Stimulation of transport of alpha-aminoisobutyric acid into the testes of immature mice in vivo by follicle-stimulating hormone

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Abstract. Groups of immature mice were injected sc with radiocarbon-labelled alpha-aminoisobutyric acid (AIB) after being given a single sc injection of hFSH or of 0.9% saline. As an index of the transport of AIB, the specific activity of isotope was measured in homogenates of testis and of liver. FSH treatment caused statistically significant increases in the specific activity of isotope in the testes and in the ratio of testicular to liver specific activity. The effect was greatest in 9-day-old mice injected with FSH 16 h before removal of the testes. Uptake of labelled AIB was not stimulated after administration of hCG or testosterone. Doses of cycloheximide sufficient to reduce the rate of protein synthesis by over 99% did not impair testicular uptake of labelled AIB or the influence of FSH on AIB uptake. These results suggest that FSH stimulates amino acid transport into cells of the immature testis and that this action is independent of the stimulatory effect of FSH on testicular protein synthesis.

Follicle-stimulating hormone (FSH) has been found to increase the rates of synthesis of ribonucleic acids and of proteins in the testes of intact immature and hypophysectomised adult rats and mice (Means et al. 1980; Davies 1980). Though the effect of FSH on proteins appears to be a result of that on RNA, FSH could additionally facilitate protein synthesis by enhancing transport of amino acids into testicular cells. Two groups of workers have studied this possibility by administering FSH to rats and then measuring testicular uptake of radioactivity-labelled alpha-aminoisobutyric acid (AIB) — a non-physiological compound which is transported into cells in a similar manner to physiological amino acids but which is not incorporated into proteins. Means & Hall (1967) detected no increase in in vitro uptake of AIB in teased testicular tissue from immature rats which had been treated with FSH. On the other hand Irusta & Wassermann (1972) reported that in vitro transport of AIB into testicular tissue was stimulated when intact immature or hypophysectomized adult rats had been treated with FSH in vivo. In the experiments reported in this paper we have re-investigated this problem, administering both FSH and radioactivity-labelled AIB to intact immature mice in vivo. This protocol was chosen because it was thought to reflect normal physiology more closely than does the uptake of AIB into testicular tissue in vitro.

Materials and Methods

Animals
Mice of the CFW strain (stock originally supplied by the Laboratory Animals Centre, Carshalton) were maintained under constant conditions of temperature (20°C) and lighting (8 h light: 16 h dark). Groups of 10 to 15 new-born male pups were fostered to pairs of lactating mothers. In each experiment foster litter mates were used to form randomized blocks for treatment. Except where specified, mice were sacrificed at 9 days of age.

Hormones
Human pituitary follicle-stimulating hormone was extracted and assayed as described by Butt & Lynch (1974). In an experiment in which the effects of chorionic gonadotrophin and testosterone were also investigated, a highly purified preparation of FSH containing 11 900 IU of FSH activity and 35 IU of LH per mg was used. The
preparation used in the other experiments contained 725 IU of FSH and 150 IU of LH per mg. Except where specified, each mouse was given a single dose of 5 IU of FSH sc in 0.1 ml of 0.9% saline 16 h before sacrifice. Control animals were injected with 0.1 ml of saline.

Human chorionic gonadotrophin (hCG) (Paines and Byrne Ltd.) contained 1500 IU of LH activity and < 15 IU of FSH activity per mg. A single 10 IU dose of hCG was given sc in 0.1 ml saline 16 h before sacrifice. A 0.5 mg dose of testosterone propionate (Evans Medical Ltd.) was given sc in 0.1 ml of arachis oil, also 16 h before sacrifice.

Isotopes
Each animal was given a single sc injection containing 1.5 μCi of alpha-amino[1-14C]isobutyric acid (specific activity 60 Ci/mol) sc in 0.1 ml saline 2 h before sacrifice. In the final experiment one group of mice received 20 μCi of L-[4,5-3H]lysine monohydrochloride (specific activity 250 Ci/mol) sc in 0.1 ml saline 2 h before sacrifice. These compounds were obtained from the Radiochemical Centre, Amersham.

Experiments
The effect of FSH on the testicular uptake of AIB was measured in groups of 12 mice killed at 5, 9, 14 or 19/20 days or at 15 weeks of age. Six animals in each group were treated with FSH 12 h before sacrifice and the rest received an injection of saline.

The time course of the action of FSH was investigated in 5 groups of 4 mice. One group was injected with saline and the remainder were treated with FSH 4, 8, 16 or 24 h before sacrifice.

The effect of different sized doses of FSH was studied in 5 groups of 6 10-day-old mice given 0.2, 0.6, 1.7, 5 or 15 IU of FSH.

Six groups of 4 mice were used to investigate effects of hCG and testosterone propionate, alone and in combination with highly purified FSH. The groups were treated as follows: i) saline, ii) FSH, iii) testosterone, iv) hCG, v) FSH and testosterone, or vi) FSH and hCG.

