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THE ADRENAL SECRETION OF PROGESTERONE STIMULATES TESTICULAR STEROIDOGENESIS IN THE RAT *IN VITRO*

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Summary—We have used a multicolumn isolated cell superfusion system to investigate the interaction between isolated rat adrenal and testicular cells in the secretion of sex steroids. The steroid response of a mixed population of adrenal and testicular cells to the administration of ACTH 100 pg/ml was compared with the responses of the separate cell types. Steroid responses from 5 separate experiments were analysed. We have demonstrated increased secretion of 17α-hydroxyprogesterone ($P < 0.0005$), androstenedione ($P < 0.05$) and testosterone ($P < 0.0005$) by a mixture of cells when compared with the responses of either cell type alone. In contrast, the secretion of progesterone ($P < 0.0005$) and corticosterone ($P < 0.005$) was reduced in the mixed cell populaton, suggesting that progesterone is preferentially converted by testicular cells to 17α-hydroxyprogesterone, androstenedione and testosterone. This is confirmed by increased secretion of 17α-hydroxyprogesterone, androstenedione and testosterone but not corticosterone by a mixed population following the administration of progesterone 10 ng/ml. These results suggest that the adrenal secretion of progesterone can stimulate testicular steroidogenesis in the male rat *in vitro*.

INTRODUCTION

We have investigated the possibility that the adrenal gland may contribute to the secretion of sex steroids by the testis. This subject has been well reviewed elsewhere [1, 2, 3]. Acute studies in pubertal (approx 40 day old) male rats *in vivo* have shown that the adrenal gland is required for normal testosterone secretion [4]. Bilateral adrenalectomy results in a rapid fall in plasma corticosterone and testosterone concentrations. However, if only one adrenal is removed the remaining adrenal undergoes sufficient compensatory hypertrophy to maintain normal testosterone secretion. In chronic studies, bilateral adrenalectomy (at 25 days) before puberty abolishes the normal pubertal (30–50 days) surge of LH and testosterone secretion and decreases spermatogenesis [5]. It has therefore been suggested that the adrenal cortex secretes an essential precursor that is utilized by the gonad in the secretion of sex steroids. Certainly the adrenal secretion of progesterone may have an important biological role in the sexual maturation of the rat but the ability of the testis to synthesise testosterone from progesterone *in vitro* is limited to the periods before (up to 20 days) and after puberty (over 50 days) [6]. However, adrenalectomy appears to have no influence over testosterone secretion *in vivo* in the adult (130–150 days) male rat [8]. The *in vitro* isolated cell superfusion approach [7] has enabled us to focus on direct interactions between adrenal and testicular cells and to discover whether the testicular secretion of sex steroids is modified by the presence of adrenal cells.

EXPERIMENTAL

Preparation of isolated cells

Whole adrenals and testes were obtained from 10 adult male Wistar rats (250–300 g). Whole adrenal glands were cleaned of adhering fat, minced and digested with collagenase (Worthington Diagnostic Systems, Flow Laboratories Ltd) at a concentration of 2 mg/ml (in Krebs–Ringer buffer containing 1% BSA and 0.2% glucose) for 60 min at 37°C in a shaking water bath [9]. Cells were mechanically dispersed with a 5 ml pipette at 30 and 60 min. Isolated adrenal cells were centrifuged at 300 *g* for 15 min, the cell pellet resuspended in buffer and 1×10^6 cells suspended in each superfusion chamber as required by the experimental protocol. Testicular cells were prepared by stripping the capsule from each whole testis with the minimum of mechanical force and then digesting the testis with collagenase (Sigma Chemicals Ltd) at a concentration of 0.25 mg/ml (in Krebs Ringer buffer containing 1% BSA and 0.2% glucose) for 10 min in a shaking water bath. The subsequent digest was suspended in 100 ml of buffer in a glass cylinder and allowed to settle for 2 min by gravity. The supernatant containing isolated testicular cells was centrifuged at 300 *g* for 15 min [10]. The pellet was resuspended in buffer and 10×10^6 testicular

cells packed into each superfusion chamber according to the experimental protocol. This corresponded to approximately 10^6 Leydig cells as estimated by 3β-hydroxysteroid dehydrogenase (3β-HSD) activity [11].

