^{3}H d.p.m./mg‖¶	^{14}C d.p.m./mg‖¶	^{3}H/^{14}C	^{3}H d.p.m. (T)**	% Purity††
Control incubates						
TES	9513.9	449.5	2.9	153.9	9000.1	94.6
AND‡	1155.8	52.7	1.9	26.9	1055.2	91.3
Testosterone incubates						
TES	8640.2	398.3	2.8	141.7	7974.9	92.3
AND‡	1207.2	53.9	1.8	29.0	1079.2	89.4

* Legend as in Table 1.

ing unlabelled progesterone, the C_{17}-C_{20}-lyase is apparently metabolizing a similar amount of the 17-hydroxyprogesterone to androstenedione, but since the 17-hydroxyprogesterone formed from pregnenolone is sufficiently lowered when unlabelled progesterone is present in the incubates, the lowered conversion of pregnenolone to 17-hydroxyprogesterone is reflected as a decreased accumulation of 17-hydroxyprogesterone in the incubates. Consequently, the percentages of androstenedione and testosterone in the incubates remain unchanged since the C_{17}-C_{20}-lyase is still converting a similar amount of 17-hydroxyprogesterone into these metabolites in both control and progesterone-treated pregnenolone incubates.

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