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STIMULATION OF PROTEIN SYNTHESIS *IN VIVO* IN IMMATURE MOUSE TESTIS BY FSH

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Summary. Human pituitary FSH was found to increase the incorporation of tritiated lysine into testicular protein in prepubertal mice *in vivo*. Radioactivity was measured in washed trichloracetic acid precipitates prepared from crude testicular homogenates. The time of maximum response was 8 to 16 hr after subcutaneous injection of the hormone. This was considerably later than the maximum response *in vitro* reported by other workers.

Neither HCG nor dibutyryl cyclic 3′,5′-adenosine monophosphate had a significant effect on the incorporation of lysine.

INTRODUCTION

Means & Hall (1967) found that FSH increased the incorporation of amino acids into testicular protein *in vitro*. This paper reports experiments designed to find out whether a similar effect occurs *in vivo*, whether HCG has this action and whether the action can be reproduced by dibutyryl cyclic 3′,5′-adenosine monophosphate (dbcAMP), as it has been shown that FSH stimulates adenyl cyclase activity in the testis (Murad, Strauch & Vaughn, 1969; Kuehl, Patanelli, Tarnoff & Humes, 1970; Dorrington, Vernon & Fritz, 1972).

MATERIALS AND METHODS

Animals and experimental design

The mice used were of the CFW strain. All male young born on the same day were fostered in groups of between ten and sixteen by pairs of lactating females. Each experiment was designed as a randomized-block with replication. The young of most uniform body weight in a foster group composed a randomized block.

Gonadotrophins

Human pituitary FSH was extracted and assayed as described by Butt, Crooke & Cunningham (1961). In Exps 1 and 2 and in two replicates of Exp. 5, the FSH used was not highly purified. The potency of this preparation was 700 i.u. FSH and 140 i.u. LH activity/mg. In the remaining two replicates of Exp. 5 and in Exps 3 and 4, a more highly purified preparation was used. This contained 2000 i.u. (250 μg) FSH and less than 120 i.u. LH/mg. The potency of

the HCG (Paines and Byrne Ltd) was greater than 1500 i.u. LH and less than 15 i.u. FSH/mg. The hormones were injected subcutaneously in 0·1 ml saline. This route was used because neither intravenous nor intraperitoneal injections were practicable in the younger mice.

Radioisotope

In every experiment, each mouse was given a single subcutaneous injection of 5 μCi L-[4,5-^{3}H]lysine monohydrochloride (sp. act. 250 mCi/mmol) in 0·15 ml saline 2 hr before autopsy.

Extraction procedure

The mice were killed between 10.00 and 12.30 hours by cervical dislocation. After removal of the tunicae albugineae, both testes were weighed and then homogenized together in 1 ml distilled water. Duplicate protein estimations were made on 0·1 ml samples of the crude homogenate (Lowry, Rosebrough, Farr & Randall, 1951). A 0·5-ml sample of the homogenate was mixed with 0·5 ml of 12·5% TCA, allowed to stand for 1 hr at 4°C, and centrifuged at 600 *g* for 10 min. The precipitate was resuspended and washed twice in 5% TCA, once in ethanol and once in an ether:ethanol mixture (1:1, v/v), and was then taken up in 0·5 ml Soluene (Packard Instrument Co.). Two 0·2-ml aliquots of the solubilized extract were placed in a toluene-ethanol PPO/POPOP mixture and the radioactivity was measured by liquid scintillation spectrometry. In all but the first experiment, a portion of liver from each mouse was treated in an identical manner. In all experiments, the results were expressed either as specific radioactivity or as ratios of testicular specific activity to liver specific activity.

Experiment 1

Five groups consisting of four mice in each group were used. Each of the four animals was allocated at random to treatment with saline, 5 i.u. FSH, 1 mg dbcAMP or 5 i.u. FSH plus 1 mg dbcAMP. These substances were given subcutaneously 22 hr and 14 hr before autopsy. The mice were killed by cervical dislocation on the 9th day after birth.

Experiment 2

Mice were killed when 6, 9, 12, 15, 18 or 21 days old. An injection of saline or 7·5 i.u. FSH, was given 16 hr before autopsy. Each age group consisted of four experimental and four control animals.

Experiment 3

Using thirty animals, five for each of the different time periods, a single dose of 5 i.u. FSH was injected at 4, 8, 12, 16, 24 or 32 hr before autopsy on the 9th day. In addition to supplying one animal for each time period, each of the five foster groups provided two or three control mice, making a total of twelve control animals. These were given an injection of saline at one of the above times.

Experiment 4

Nine-day-old mice received either 10·0, 5·0, 2·5, 1·25, 0·63 or 0·31 i.u. FSH, or an injection of saline, 16 hr before autopsy. Each dose of FSH was given to three animals.