Experimental protocol

The superfusion system employs a multichannel peristaltic pump (Desaga PLG, Uniscience London), incubator (Gallenkamp) and fraction collector (LKB 2070 Ultrorac II). The steroid output from 10 parallel superfusion columns is compared in each experiment. Each superfusion chamber contains 0.4 g polyacrylamide gel based upon 20 μ nylon mesh (Henry Simon Ltd, Cheshire). Initial experiments used 2 ml disposable plastic syringes (Gillette Sabre) as superfusion columns. Later experiments used superfusion columns manufactured from polytetrafluoroethylene [12]. Isolated adrenal and testicular cells were either packed alone into superfusion columns or together as a mixed cell population. Cells were superfused with Krebs–Ringer buffer containing 0.2% BSA and 0.2% glucose at a rate of 0.5 ml/min for 1 h at a temperature of 37°C before the start of each experiment. 1, 10, 100 pg/ml synthetic ACTH (Tetracosactrin, CIBA), 10 ng/ml progesterone (Sigma) and 50 ng/ml hCG (Pregnyl, Organon) were infused over 10 min periods and the superfusate from each chamber collected in 5 min fractions for steroid hormone analysis.

Steroid hormone analysis

Steroid analysis was performed by direct radioimmunoassay for corticosterone [13], progesterone [14] and testosterone [15] but with the following modifications. All assays were performed in incubation medium. The testosterone assay used testosterone-3-(*O*-carboxymethyl) oxime-[^{124}I]iodohistamine as a radioligand.

Androstenedione was measured using sheep antisera (Guildhay HP/S/6731/A, University of Surrey) raised against androstenedione-7-α-carboxyethylthioether-ovalbumin with androstenedione-3-(*O*-carboxy-methyl)oxime.[^{125}I] iodohistamine as radioligand. Incubation was for 4 h prior to separation with a second antibody (Scottish Antibody Production Unit). Seventeen-α-hydroxyprogesterone was measured using rabbit antisera raised against 17α-hydroxyprogesterone-3-(*O*-carboxymethyl)oxime-BSA and [^{125}I]iodohistamine derivative as radioligand. The antibody was encapsulated within a semipermeable microcapsule and separation was by centrifugation after 45 min incubation [16].

All assays demonstrated parallelism to the standard curve when samples were serially diluted. Cross-reactivities to possible interfering steroids performed in incubation medium were in good agreement with those previously published.

Statistics and data analysis

To permit direct comparison of changes in steroid hormone concentrations between experiments and between cell populations, responses were converted to the natural logarithm transforming data to a normal distribution [17]. Data was then analysed by Student's *t*-test.

RESULTS

Superfusion of isolated testicular cells with eluate from stimulated adrenal cells

In one experiment, stimulation of adrenal cells with ACTH (100 pg/ml) followed by superfusion of the adrenal eluate through testicular cell columns also stimulated testosterone production (Fig. 1).

Dose–response of isolated cells to ACTH 1–100 pg/ml

Steroid responses to the administration of ACTH are illustrated in Fig. 2. In two experiments, ACTH in the dose range 1–100 pg/ml increased secretion of 17α-hydroxyprogesterone, androstenedione and testosterone from a suspension of both cell types ($n = 6$ columns per experiment) when compared with the superfusion of adrenal ($n = 2$) and testicular ($n = 2$) cells alone. However, the secretion of progesterone and corticosterone were concomitantly decreased in the mixed cell population when compared with that of adrenal cells alone. Secretion of these hormones by isolated testicular cells alone was negligible.

