

# Aspirin inhibits androgen response to chorionic gonadotropin in humans

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**Conte, Domenico, Francesco Romanelli, Silvia Fillo, Laura Guidetti, Aldo Isidori, Francesco Franceschi, Maurizio Latini, and Luigi Di Luigi.** Aspirin inhibits androgen response to chorionic gonadotropin in humans. *Am. J. Physiol.* 277 (*Endocrinol. Metab.* 40): E1032–E1037, 1999.—Eicosanoids play an important role in the regulation of the hypothalamic-pituitary axis; less clear is their role in testicular steroidogenesis. To evaluate the involvement of cyclooxygenase metabolites, such as prostaglandins, in the regulation of human testicular steroidogenesis, we examined the effects of a prostaglandin-blocker, aspirin, on plasma testosterone, pregnenolone, progesterone, 17OH-progesterone, androstenedione, dehydroepiandrosterone, and 17 $\beta$ -estradiol response to human chorionic gonadotropin (hCG) in normal male volunteers in a placebo-controlled, single-blinded study. To test the efficacy of aspirin, seminal prostaglandin E<sub>2</sub> levels were also determined. hCG stimulation increased peripheral levels of testosterone, 17OH-progesterone, androstenedione, dehydroepiandrosterone, and 17 $\beta$ -estradiol, without affecting circulating pregnenolone and progesterone values. Aspirin significantly lowered seminal prostaglandin E<sub>2</sub> levels, whereas it did not modify steroid concentrations not exposed to exogenous hCG. Moreover, the drug significantly reduced the response of testosterone, 17OH-progesterone, androstenedione, and dehydroepiandrosterone to hCG, as assessed by the mean integrated area under the curve, whereas it did not influence 17 $\beta$ -estradiol response. In conclusion, aspirin treatment inhibits androgen response to chorionic gonadotropin stimulation in normal humans. The action of aspirin is probably mediated via an effective arachidonate cyclooxygenase block.

human chorionic gonadotropin; prostaglandin; androgens; testis

PROSTAGLANDINS and other arachidonate metabolites, generically named eicosanoids, play an important role in the regulation of the hormonal secretions of the hypothalamic-pituitary-testicular axis, as shown by in vivo and in vitro studies in animals. Indeed, it is well established that at the hypothalamic level prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), synthesized by cyclooxygenase enzyme, acts as an intracellular mediator of gonadotropin-releasing hormone (GnRH) release (20, 21). In this regard, one of our previous studies, which showed that aspirin, a prostaglandin-blocker, inhibits luteinizing

hormone (LH) response to naloxone in humans, suggested also that this activity of eicosanoids of the cyclooxygenase pathway operates in humans (2). It is well-known that at the pituitary step, the arachidonate lipoyxygenase pathway is required for LH secretion (1, 19). Less clear is the role of eicosanoids at the testis site, in the regulation of steroidogenesis. Indeed, it has been reported that cyclooxygenase (29) or lipoyxygenase metabolites (7) or arachidonic acid (AA) itself (12) could be directly involved in the process of testosterone (T) production. In a previous study, we showed that exogenous AA stimulates T production in rat Leydig cells and that its conversion to cyclooxygenated or lipoyxygenated metabolites is not required for the steroidogenic action (23).

Therefore, to verify the involvement of eicosanoids, particularly cyclooxygenated compounds such as prostaglandins, in the regulation of testicular steroidogenesis and to evaluate whether it also operates in humans, the present study was designed to examine the effect of a treatment with oral aspirin, an inhibitor of the cyclooxygenase pathway of AA metabolism, on testicular steroidogenesis. To this end, during short-term aspirin administration, when the subjects were not exposed to exogenous human chorionic gonadotropin (hCG) and were under acute stimulation with chorionic gonadotropin, the following hormone levels were determined: plasma T, pregnenolone, 17 $\beta$ -estradiol,  $\Delta^4$  [progesterone; 17OH-progesterone (17OH-P); androstenedione (A)], and  $\Delta^5$  [dehydroepiandrosterone (DHEA)] T precursors. To test the efficacy of aspirin as a prostaglandin-blocker at the dose and times used in the experimental protocol, seminal PGE<sub>2</sub> levels were also determined.