Experiment 5

Single injections containing various ratios of FSH, HCG and saline were given to mice 16 hr before autopsy on the 9th day. Thirty-six animals were used. Four animals were given one of the treatments and each one received either 5·0, 0·5 or 0 i.u. FSH and either 10, 1 or 0 i.u. HCG in a 3×3 randomized-block design.

Experiment 6

Each mouse received a single injection of saline, or 2 mg dbcAMP or 0·5 mg theophylline, or 2 mg dbcAMP plus 0·5 mg theophylline, $\frac{1}{2}$, $1\frac{1}{2}$, $4\frac{1}{2}$ or $13\frac{1}{2}$ hr before autopsy on the 9th day. Each of these sixteen treatments was given to two animals.

RESULTS

Experiment 1

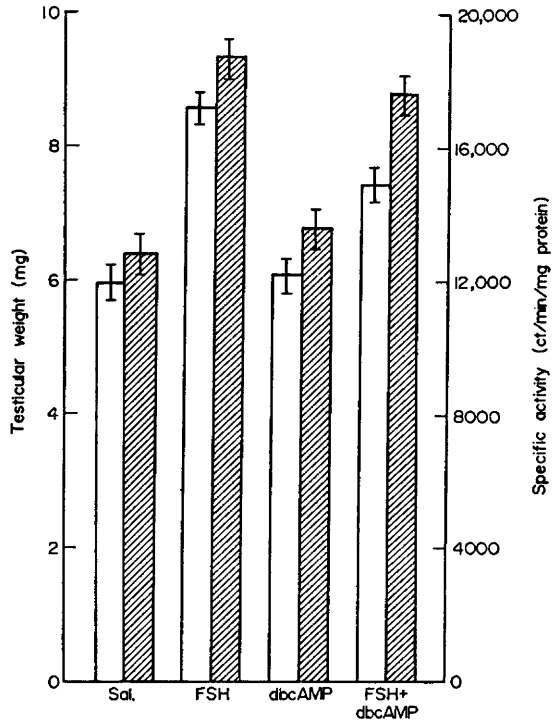
Comparison of the results from mice treated with FSH alone and the controls treated with saline showed that FSH increased the mean testicular weight by 43%, the total weight of testicular protein by 28% and the specific radioactivity of testicular protein by 46% (Text-fig. 1). Analyses of variance showed that the overall effects of FSH were statistically significant ($P<0·001$ for testicular weight and protein sp. act.; $P<0·01$ for total testicular protein). Cyclic AMP did not have a significant effect, and there was no significant interaction between FSH and cAMP.

Experiment 2

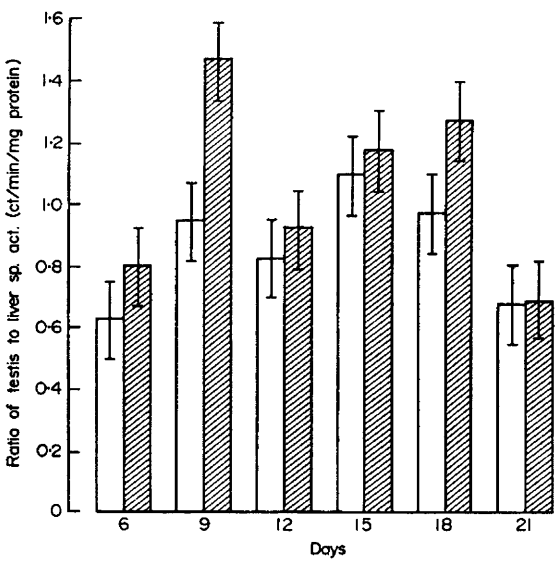
In this experiment to determine the effect of age on the response to FSH, FSH treatment caused a mean overall increase of 16% in the specific radioactivity of testicular protein ($P<0·01$). As FSH did not increase lysine incorporation into liver protein, the results of this and subsequent experiments were expressed as the ratio of the testicular protein specific radioactivity to liver protein specific radioactivity. This was done to minimize the effect of error in the amount of isotope injected. The mean effect of FSH was a 22% increase in this ratio. The testis appeared to be most sensitive to exogenous FSH at 9 days, at which age the hormone caused a 54% increase in the ratio (Text-fig. 2).