Pooled data of isolated cell response to ACTH stimulation

Steroid responses to the administration of ACTH 100 pg/ml pooled from 5 separate experiments are analysed. Increased secretion of 17α-hydroxyprogesterone ($P < 0.0005$), androstenedione ($P < 0.05$) and testosterone ($P < 0.0005$) by the mixed cell population ($n = 6$ columns per experiment) was accompanied by decreased secretion of progesterone ($P < 0.0005$) and corticosterone ($P < 0.005$) when

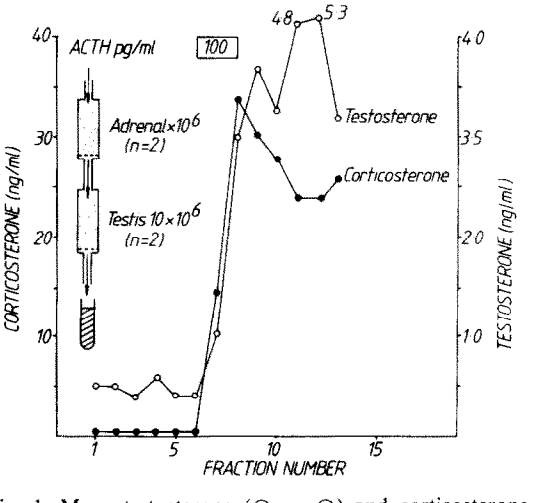
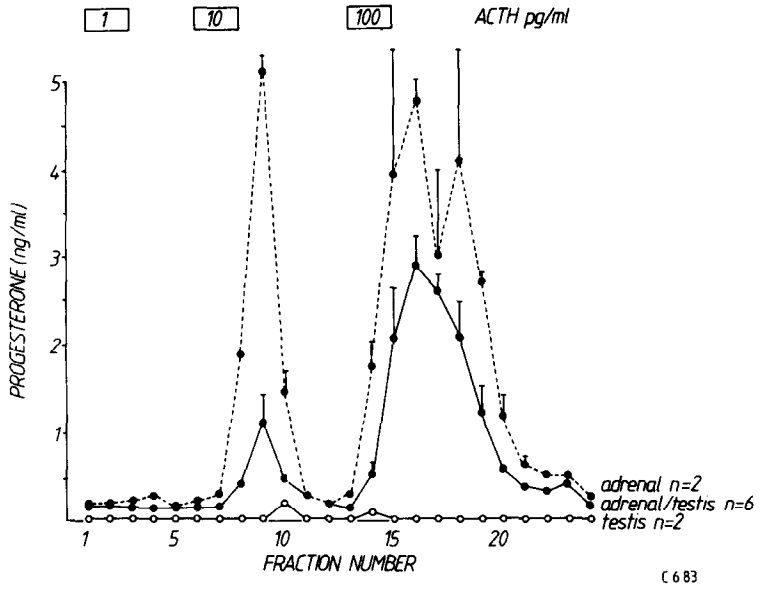
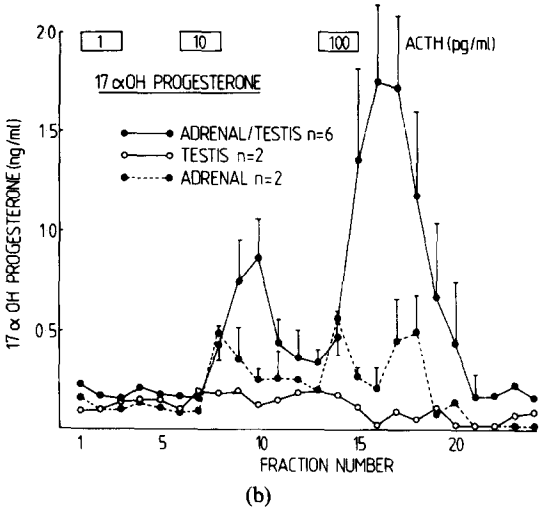


Fig. 1. Mean testosterone (○——○) and corticosterone (●——●) responses to ACTH 100 pg/ml following the perfusion of eluate from isolated superfused adrenal cells ($n = 2$ columns) through isolated superfused testicular cells ($n = 2$).