## MATERIALS AND METHODS

**Subjects.** Eight healthy male volunteers, aged 20–30 years, entered the study after giving written informed consent according to the Helsinki II declaration. They were nonsmokers and had been free of medication for at least 4 wk. All had normal medical histories, physical examinations, serum chemistries, full blood counts, urinalyses, and hormonal evaluations, including dynamic pituitary-testis axis function tests (plasma LH levels before and after 100  $\mu$ g iv injection of GnRH at –30, –15, 0, +15, +30, +45, +60, and +90 min were the following: 5.2  $\pm$  0.6, 4.8  $\pm$  0.5, 6.1  $\pm$  0.9, 11.3  $\pm$  1.2, 21.2  $\pm$  2.7, 19.1  $\pm$  1.6, 14.3  $\pm$  0.9, and 9.1  $\pm$  0.8 mIU/ml, respectively).

Each subject received the following treatments, separated by an interval of at least 1 mo. For the experimental study: 1) placebo aspirin plus hCG, 2) aspirin plus hCG; for the control

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study, 3) aspirin plus placebo hCG, and 4) placebo aspirin plus placebo hCG.

**Study design.** All the treatments were placebo controlled, single blinded, and approved by the local Ethical Committee. Aspirin (Cemirit, Bayer, Milan, Italy) or placebo was administered in an oral dose of 800 mg (1 tablet) two times daily for 7 days (i.e., 2 days before and 5 days after hCG or placebo administration). For the hCG test, the dose administered, the sampling times, and the steroids evaluated were as reported in previous studies (8, 25).

On the morning of the experiment, between 0800–0900, an indwelling catheter was inserted after an overnight fast and basal blood sampling was begun at –60 min and was continued at –20-min intervals until intramuscular administration of 5,000 IU hCG (Profasi HP, Serono, Rome, Italy) or normal saline at 0 min. Further blood samples were drawn at 2, 24, 48, 72, and 96 h. Before aspirin (or placebo) ingestion on the morning of hCG administration (2 h before the test) and 96 h after chorionic gonadotropin injection (after the last blood sample), the subjects were asked to collect semen specimens by masturbation for seminal PGE<sub>2</sub> measurement.

**Hormonal assay.** PGE<sub>2</sub> was extracted from seminal plasma, as previously described (3), and measured by RIA (NEN-Du Pont, Wilmington, DE). The intra- and interassay coefficients of variation (CVs) were 6.4 and 8.2%, respectively ( $n = 10$ ).

T, pregnenolone, progesterone, 17OH-P, DHEA, A, and 17 $\beta$ -estradiol were determined by RIA. T, progesterone, DHEA, and A kits were purchased from DSL (Webster, TX). The intra- and interassay CVs were 7.8 and 8.1% for T, 6.6 and 11.7% for progesterone, 3.1 and 6.9% for DHEA, and 4.3 and 7.7% for A, respectively ( $n = 10$ ). The sensitivity was 0.08 ng/ml for T, 0.12 ng/ml for progesterone, 0.009 ng/ml for DHEA, and 0.03 ng/ml for A. The pregnenolone kit was purchased from Diagnostics Biochem (London, ON, Canada). The intra- and interassay CVs were 13 and 15%, respectively ( $n = 10$ ). The sensitivity for pregnenolone was 0.15 ng/ml. 17OH-P and 17 $\beta$ -estradiol kits were purchased from CIS (Cedex, France). The intra- and interassay CVs were 5.9 and 6.9% for 17OH-P, respectively, and 6.5 and 10.5% for 17 $\beta$ -estradiol, respectively ( $n = 10$ ). The sensitivity for 17OH-P and 17 $\beta$ -estradiol was 0.02 ng/ml and 1.35 pg/ml, respectively.

**Statistical methods.** Steroid levels after hCG (or placebo) administration are expressed as a percentage of the basal values. Moreover, cumulative secretory areas under the curve from 0 to 96 h (AUC) were calculated by the trapezoidal method and expressed as percent differences between either placebo aspirin + hCG or aspirin + hCG tests and placebo aspirin + placebo hCG test. Results are expressed as means  $\pm$  SE.

Seminal PGE<sub>2</sub> level comparisons were performed by paired Student's *t*-test. For all hormones, one-way ANOVA with repeated measures was employed to determine the overall response to hCG stimulation. The Dunnett's statistical test was used for post hoc multiple comparison analysis against values before hCG administration. Comparisons of the differences between tests were performed by paired Student's *t*-test. Statistical significance was considered as  $P < 0.05$ .