Experiment 3

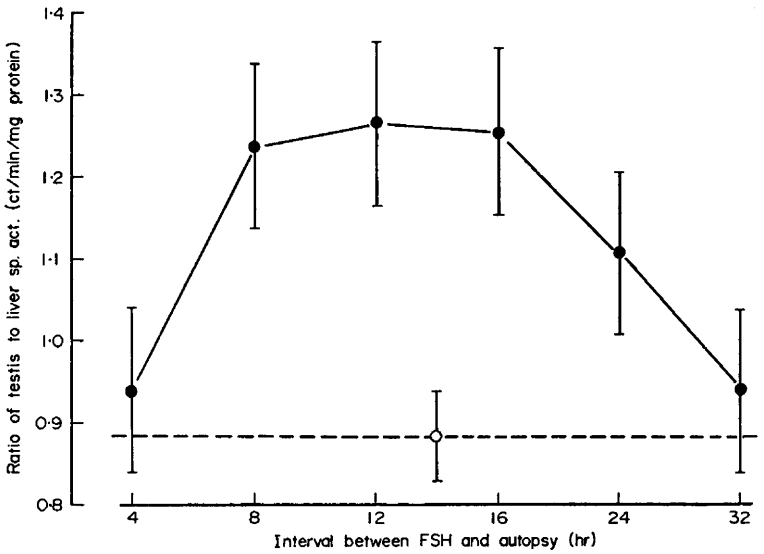
From Text-fig. 3, it can be seen that FSH takes approximately 8 hr to stimulate an increase in protein synthesis and that this effect rapidly falls off after about 16 hr.



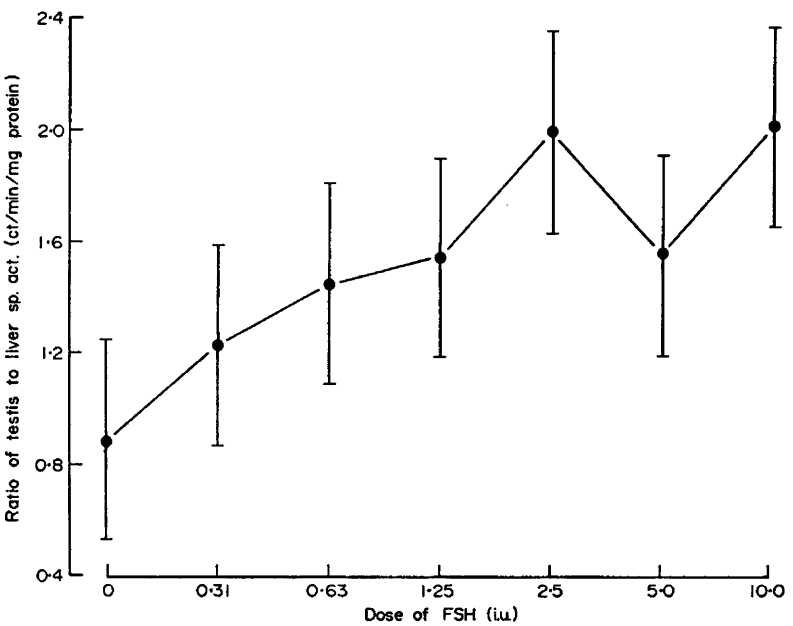
TEXT-FIG. 1. Experiment 1. The effect of FSH on the weight of the testes minus tunicae albugineae (open columns) and tritium activity/mg testicular protein (hatched columns). Vertical bars represent the Mean ±one S.E.M. Sal., saline; dbcAMP, dibutyryl cyclic AMP.



TEXT-FIG. 2. Experiment 2. The ratio of [^{3}H]lysine incorporation into testicular protein to incorporation into liver protein in mice treated with FSH (hatched columns) and control mice injected with saline (open columns). Vertical bars represent the Mean ±one S.E.M.



TEXT-FIG. 3. Experiment 3. The ratio of incorporation of [^{3}H]lysine into testicular and liver protein at different times after a single injection of FSH (———) and in control mice (— — —). Vertical bars represent the Mean ± one S.E.M.



TEXT-FIG. 4. Experiment 4. The effect of different doses of FSH on the ratio of radioactivity in testicular protein to radioactivity in liver protein. Vertical bars represent the Mean ± one S.E.M.

Experiment 4

The responses to different doses of FSH are shown in Text-fig. 4. The mean ratio of radioactivity in testicular protein to activity in liver protein was higher in tissue from mice which had received the larger doses of FSH.

Experiment 5

The mean ratios of testis to liver protein specific radioactivities for each treatment to investigate the effect of HCG and various preparations of FSH are given in Table 1. By analysis of variance the overall effect of FSH was found to be significant ($P<0{\cdot}05$) and so was the difference between the means of control mice and those of mice given 5 i.u. FSH ($P<0{\cdot}025$). The replicate effect, which included effects due to the two different preparations of FSH used as well as to differences between foster groups, was not significant, and neither was the effect of HCG nor any of the interactions.

