The effect of a synthetic polypeptide, threonyl-prolyl-arginyl-lysine, on ovulation in the squirrel monkey (Saimiri sciureus) and hamster

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Summary. Synthetic TPAL, a polypeptide reported to occur naturally in hamster zygotes, was tested at doses of 3 or 18 µg/day for antiovulatory activity in cyclic hamsters (for 4 days) and squirrel monkeys (for 5 days) induced to ovulate. The TPAL treatment did not alter the ovulatory response in hamsters or monkeys.

Introduction

Kent (1973) has argued that an embryonic factor prevents subsequent ovulation in mated hamsters. Such a factor would have to come from the preimplantation embryo because hamsters have a 4-day oestrous cycle but implantation does not begin until 5 days post coitum and the placental knob is first formed about the 6th day (Graves, 1945; Boyer, 1968). The factor, extracted from 2- to 8-cell zygotes, had antiovulatory activity in the nongravid Syrian hamster but did not prevent oestrus (Kent, 1973) and has been characterized as a polypeptide, threonyl-prolyl-arginyl-lysine (TPAL) (Kent, 1975a, b). Although 3 µg TPAL/day blocks 90% of ovulations in hamsters (Kent, 1975a), its exact mechanism of action is unknown. The antiovulatory activity of synthetic TPAL in the hamster has not been verified or shown for any other species.

The present study was therefore conducted to test the activity of synthetic TPAL in hamsters and squirrel monkeys. Squirrel monkeys were chosen to provide a primate model because of previous success with this species for studies of ovulation induction (Dukelow, 1970; Harrison & Dukelow, 1973; Kuehl & Dukelow, 1975) and of the contraceptive action of megestrol acetate (Harrison & Dukelow, 1971; Harrison, Rawson & Dukelow, 1974).

Materials and Methods

The synthetic TPAL was purchased as a freeze-dried powder (H-Thr-Pro-Agr-Lys-OH; Calbiochem, La Jolla, California) and dissolved in 0·15 M-NaCl. Kent (1975a) showed that a dose of 3 µg TPAL extract/day was effective for reducing the number of ovulations in the hamster, and aliquots were therefore prepared for doses of 3 or 18 µg TPAL/0·1 ml 0·15 M-NaCl.

Hamster tests

Seventeen female Syrian hamsters (Mesocricetus auratus) from our inbred colony were allowed unrestricted access to Wayne Lab-Blox (Allied Mills, Inc., Chicago, Illinois) and water. Nulliparous females, 100–140 g, were used after they had experienced two consecutive 4-day oestrous cycles. A copious, grey-white discharge was considered indicative of the day after oestrus (Orsini, 1961) and this day was the 1st of treatment. Females were randomly assigned to one of three groups and were given subcutaneous injections of 3 or 18 µg TPAL or 0·15 M-NaCl (controls) between 09:00 and 10:00 h daily for 4 days. Between 09:30 and 11:00 h on Day 5 the animals were killed and the ovaries and oviducts removed for examination. Counts were made of the corpora haemorrhagica and the ova flushed from the oviducts with saline to determine the ovulation number. The data were examined for the effect of dose of TPAL on the number of ovulations per animal by analysis of variance.

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Squirrel monkey tests

Twelve female squirrel monkeys (Saimiri sciureus) of either the Brazilian (export point of origin: Leticia, Colombia) or Bolivian (export point of origin: Santa Cruz, Bolivia) type were maintained on Wayne Monkey Diet (Allied Mills, Inc.) with fruit and water. All 12 females were of the same karyotype with the Columbian characteristic of 6 pairs of acrocentric chromosomes (Jones & Ma, 1975). The females, weighing from 575 to 800 g, were assigned to one of three groups (A, B or C). Each animal received 3 trials: in the first trial, Group A received the vehicle and Groups B and C the 3 and 18 µg TPAL/day respectively; 3 weeks later Groups A and B were treated with TPAL and Group C the vehicle; and in the third trial, 3 weeks after the end of the second trial, Group B received vehicle and Groups C and A the 3 and 18 µg doses of TPAL. Within each trial, Days 1–5 and 08:00–11:00 h, the monkeys were also given i.m. injections of 1 mg FSH (FSH-P: Armour-Baldwin, Omaha, Nebraska) in 0·2 ml 0·15 m-NaCl on Days 1, 2, 3 and 4 (09:00–11:00 h) and 500 i.u. hCG (Chorionic Gonadotropin: Calbiochem, La Jolla, California) in 0·25 ml 0·15 m-NaCl on Day 4 (09:00 h). The squirrel monkeys were induced to ovulate without regard to their natural cycle by the regimen described by Dukelow (1970). All the animals had responded at least once to this treatment before the start of this study. The procedure for laparoscopy, Days 5 and 6, and the examination for ovulation have been described previously (Dukelow, Jarosz, Jewett & Harrison, 1971; Harrison & Dukelow, 1974; Dukelow & Ariga, 1976). Ovulation was defined by the presence of corpora haemorrhagica. At laparoscopy the follicular response was determined from the number of follicles ≥ 2 mm in diameter by 12 h after hCG. Ovulation and the follicular responses were assessed by χ² statistics and analysis of variance, respectively.