(a)



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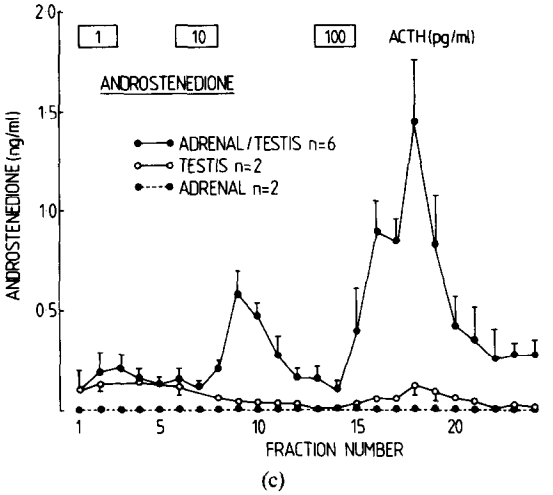
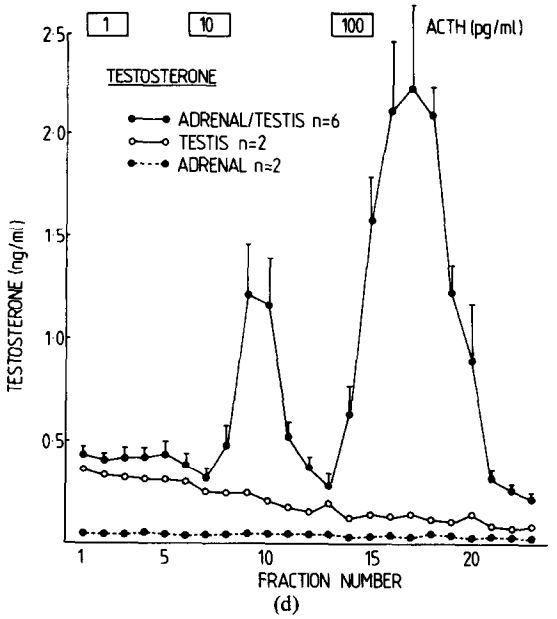
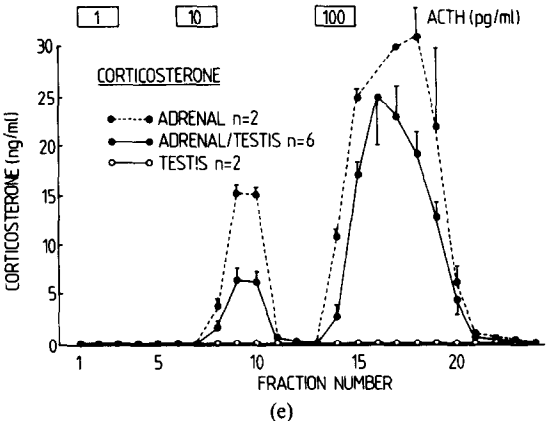


(c)



(d)