## RESULTS

Aspirin induced a significant reduction of seminal PGE<sub>2</sub> levels in the aspirin + hCG test from  $86 \pm 5$   $\mu$ g/ml before the treatment to  $12 \pm 2$  and  $14 \pm 3$   $\mu$ g/ml, during and at the end of its administration, respectively ( $P < 0.001$ ). Seminal PGE<sub>2</sub> levels were not modified in the placebo aspirin + hCG test ( $85 \pm 4$ ,

$80 \pm 5$ , and  $89 \pm 4$   $\mu$ g/ml before, during, and at the end of treatment, respectively).

During basal sampling (from –60 to 0 min), all steroids measured were in the normal range and did not show significant differences for the four tests (data not shown). Furthermore, no differences in steroids and seminal PGE<sub>2</sub> levels were observed before and after placebo hCG administration (in aspirin + placebo hCG and placebo aspirin + placebo hCG tests; data not shown).

Because hormonal concentrations varied from one subject to the other, to give more homogeneity to the results, the variations of plasma steroid values after hCG injection were expressed as a percentage of the levels not exposed to exogenous chorionic gonadotropin (each subject as its own control).

After hCG administration, significant percent increases in plasma T occurred at 2, 24, 48, 72, and 96 h ( $25.9 \pm 5.8$ ,  $39.7 \pm 11.7$ ,  $87.2 \pm 16.8$ ,  $72.8 \pm 19.3$ , and  $76.3 \pm 25.6$ , respectively;  $P < 0.01$ ) in the placebo aspirin + hCG test and at 48, 72, and 96 h ( $38.3 \pm 19.9$ ,  $43.3 \pm 15.2$ , and  $29.3 \pm 19.9$ , respectively;  $P < 0.01$ ) in the aspirin + hCG test, with significant decreases ( $P < 0.01$ ) of T values in the latter test, at any time considered (Fig. 1).

With regard to plasma pregnenolone and progesterone, hCG administration did not induce significant variations in either placebo aspirin + hCG or aspirin + hCG tests at any time considered (data not shown).

As for the effect of hCG administration on 17OH-P levels, significant percent increases were observed at 24 and 48 h in both the placebo aspirin + hCG ( $92.7 \pm 36.2$  and  $81.4 \pm 49.7$ , respectively;  $P < 0.01$ ) and the aspirin + hCG tests ( $55.5 \pm 7.0$  and  $43.9 \pm 12.6$ ,

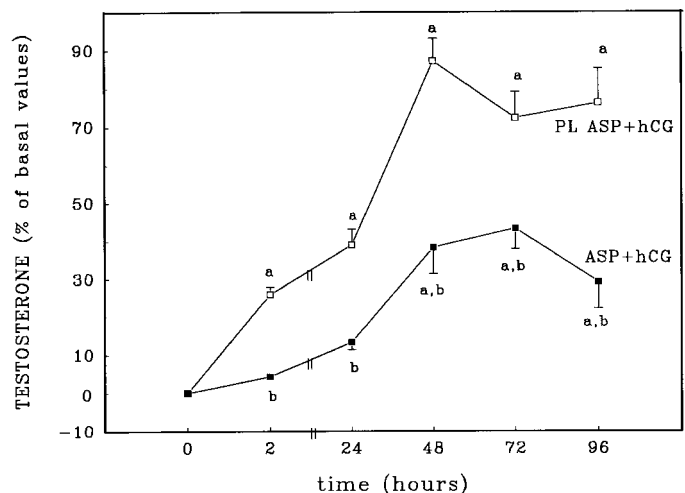


Fig. 1. Effects of oral aspirin (or placebo) treatment on means  $\pm$  SE percent increment of plasma testosterone concentrations after im human chorionic gonadotropin (hCG) administration to 8 normal volunteers. Absolute values (means  $\pm$  SE) at 0, 2, 24, 48, 72, and 96 h are the following:  $494.8 \pm 30.8$ ,  $621.6 \pm 37$ ,  $686.1 \pm 33.8$ ,  $920.6 \pm 53.9$ ,  $846.8 \pm 45$ , and  $870.1 \pm 69$  ng/ml, respectively, in placebo (PL) aspirin (ASP) + hCG test and  $569.6 \pm 43.1$ ,  $594.6 \pm 47.2$ ,  $653.6 \pm 61.7$ ,  $781.1 \pm 53.6$ ,  $803.4 \pm 31.3$ , and  $723.4 \pm 35.4$  ng/ml, respectively, in aspirin + hCG test. a:  $P < 0.01$ , placebo aspirin + hCG and aspirin + hCG vs. respective basal values. b:  $P < 0.01$ , aspirin + hCG vs. placebo aspirin + hCG.

respectively;  $P < 0.01$ ), with a significant decrease ( $P < 0.05$ ) in steroid values at 24 h in the latter test (Fig. 2).