Results

In the hamster tests, on Day 5 only 1 of the 17 females had not ovulated by midmorning and the rest presented a normal vaginal discharge indicative of the day oestrus. At autopsy on Day 5, the animals receiving 0·15 m-NaCl had 17·2 ± 1·2 (s.e.m.) ovulations/hamster and those receiving 3 and

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>No. of animals</th>
<th>No. of animals ovulating* (%)</th>
<th>No. of ovulations/animal ovulating</th>
<th>No. of follicles/animal (mean ± s.e.m.)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0·15 m-NaCl only)</td>
<td>12</td>
<td>6 (50)</td>
<td>1·5</td>
<td>5·7 ± 2·0</td>
</tr>
<tr>
<td>3 µg TPAL</td>
<td>12</td>
<td>6 (50)</td>
<td>1·2</td>
<td>5·3 ± 3·0</td>
</tr>
<tr>
<td>18 µg TPAL</td>
<td>12</td>
<td>8 (67)</td>
<td>1·3</td>
<td>5·7 ± 4·2</td>
</tr>
<tr>
<td>Trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First (October)</td>
<td>12</td>
<td>8 (67)</td>
<td>1·1</td>
<td>6·3 ± 3·2</td>
</tr>
<tr>
<td>Second (November)</td>
<td>12</td>
<td>6 (50)</td>
<td>1·3</td>
<td>5·0 ± 3·0</td>
</tr>
<tr>
<td>Third (December)</td>
<td>12</td>
<td>6 (50)</td>
<td>1·5</td>
<td>5·4 ± 3·1</td>
</tr>
</tbody>
</table>

* By 36 h after hCG.
† Follicles ≥ 2 mm in diameter at laparoscopy 12 h after hCG.

Table 1. Effect of TPAL (3 or 18 µg/day for 5 days) on induced ovulation and follicular response in the squirrel monkey

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F ratio</th>
<th>Significance</th>
</tr>
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<tr>
<td>Animals</td>
<td>11</td>
<td>251·556</td>
<td>22·869</td>
<td>4·93</td>
<td>P &lt; 0·05</td>
</tr>
<tr>
<td>Doses</td>
<td>2</td>
<td>0·889</td>
<td>0·444</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Months</td>
<td>2</td>
<td>9·722</td>
<td>4·861</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>92·722</td>
<td>4·636</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>354·889</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
18 μg TPAL/day (6/group) had 14.2 ± 2.5 and 14.3 ± 7.1 ovulations/hamster. The hamster which did not ovulate (18 μg group) had 14 large follicles (>1 mm diam.) with mature ova. One female in the 3 μg dose group had 11 corpora haemorrhagica and 6 large follicles in her ovaries, suggesting incomplete ovulation. The 231 ova recovered in oviduct flushings corresponded to 257 corpora haemorrhagica (90%). There was no statistically significant difference between the ovulation numbers in the treated and untreated hamsters.

The results of the squirrel monkey tests are shown in Table 1. There was no difference in the proportion of females ovulating in response to each treatment or at each trial. Nine animals ovulated at least once and 75% of the ovulations were single. Seven ovulations had occurred by 12 h and 26 by 36 h after hCG injection. There was also no difference in the follicular response for dosage of TPAL or month of trial. There was, however, a significant difference (P < 0.05) between individual squirrel monkeys, justifying the use of this design.

Discussion

Previous work (Kent, 1975a, b) had identified TPAL as an active material extracted from hamster zygotes which signalled a successful mating and halted follicular growth and ovulation. It was anticipated that synthetic TPAL would have a similar effect. However, when synthetic TPAL was administered to hamsters in a manner similar to that used by Kent (1973, 1975a) there was no significant reduction of the number of ovulations, even with a 6-fold increase in dose. These results suggest that the tetrapeptide in the material extracted from hamster zygotes is not the active factor or that another factor, possibly the carrier protein to which Kent (1975b) alluded, is necessary for biological activity.

The results of a similar trial in squirrel monkeys were also conclusive: at the doses tested, synthetic TPAL did not significantly alter the proportion of animals ovulating or the follicular response to a gonadotrophin treatment regimen known to induce ovulation. Squirrel monkeys undergo seasonal changes in their responsiveness to the standard ovulation induction regimen (Harrison & Dukelow, 1973). The results of the present study showed no statistically significant difference in the proportion of animals ovulating and the number of ovulations/ovulating female and these values are consistent with those of previous studies for this season of the year (Harrison & Dukelow, 1971, 1973; Kuehl & Dukelow, 1975). This 50–67% response is also sufficient to test for contraceptive action (Harrison & Dukelow, 1971; Harrison et al., 1974).

The female squirrel monkey has a short cycle of 8–10 days (Rosenblum, 1968), and preimplantation blastocysts can be recovered from the uterus 5 days after the hCG injection when ovulation is induced by gonadotrophins (Ariga & Dukelow, 1977). Travis & Holmes (1974) have shown that progesterone secretion by the corpus luteum declines at about this time and an embryonic signal to prevent this seems possible. While TPAL does not appear to be the message in the squirrel monkey, a similar compound may exist. It is also possible that the mode of action of TPAL involves a decrease in the pituitary secretion of gonadotrophins because the use of exogenous gonadotrophins to induce ovulation would mask such an effect. However, the small amounts of gonadotrophins used in this regimen (Dukelow, 1970) and the hamster results in the present study seem to preclude this possibility.

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