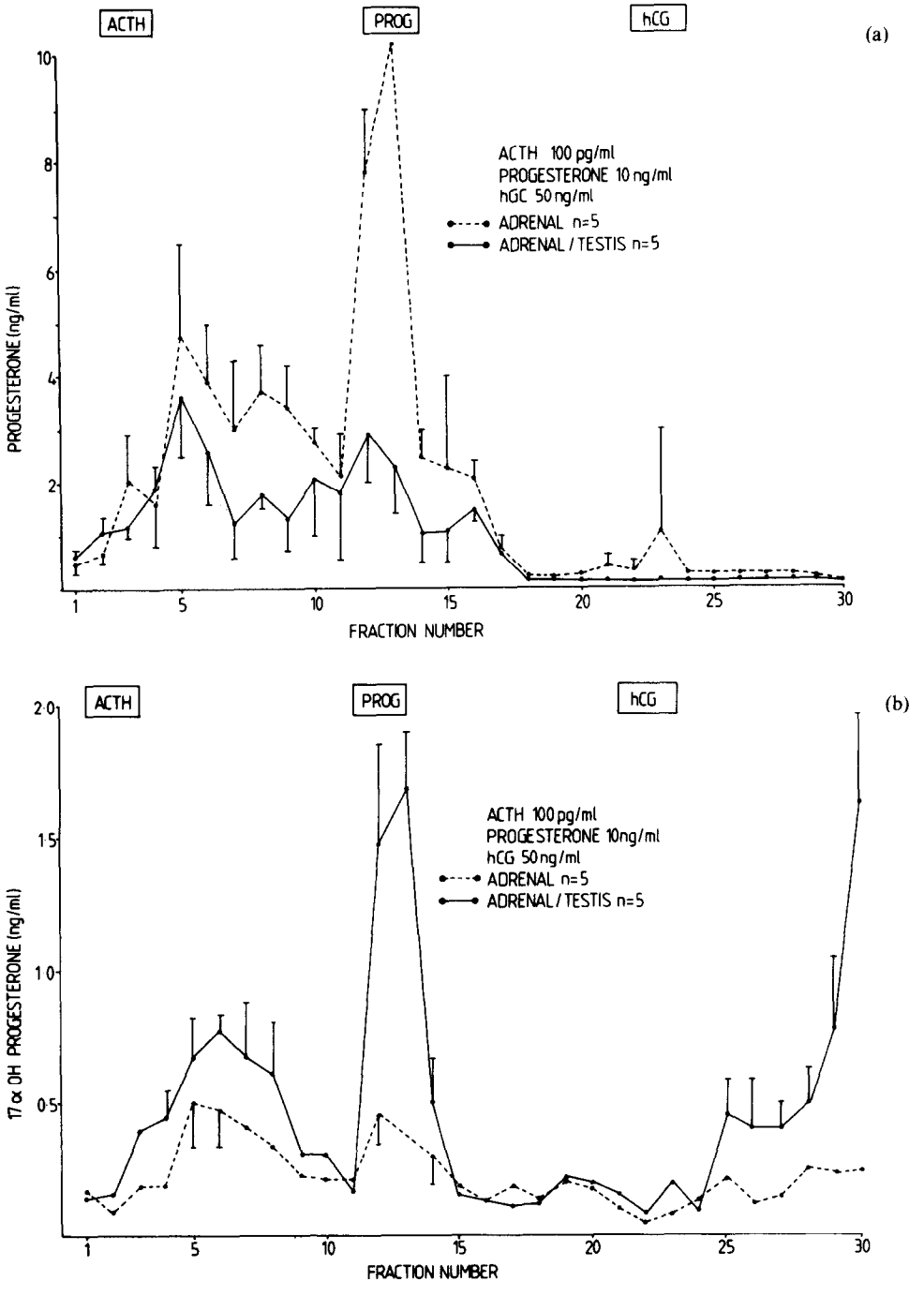
(e)