Regarding plasma A levels after hCG administration, significant percent increases occurred in the placebo aspirin + hCG test at any time considered ( $52.9 \pm 50.9$ ,  $50.6 \pm 48.7$ ,  $58.8 \pm 33.7$ ,  $67.5 \pm 18.8$ , and  $63.0 \pm 51.3$ ;  $P < 0.05$ ), whereas no increases were observed in the aspirin + hCG test, with a significant decrease ( $P < 0.01$ ) at 2, 72, and 96 h in the latter test (Fig. 3).

When the effects of hCG stimulation on DHEA levels were evaluated, significant percent increases were observed at 48 h in both the placebo aspirin + hCG ( $67.8 \pm 44.8$ ;  $P < 0.05$ ) and the aspirin + hCG tests ( $31.6 \pm 20.9$ ;  $P < 0.05$ ), with a significant decrease ( $P < 0.05$ ) at 2, 48, 72, and 96 h in the latter test (Fig. 4).

With regard to plasma  $17\beta$ -estradiol response to chorionic gonadotropin, significant percent increases occurred at 24, 48, 72, and 96 h in the placebo aspirin + hCG test ( $241.8 \pm 167.9$ ,  $370 \pm 142.2$ ,  $253.0 \pm 107.8$ , and  $209.2 \pm 119.3$ , respectively;  $P < 0.01$ ) and at 24, 48, and 72 h in the aspirin + hCG test ( $354.3 \pm 184.9$ ,  $279.8 \pm 163.0$ , and  $180.7 \pm 131.2$ , respectively;  $P < 0.05$ ), without any significant difference between the two tests (Fig. 5).

To better evaluate and also to quantify the effects of aspirin on hCG-induced testicular steroidogenesis, the percent differences (% $\Delta$ ) of the cumulative AUCs of the steroids after chorionic gonadotropin stimulation were also calculated (Fig. 6). % $\Delta$  of T AUC response to hCG was significantly decreased by aspirin treatment ( $148.23 \pm 3.89$  vs.  $126.10 \pm 3.53$  placebo aspirin + hCG vs. aspirin + hCG test, respectively;  $P < 0.01$ ). In the same way, % $\Delta$  of  $17\text{OH-P}$  AUC response to hCG was significantly decreased by aspirin ( $144.32 \pm 5.30$  vs.