Table 1. Mean ratios of testis to liver radioactivity per mg protein

Dose of HCG (i.u.)	*Dose of FSH (i.u.)* 0	0·5	5	*Mean*
0	1·009	1·047	1·319	1·125
1	1·186	1·105	1·410	1·234
10	1·050	1·231	1·328	1·203
Mean	1·082	1·128	1·352	

Experiment 6

In this experiment to study the effects of dbcAMP and theophylline, no treatment had a significant effect on the incorporation of lysine into testicular protein or on the ratio of testicular to liver specific radioactivities. The mice injected with dbcAMP plus theophylline died before they could be killed at 4½ or 13½ hr after treatment.

DISCUSSION

Treatment of immature mice with FSH increased testicular weight, total testicular protein and the incorporation of labelled lysine into testicular protein *in vivo*. The effect was greatest 8 to 16 hr after injection of the hormone. This latency is much longer than the 1 to 2 hr found by Means & Hall (1967), but the conditions of the experiments differed in a number of ways. These workers injected ovine FSH intraperitoneally into 20-day-old rats and then incubated testicular tissue in a medium containing labelled amino acid. It is probable that the most important factor causing the difference in latency was that Means & Hall measured amino acid incorporation into testis fragments *in vitro* whereas our experiments were on intact testes *in vivo*.

As the loss of a small amount of precipitate was inevitable during washing, we wished to sample the tissue extracts for both radioactivity counting and protein estimation. This was not possible, however, because the solubilizer interfered with the measurement of protein.

Despite the delay in the maximum effect *in vivo*, the response to different doses of FSH was comparable with that reported by Means & Hall (1967), making allowance for the different body weights of the animals. There was little effect

of HCG in either system, though our results suggest that it may have augmented the effect of 0·5 i.u. FSH in 9-day-old mice.

In 20-day-old rats, FSH binds to intratubular cells but not to interstitial tissue (Means, 1973). Dorrington *et al.* (1972) have shown that the action of FSH in stimulating cyclic AMP production is largely or entirely confined to the tubules. In 9-day-old mice, FSH treatment increases the numbers of supporting cells and spermatogonia (Davies, 1971). As these types of cell form 77% and 21%, respectively, of the intratubular cells at this stage, and FSH treatment increases both types in proportion to their numbers, it is probable that most of the lysine was incorporated into the supporting cells and a smaller proportion into the spermatogonia. Although the stimulatory action of FSH on testicular protein synthesis was most marked in the 9-day-old mice, it also appeared to be present in the 6-, 12-, 15- and 18-day-old animals. In 18-day-old mice, the germinal epithelium is at the same stage of development as that of the 20-day-old rats used by Means & Hall (Nebel, Amarose & Hackett, 1961). At this stage, primary spermatocytes are the most numerous intratubular cells and their number increases rapidly (Widmaier, 1963); FSH may, therefore, be stimulating lysine uptake also into this type of cell. The site of the effect of FSH on protein synthesis needs to be investigated by other methods such as cell separation techniques and histoautoradiography.

It is not yet known whether FSH acts on germinal cells directly or whether its effects on germinal cells are mediated by the supporting cells in the immature testis and the Sertoli cells in the adult testis. The anatomical arrangement of the intratubular cells suggest this latter possibility, because Dym & Fawcett (1970) have shown that germinal cells are separated from the tubular wall by Sertoli cell cytoplasm. Furthermore, fluorescein- or ferritin-tagged FSH labels Sertoli rather than germinal cells (Mancini, Castro & Seiguer, 1967; Castro, Seiguer & Mancini, 1970). Recently, Lacy (1973) has presented evidence to support the hypothesis that FSH acts by way of the Sertoli cells by stimulating them to produce androgen that promotes spermatogenesis.

Although FSH appears to play an important rôle in the prepubertal development of the testis, its effects on spermatogenesis in mature animals is less important than that of androgen (Woods & Simpson, 1961; Clermont & Harvey, 1967; Steinberger & Duckett, 1967; Ortavant, Courot & de Reviers, 1969; Davies, Courot & Gresham, 1974). Injections of FSH do stimulate protein synthesis in testicular tissue from hypophysectomized adult rats, but the effect does not start until 18 hr after hypophysectomy (Means & Hall, 1968). In the adult, therefore, it is possible that FSH acts on regressing but not on normal germinal epithelium. Alternatively, the effect of exogenous FSH may be masked by endogenous FSH present immediately after hypophysectomy.

Means and his colleagues have investigated the sequence of metabolic effects of FSH *in vitro* in testicular tissue from immature rats, and Means (1973) suggests that binding of the hormone to the cell membrane is followed very rapidly by activation of adenylate cyclase and protein kinase. Shortly afterwards, there is enhancement of nuclear RNA synthesis which precedes the increase in protein synthesis. If cyclic adenosine 3′,5′-monophosphate mediates the effect of FSH on RNA synthesis, it should be possible to stimulate amino acid

incorporation by administering dbcAMP. Our failure to accomplish this suggests, but does not prove, that cyclic AMP is not involved in the action of FSH on testicular protein synthesis.

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