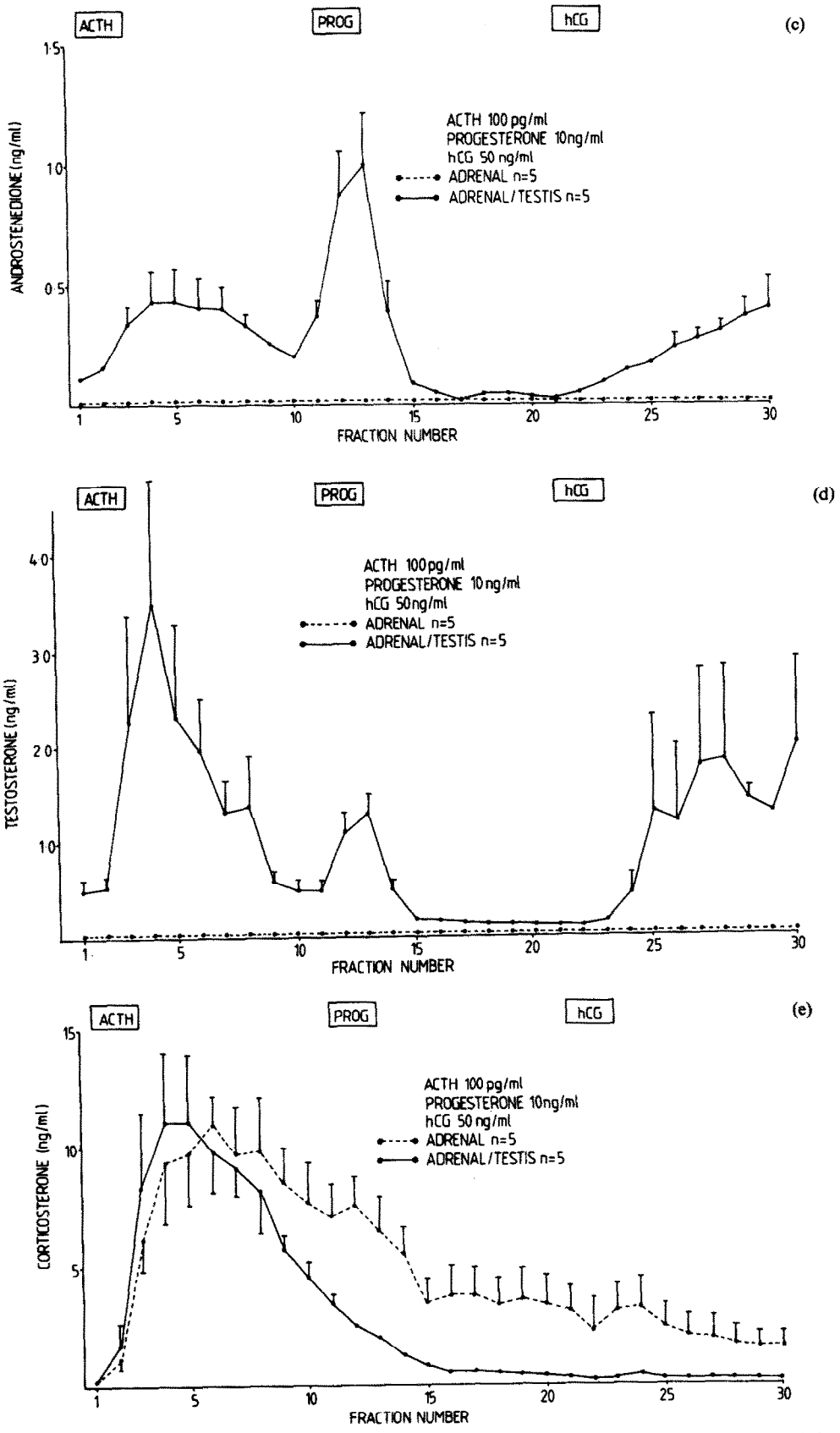
Figs 2(a–e). Mean ± SD steroid responses to ACTH over the dose range 1–100 pg/ml by isolated superfused adrenal cells (●---●, $n = 2$ columns), testicular cells (○——○, $n = 2$) and a mixed cell population (●——●, $n = 6$).

compared with the secretion of adrenal cells alone ($n = 2$), despite increased basal corticosterone secretion by the mixed cell population. Secretion of these hormones by testicular cells alone ($n = 2$) was negligible.

Response of isolated cells to ACTH, progesterone and hCG

Steroid responses in 1 experiment to the administration of ACTH 100 pg/ml, progesterone 10 ng/ml and hCG 50 ng/ml are illustrated in Fig. 3. In one experiment both ACTH and progesterone stimulated the secretion of 17α-hydroxyprogesterone, androstenedione and testosterone by the mixed cell population ($n = 5$ columns) whereas the concentrations of progesterone and corticosterone were decreased when compared with that of adrenal cells alone ($n = 5$). Viability of the testicular cells is indicated by the responsiveness of these cells to the administration of hCG 50 ng/ml. Stimulation of testicular steroidogenesis by hCG did not stimulate adrenal steroidogenesis.