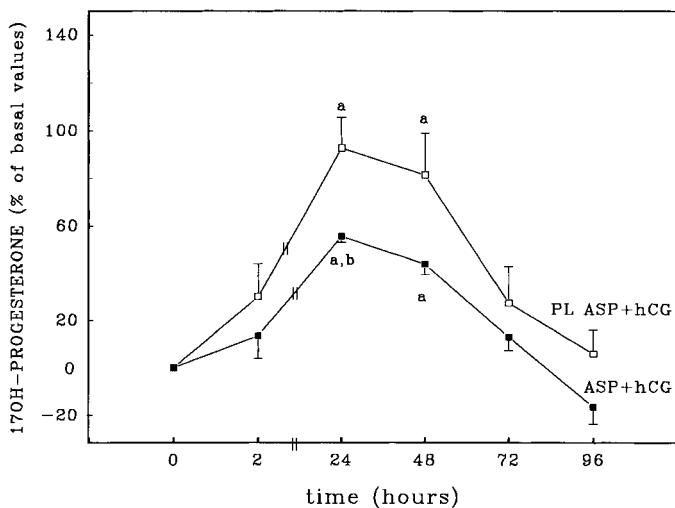


Fig. 2. Effects of oral aspirin (or placebo) treatment on means  $\pm$  SE percent increment of plasma  $17\text{OH}$ -progesterone concentrations after im hCG administration to 8 normal volunteers. Absolute values (means  $\pm$  SE) at 0, 2, 24, 48, 72, and 96 h are the following:  $2.3 \pm 0.1$ ,  $3.2 \pm 0.3$ ,  $4.4 \pm 0.1$ ,  $4.1 \pm 0.2$ ,  $2.9 \pm 0.3$ , and  $2.4 \pm 0.2$  pg/ml, respectively, in placebo aspirin + hCG test and  $2.7 \pm 0.1$ ,  $2.9 \pm 0.1$ ,  $4.1 \pm 0.2$ ,  $3.8 \pm 0.2$ ,  $3 \pm 0.1$ , and  $2.2 \pm 0.1$  pg/ml, respectively, in aspirin + hCG test. a:  $P < 0.01$ , placebo aspirin + hCG and aspirin + hCG vs. respective basal values. b:  $P < 0.01$ , aspirin + hCG vs. placebo aspirin + hCG.

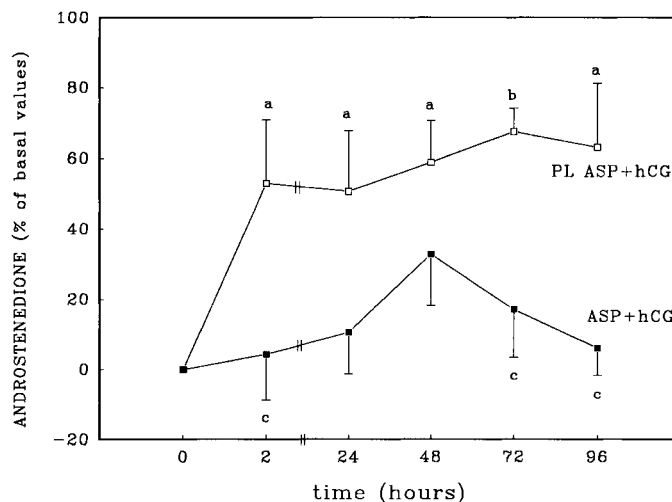


Fig. 3. Effects of oral aspirin (or placebo) treatment on means  $\pm$  SE percent increment of plasma androstenedione concentrations after im hCG administration to 8 normal volunteers. Absolute values (means  $\pm$  SE) at 0, 2, 24, 48, 72, and 96 h are the following:  $2.2 \pm 0.2$ ,  $3.1 \pm 0.1$ ,  $3.1 \pm 0.2$ ,  $3.3 \pm 0.2$ ,  $3.7 \pm 0.5$ , and  $3.4 \pm 0.3$  ng/ml, respectively, in placebo aspirin + hCG test and  $2.6 \pm 0.2$ ,  $2.4 \pm 0.1$ ,  $2.6 \pm 0.1$ ,  $3.2 \pm 0.2$ ,  $2.9 \pm 0.4$ , and  $2.7 \pm 0.3$  ng/ml, respectively, in aspirin + hCG test. a:  $P < 0.05$ , placebo aspirin + hCG vs. basal values. b:  $P < 0.01$ , placebo aspirin + hCG vs. aspirin + hCG. c:  $P < 0.01$ , aspirin + hCG vs. placebo aspirin + hCG.

$129.05 \pm 3.18$  placebo aspirin + hCG vs. aspirin + hCG test, respectively;  $P < 0.05$ ). A significant decrease was also observed in % $\Delta$  of A AUC ( $138.13 \pm 3.98$  vs.  $126.71 \pm 7.42$  placebo aspirin + hCG vs. aspirin + hCG test, respectively;  $P < 0.05$ ) and in % $\Delta$  of DHEA AUC ( $141.43 \pm 11.67$  vs.  $112.40 \pm 5.30$  placebo aspirin + hCG vs. aspirin + hCG test, respectively;  $P < 0.05$ )