To investigate whether the effect of FSH on AIB uptake was mediated by newly synthesized protein, 4 groups of 5 mice were treated with saline, FSH, cycloheximide, or FSH and cycloheximide. Two doses of cycloheximide (Sigma Chemical Co.) were given sc in 0.1 ml saline, the first of 13 μg/g body weight, 16.5 h before killing and the second of 33 μg/h, 2.5 h before killing.

Measurement of AIB uptake
After decapsulation, both testes from each animal were homogenized in 1 ml distilled water. One ml of solubilizing solution (8 g sodium hydroxide in 100 ml of 33% methanol) was added to the homogenate which was then heated to 60°C over 20 min. After neutralization with 0.5 ml of 4 M nitric acid, duplicate 1 ml aliquots of solubilized homogenate were placed in 9 ml of a xylene-based scintillation cocktail (Fisofluor 1, Fisons Ltd., Loughborough) and the beta emission was measured for 10 min by scintillation spectrometry. The protein concentration in triplicate 0.1 ml aliquots of neutralized sample was measured by the method of Lowry et al. (1951). A

| Table 1. |
| Testicular uptake of [14C]AIB in mice of different ages. Specific radioactivity in DPM/μg of protein. (Mean ± SEM). |

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th>FSH</th>
<th>% increase</th>
</tr>
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<tbody>
<tr>
<td>DPM/μg testicular protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>16.4 ± 2.5</td>
<td>17.5 ± 1.7</td>
<td>7</td>
</tr>
<tr>
<td>9 days</td>
<td>3.80 ± 0.46</td>
<td>5.57 ± 1.12</td>
<td>47</td>
</tr>
<tr>
<td>14 days</td>
<td>4.74 ± 0.16</td>
<td>5.85 ± 0.34</td>
<td>23</td>
</tr>
<tr>
<td>19/20 days</td>
<td>2.54 ± 0.16</td>
<td>3.09 ± 0.18</td>
<td>22</td>
</tr>
<tr>
<td>adult</td>
<td>0.361 ± 0.017</td>
<td>0.387 ± 0.025</td>
<td>7</td>
</tr>
</tbody>
</table>

| Ratio of testicular to liver specific activity |             |             |            |
| 5 days   | 1.35 ± 0.19 | 1.62 ± 0.18 | 20         |
| 9 days   | 0.93 ± 0.12 | 1.60 ± 0.28 | 72         |
| 14 days  | 1.18 ± 0.03 | 1.54 ± 0.07 | 31         |
| 19/20 days | 1.22 ± 0.13 | 1.45 ± 0.13 | 19         |
| adult    | 0.36 ± 0.02 | 0.34 ± 0.03 | −6         |
The effect of cycloheximide on protein synthesis
The rate of protein synthesis in the testes of 11 cycloheximide-treated 9-day mice was compared with that in 10 mice which had not received cycloheximide. The method has been described previously (Lawrence & Davies 1977).

Results
The specific activity of tritium in testicular homogenates is shown in Table 1. It is evident that FSH increased the uptake of [14C]AIB into the testes of immature mice, particularly in 9-day-old animals, but the effect was negligible in adult mice. A two-way analysis of variance was performed after log transformation of the data so that the variances at each age should be of similar magnitude. The overall effect of FSH on the uptake of [14C]AIB into the testis was significant ($P < 0.025$). FSH did not influence uptake into the liver (control mean 3.19 DPM/µg, FSH mean 3.12, $P < 0.10$ on log transformed data).

As FSH did not affect the uptake of AIB in liver tissue the ratio of the specific activity in the testis to that in the liver was used in subsequent experiments. The ratio also showed that the effect of FSH was greatest in the 9-day-old mice (Table 1).

Time of administration of FSH
Fig. 1 shows that the effect of FSH on AIB uptake had an appreciable latency. There was no stimulation at 4 h, moderate stimulation at 8 h and the greatest increase was at 16 h.

Dose of FSH
A single dose of 5 IU or 15 IU of FSH increased the ratio of testicular to liver specific activity whereas smaller doses did not do so (Fig. 2). The reason for the rather high ratio found in this experiment is not known. It may have been related to the fact that the animals were 10 days old rather than 9 days as in other experiments and were consequently nearly a gram heavier.

Purified FSH and other hormones
From Fig. 3 it can be seen that highly purified FSH increased the ratio of testis to liver specific activity.

Fig. 1.
Ratio of testis to liver specific radioactivity 2 h after administration of 1.5 µCi of [14C]AIB. Four 9-day-old mice were killed at the times indicated after administration of 5 IU of FSH.
Fig. 2.
Testis to liver specific activity ratio 2 h after administration of 1.5 μCi of [14C]AIB. Groups of 6 10-day-old mice were killed 16 h after receiving the doses of FSH indicated.