Figs 3(a–e). Mean ± SD steroid responses to ACTH 100 pg/ml, progesterone 10 ng/ml and hCG 50 ng/ml by isolated superfused adrenal cells (●——●, $n = 5$ columns) and a mixed adrenal–testicular cell population (●——●, $n = 5$).

Reproducibility characteristics of the multichannel superfusion system

The coefficient of variation in flow rate between the 10 columns was 1.5% at 0.5 ml min. The between column coefficient of variation of steroidogenesis for each hormone was obtained for a mixed cell population and was 15.4% for testosterone, 20.3% for corticosterone, 25.1% for progesterone, 32.4% for androstenedione and 34.2% for 17α-hydroxyprogesterone in response to the administration of ACTH over the dose range 1–100 pg/ml.

DISCUSSION

We have used a multicolumn isolated superfusion system to study a direct interaction between adrenal cells and testicular cells. Previous work using *in vitro* static incubations failed to provide evidence of direct adrenal–gonad interaction unless both cell types were fused by electrical-field-induced fusion [18]. We have shown that stimulation of adrenal cells with ACTH followed by superfusion of the elutate through testicular cells stimulates testosterone production, suggesting that an adrenal product can promote testosterone secretion by rat testicular cells *in vitro*. Comparison of steroid secretion by a mixed population of superfused adrenal and testicular cells with the secretion from each cell type alone suggests that the adrenal secretion of progesterone is responsible for stimulating testicular steroidogenesis. This is confirmed by the infusion of progesterone which directly stimulates testicular steroidogenesis by the conversion to 17α-hydroxyprogesterone, androstenedione and testosterone. However, the peak steroid response to the infusion of progesterone was less than that achieved with ACTH-stimulated cells (mean ± SD testosterone concentration 1.3 ± 0.2 ng/ml for progesterone infusion compared with 3.52 ± 1.3 ng/ml for ACTH-stimulated cells), despite a higher concentration of progesterone infused (10 ng/ml) when compared with that achieved by ACTH-stimulated cells (3.66 ± 1.33 ng/ml). The adrenal secretion of pregnenolone and 17-hydroxypregnenolone may also be important steroid precursors for testicular steroidogenesis. Whilst progesterone appears to promote testicular steroidogenesis, the administraton of progesterone to adrenal cells fails to promote adrenal steroidogenesis. Further, the superfusate from hCG-stimulated testicular cells does not appear to stimulate adrenal steroid production. This implies that the direction of adrenal–testicular cooperation is one-way from the adrenal to the testis. The decrease in progesterone and corticosterone concentrations in the superfusate of the ACTH-stimulated mixed cell population, when compared with that of adrenal cells alone, suggests preferential utilisation of adrenal progesterone by testicular cells. This presumably reflects differing transport mechanisms within the membranes of adrenal and testicular cells. Glucocorticoid receptors have been demonstrated in interstitial cells of the rat testis [19]. Transport of progesterone across testicular cell membranes appears to be rapid, permitting utilisation by microsomal enzymes. In comparison, the testicular response to LH or hCG is much slower, implying that the rate limiting step in LH- or hCG-mediated steroidogenesis occurs prior to progesterone biosynthesis at the level of either side chain cleavage of 3β-HSD [20]. These results suggest that the adrenal secretion of progesterone stimulates testicular steroidogenesis in the rat *in vitro* supporting the suggestion that the adrenal gland may contribute significantly to the function of the rat testis *in vivo*. Whilst there is no direct adrenal–testicular circulation *in vivo*, the testis can utilise adrenal progesterone reaching its arterial supply after hepatic metabolism. Plasma progesterone levels in the male rat are approx 1–25 ng/ml according to the degree of stress [21]. The levels of progesterone achieved *in vitro* are well within this range. However, caution should be exercised in extrapolating results obtained from *in vitro* experiments to the whole animal.

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