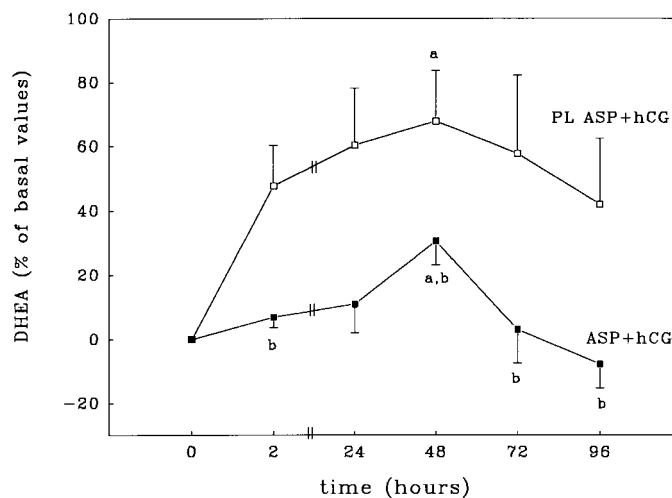


Fig. 4. Effects of oral aspirin (or placebo) treatment on means  $\pm$  SE percent increment of plasma dehydroepiandrosterone (DHEA) concentrations after im hCG administration to 8 normal volunteers. Absolute values (means  $\pm$  SE) at 0, 2, 24, 48, 72, and 96 h are the following:  $6.5 \pm 0.5$ ,  $9.5 \pm 1.1$ ,  $10.3 \pm 1.2$ ,  $10.8 \pm 1.1$ ,  $10.1 \pm 1.4$ , and  $9.1 \pm 1.2$  ng/ml, respectively, in placebo aspirin + hCG test and  $9.2 \pm 0.8$ ,  $9.6 \pm 0.6$ ,  $9.9 \pm 0.9$ ,  $12.2 \pm 1.4$ ,  $9.7 \pm 1.6$ , and  $8.3 \pm 0.8$  ng/ml, respectively, in aspirin + hCG test. a:  $P < 0.05$ , placebo aspirin + hCG and aspirin + hCG vs. respective basal values. b:  $P < 0.05$ , aspirin + hCG vs. placebo aspirin + hCG.

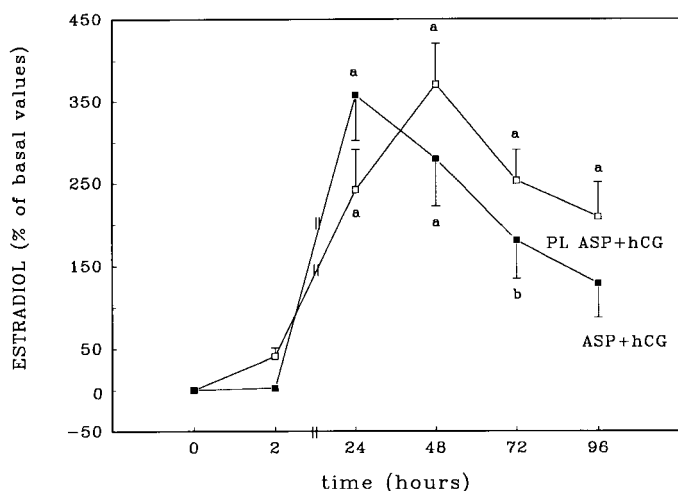


Fig. 5. Effects of oral aspirin (or placebo) treatment on means  $\pm$  SE percent increment of plasma 17 $\beta$ -estradiol concentrations after im hCG administration to 8 normal volunteers. Absolute values (means  $\pm$  SE) at 0, 2, 24, 48, 72, and 96 h are the following: 18.3  $\pm$  2.2, 24.8  $\pm$  2.2, 64.1  $\pm$  12.8, 80.2  $\pm$  4.7, 59.4  $\pm$  2.5, and 51.6  $\pm$  3.2 pg/ml, respectively, in placebo aspirin + hCG test and 19.1  $\pm$  3.3, 18.8  $\pm$  2.7, 74.1  $\pm$  3.3, 61.9  $\pm$  4.3, 45.5  $\pm$  3.5, and 36.6  $\pm$  3.1 pg/ml, respectively, in aspirin + hCG test. a:  $P < 0.01$ , placebo aspirin + hCG and aspirin + hCG vs. respective basal values. b:  $P < 0.01$ , aspirin + hCG vs. placebo aspirin + hCG.

response to hCG. In contrast, % $\Delta$  of 17 $\beta$ -estradiol AUC was not modified by aspirin treatment.