Fig. 3.
Testis to liver specific activity ratio 2 h after administration of 1.5 μCi of [14C]AIB. Hormone treatments were given to 6 groups of 4 9-day-old mice 16 h before sacrifice (see text for details).
The effect of FSH was neither augmented nor decreased by the addition of testosterone or hCG. Separate analyses of variance of the FSH and testosterone data and of the FSH and hCG data showed that FSH affected the ratio significantly ($P < 0.001$) but the other hormones did not do so.

**FSH and cycloheximide**

Treatment with cycloheximide reduced the rate of testicular protein synthesis by 99.4%. Nevertheless cycloheximide in the same dosage did not significantly affect the ratio of testis to liver specific radioactivity after administration of $[^14]C$AIB, neither did cycloheximide diminish the effect of FSH on the ratio (Table 2). An analysis of variance of the ratio data indicated that the effect of FSH was still significant ($P < 0.01$) but the effect of cycloheximide and the interaction between FSH and cycloheximide were not significant ($P > 0.05$). Cycloheximide increased the uptake of $[^14]C$AIB into both testicular and liver tissue by 48%. It is thought that this may have been due to an increase in the circulating level of AIB resulting from impairment of catabolism or of excretion.

**Discussion**

The stimulatory effect of FSH on testicular protein synthesis in intact immature and hypophysectomized adult rodents is well documented (Means 1975; Davies & Lawrence 1978). Protein synthesis has been shown to be a consequence of the influence of FSH on RNA synthesis. However the results reported in this paper suggest that FSH additionally promotes protein synthesis by enhancing the availability of amino acids as a result of increasing their transport into testicular cells. The results of our experiments thus confirm those of Irusta & Wassermann (1974) who measured the in vitro uptake of $[^14]C$AIB in decapsulated testes from 22-day-old rats given an ip injection of FSH 4 h before sacrifice. FSH injected ip into 20-day-old rats 1 h before testicular tissue was removed had no effect on the uptake of AIB in vitro (Means 1975). Presumably this negative result was because there is a latency in the effect of FSH on amino acid uptake. In the in vivo expriments in mice an effect was seen 8 h after sc administration of FSH but not at 4 h.

The ratio of specific activity in the testis to that in the liver was thought to be a better index of the effect of FSH on the testis than specific activity in the testis because the ratio was not affected by experimental error in the amount of isotope injected. The ratio also compensated for differences in the rate of absorption of isotope and in the size of the animals. The ratio did change with age but, with the exception of the age and dose-response experiments, only 9-day-old mice were used.

From the dose response graph it was evident that stimulation of AIB transport occurred only if the dose of FSH was moderately large. Doses of similar size are needed for stimulation of RNA or protein synthesis in immature mice (Davies & Lawrence 1978). One reason why a dose of 5 IU was needed may have been that as intact mice were used a low concentration of endogenous FSH was already present in the circulation and this would have caused some stimulation of amino acid transport even in the control animals. It is also possible that some of the exogenous hormone was destroyed at the site of injection before entering the circulation.

Highly purified FSH stimulated AIB uptake whereas hCG and testosterone did not. Similarly uptake was not affected by prolactin (10 IU sc 16 h before killing) or hGH (0.1 mg sc 16 h before killing), nor did these hormones interact with FSH (results not shown). Likewise Irusta & Wassermann (1972, 1974) reported that LH and testosterone

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**Table 2.**

Effects of FSH and cycloheximide on uptake of $[^14]C$AIB. Ratio of specific activity in testis to that in liver (Mean ± SEM).

<table>
<thead>
<tr>
<th>Control</th>
<th>FSH</th>
<th>Cycloheximide</th>
<th>FSH + Cyclo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.18 ± 0.13</td>
<td>1.60 ± 0.17</td>
<td>1.12 ± 0.12</td>
<td>1.72 ± 0.20</td>
</tr>
</tbody>
</table>
failed to increase uptake of AIB. These findings suggest that the response is hormone specific.

The lack of effect of cycloheximide on the stimulation of testicular AIB uptake by FSH in mice is in contrast to the observation of Irusta & Wassermann (1972, 1974) that pre-incubation of rat testicular tissue with cycloheximide reduced AIB uptake. In experiments with isolated immature rat ovaries, Ahrén et al. (1967) found that addition of puromycin to the incubation medium reduced but did not abolish FSH-stimulated AIB uptake, whereas treating the rats with puromycin before administration of FSH inhibited uptake completely. Both these groups of workers suggested that the effect of FSH on gonadal AIB uptake might result from stimulation of production of a carrier protein involved in amino acid transport, but the result of our experiment in which cycloheximide was given before treatment with FSH and again before administration of AIB does not support this conclusion. If FSH does stimulate production of a carrier protein this action must account for only part of the hormone's effect on amino acid transport.

Acknowledgments

We wish to express our thanks to the Wellcome Foundation and to the Mason Trust for supporting this work.

References


Received on March 31st, 1981.