## DISCUSSION

First of all, the finding that chorionic gonadotropin induced the expected increase of peripheral levels of T, its  $\Delta^4$  (17OH-P and A) and  $\Delta^5$  (DHEA) precursors, as well as 17 $\beta$ -estradiol, confirmed previous numerous reports present in literature (8, 9, 14, 25). With regard to progesterone and pregnenolone, the finding that their plasma levels were not increased by hCG stimulation was also in agreement with a previous report (14). The lack of chorionic gonadotropin effect on circulating

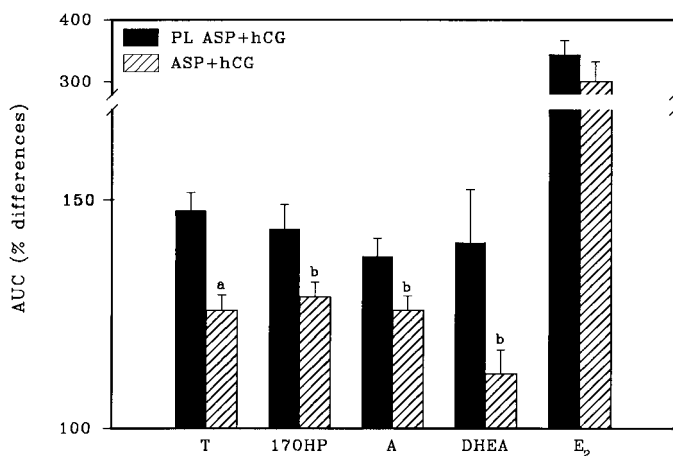


Fig. 6. Effects of oral aspirin (or placebo) treatment on means  $\pm$  SE percent differences of cumulative areas under the curves (AUC) of steroid response after im hCG administration to 8 normal volunteers. T, testosterone; 17OHP, 17OH-progesterone; A, androstenedione; E<sub>2</sub>, 17 $\beta$ -estradiol. a:  $P < 0.01$ , aspirin + hCG vs. placebo aspirin + hCG. b:  $P < 0.05$ , aspirin + hCG vs. placebo aspirin + hCG.

progesterone and pregnenolone values may have been due to their rapid conversion into their metabolites under hCG stimulation inside the Leydig cells.

Furthermore, the results of the present study indicate that aspirin treatment, at the dose and times used in the experimental protocol, is effective as a cyclooxygenase inhibitor because it significantly reduced seminal PGE<sub>2</sub> levels. In addition, the inhibitory activity of the drug on hCG-induced T production seems to suggest that the cyclooxygenase pathway of the AA metabolism could be involved in hCG-stimulated testicular steroidogenesis in humans, whereas it was not required when the subjects were not exposed to exogenous chorionic gonadotropin, as shown by the lack of aspirin effect in this condition. Moreover, aspirin treatment inhibited the  $\Delta^4$  T precursors, such as 17OH-P and A, and also the  $\Delta^5$  T precursor, DHEA, whereas it did not modify the 17 $\beta$ -estradiol response to chorionic gonadotropin. This lack of aspirin effect could reflect the small testicular contribution to the overall amount of plasma 17 $\beta$ -estradiol levels (30). Theoretically, the simultaneous inhibition of T and some of its  $\Delta^4$  and  $\Delta^5$  precursor responses to hCG induced by aspirin treatment could be due to a specific block of the enzymatic activities involved in the production of 17OH-P, DHEA, A, and T (i.e., 17 $\alpha$ -hydroxylase, 17,20-lyase, and 17 $\beta$ -hydroxysteroid dehydrogenase) and/or to higher enzymatic inhibitions. In this regard, it has been reported that another cyclooxygenase inhibitor, indomethacin, is able to inhibit 3 $\beta$ -hydroxysteroid dehydrogenase and 17 $\beta$ -hydroxysteroid dehydrogenase activities in rat testis (22). On the other hand, more recently, it has been shown that AA by itself exerts a specific inhibitory effect on 17 $\beta$ -hydroxysteroid dehydrogenase in rat Leydig cells (13). However, with regard to human testis, these possibilities seem to be unlikely because the present study indicates that aspirin treatment does not affect steroidogenesis in conditions of nonexposure to exogenous hCG. As a consequence, the aspirin inhibition of androgen response to chorionic gonadotropin may more likely be explained by the drug's interference with the LH-hCG action on the plasma membrane of Leydig cells. It is well established that steroidogenesis in Leydig cells is mainly regulated by LH, via the interaction with its receptors coupled to the adenylate cyclase-cAMP signaling pathway. It is also well-known that occupied LH receptors can activate a phospholipase C signaling cascade, but only at high hormone concentrations, with consequent stimulation of phosphatidylinositol turnover leading to intracellular release of diacylglycerol, inositol triphosphate, calcium, AA, and its metabolites (4, 10, 24, 28). This fatty acid, once released into the cytosol, may act itself directly as a signaling molecule and/or via its metabolic pathways (18). Indeed, AA may undergo different metabolizations via cyclooxygenase, lipoxygenase, and cytochrome P-450-dependent epoxygenase pathways, leading to the formation of 1) prostaglandins and thromboxanes, 2) hydroxyeicosatetraenoic acids and leukotrienes, and 3) epoxyeicosatetraenoic acids, respectively. Therefore, it is possible that aspirin can block the coupling of

occupied LH receptors to phospholipase C, considering that the dose of chorionic gonadotropin used in the present study was high, compared with the endogenous LH levels. Moreover, this hypothesis could explain the discrepancy between the results obtained when the subjects were not exposed to exogenous hCG and under chorionic gonadotropin stimulation.

Several experimental evidences, obtained in animals, indicate the involvement of AA and its metabolites in gonadal function. In particular, it has been shown that these compounds seem to be required in the regulation of testicular steroidogenesis. Indeed, it has been reported that LH induces a rapid release of AA from Leydig cells (5), which is dependent on and directly proportional to the membrane concentration of LH-hCG receptors (17). Moreover, different findings suggest that this fatty acid displays a steroidogenic effect that could be either direct or mediated by its metabolic pathways. The results of studies on the effect of AA itself on testicular steroidogenesis seem to be quite variable and sometimes contradictory, depending on the different experimental conditions. In fact, it has been reported that exogenous AA is able to stimulate basal T production (6, 7). When the effect of exogenous AA on LH-hCG-induced T secretion was evaluated, a direct biphasic (stimulatory/inhibitory) activity was observed, depending on the time of incubation (12) or its concentrations (6). However, because LH induces AA release from Leydig cells, as previously mentioned, when exogenous AA was added to Leydig cells stimulated by LH-hCG, the effect on T production appeared to be cumulative to that of its endogenously released amount, so that physiological considerations must be taken into account with caution. Furthermore, several studies have been carried out to evaluate the role of AA metabolites on testicular steroidogenesis. (7, 11, 15, 22, 23, 29) However, the results of these studies are conflicting. Indeed, it has been reported that eicosanoids of both cyclooxygenase (29) and lipoxygenase (7, 15) pathways may be involved in the stimulation of T production, whereas other authors (11, 23) indicate that the conversion of AA to the cyclooxygenase or lipoxygenase metabolites is not required for its steroidogenic effect. On the other hand, evidence has been provided suggesting the involvement of both cyclooxygenase and lipoxygenase metabolites in the stimulation of testicular steroidogenesis (22). However, most of these in vitro studies were carried out with arachidonate pathway inhibitors, such as indomethacin and nordihydroguaiaretic acid (NDGA, a lipoxygenase blocker). In this regard, it has been reported that indomethacin, which at low doses is an inhibitor of the cyclooxygenase pathway, at higher concentrations inhibits lipoxygenase activity (26). In the same way, in a previous study we observed that NDGA at low concentrations is a specific lipoxygenase inhibitor, whereas at higher concentrations it also inhibits cyclooxygenase activity (23). Because in most of these reports indomethacin and NDGA were used at higher concentrations, caution is required when interpreting the results of these studies.

The present study indicates that in humans the conversion of AA to its cyclooxygenase metabolites could be required for the expression of its steroidogenic effect. An alternative explanation of the effect of aspirin treatment observed in this study could be theoretically considered: the drug-induced inhibition of androgen response to hCG might not be related to a direct cyclooxygenase block but rather to an indirect stimulation of other arachidonate metabolic pathways (i.e., lipoxygenase and/or epoxygenase), caused by increased substrate availability, and consequent production of some metabolite(s) with inhibitory activity on Leydig cells steroidogenesis. However, this possibility should be excluded because has been shown that lipoxygenase metabolites display stimulatory rather than inhibitory effects on T production (22, 27), and no evidence of stimulatory activity of the epoxygenase pathway has been obtained (13). Finally, the results observed might be explained by a prostaglandin-independent action of aspirin. This hypothesis, however, raises certain difficulties in that 1) the drug effects were observed only under hCG stimulation and not in conditions of nonexposure to exogenous chorionic gonadotropin and 2) considerably higher doses of aspirin than those used in the present study are needed to induce effects not mediated by cyclooxygenase block (16).

In conclusion, the present study shows, for the first time to our knowledge, that in humans androgen response to chorionic gonadotropin is inhibited by aspirin treatment and that the action of the drug is probably mediated via an effective arachidonate cyclooxygenase block.

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