Effects of delayed transfer and treatment with oestrogen on the transport of microspheres by the rat oviduct

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Summary. Starch or dextran blue microspheres were transferred microsurgically to the infundibulum of the oviduct on Days 1, 2, or 3 of pregnancy of control and oestradiol-treated rats. The animals were killed a few hours to several days after transfer to assess the number and distribution of ova and microspheres in the tract.

After transfer on Day 1 of pregnancy, microspheres and eggs crossed the ampullary–isthmic junction (AIJ) 18 h after ovulation. After transfer on Day 2 of pregnancy, more than 50% of microspheres were retained in the ampulla, indicating that the AIJ changes again 34 h after ovulation. Treatment with oestradiol did not advance the passage of eggs or microspheres across the AIJ but caused accelerated transport through the isthmus as soon as the eggs or microspheres reached this segment. Dextran blue microspheres were seen to move back and forth in the isthmus of control anaesthetized rats at a frequency of 5–6 times/min. Between 7 and 20 h after treatment with oestradiol the frequency of these movements was significantly augmented, indicating that increased frequency of contractions of the smooth muscle of the isthmus precedes and accompanies accelerated transport of ova through this segment.

Keywords: rat; oviduct; embryo transport; oestradiol; microspheres

Introduction

Current understanding of the mechanical function of the oviduct has derived principally from the study of its muscle activity (for reviews see Harper et al., 1976) and ciliary activity (Gaddum-Rosse & Blandau, 1973, 1976; Gaddum-Rosse et al., 1975) and from a detailed assessment of the movements of ova (Talo, 1980) or ovum surrogates (Pauerstein et al., 1975; Bourdage & Halbert, 1984). The hormonal control of this function has been inferred from ovum transport alterations that result from an experimentally induced excess or deficit of various hormones. None the less, a paucity of integration of all these elements reduces understanding of how embryo transport is accomplished.

An excess of oestradiol consistently accelerates the transport of eggs through the rat oviduct, but it is not known what mechanical changes account for this effect and in which oviducal segments they take place. The observation that microspheres transferred to the infundibulum of the rat oviduct on Day 1 of pregnancy reproduce the transport of native eggs without disturbing it (Moore & Croxatto, 1988) prompted us to apply this technique to resolve some of these questions.

The aims of the present study were to: (1) characterize the gate-like behaviour of the ampullary–isthmic junction of the rat, and (2) determine the nature and location of functional changes associated with oestrogen-induced accelerated transport.

Materials and Methods

Animals. Adult virgin Sprague–Dawley rats were kept under conditions of controlled temperature (21–24°C) and light (07:00–21:00 h). Water and pelleted food were supplied ad libitum. Vaginal smears (lavage) were taken daily and
pro-oestrous females were caged overnight with fertile males. The following day was designated Day 1 of pregnancy if the presence of spermatozoa in the vaginal smear was verified.

**Treatment.** The microspheres were of starch, 80–100 µm in diameter (Lot DK 850810, Pharmacia, Uppsala, Sweden) or dextran blue, 120–140 µm in diameter (Density Marker Beads, DMB-1, Lot HF 26353 Pharmacia). Trypan blue-stained cumuli or microspheres (4 dextran blue, 6 starch) were transferred to the infundibulum of the oviduct on Day 1, 2 or 3 of pregnancy with the aid of a microsyringe and a surgical microscope, as described by Moore & Croxatto (1988). Oestradiol, 1 or 5 mg dissolved in 0.5 ml olive oil, was given as a single s.c. injection as described in the ‘Results’.

**Assessment of oviducal transport of eggs, microspheres and stained cumuli.** Animals were killed with an overdose of ether at the times indicated. Oviducts and uteri were removed free of fat tissue and were flushed separately. In some experiments the oviducts were divided into 2 or 3 segments. Cuts were done at the ampullary–isthmic junction when two segments were required and also at mid-isthmus, when 3 segments were required. Each segment was flushed separately with saline solution (9 g NaCl/l) and the flushings were examined under low-power magnification to assess the number of microspheres and eggs.

Stained cumuli and dextran blue microspheres were used to characterize the pattern of transport through the ampulla. Movement of these particles from the ostium abdominale to the ampullary–isthmic junction was observed continuously under the operating microscope and the time taken was recorded.

In another group of animals the frequency of back and forth movements of dextran blue microspheres in the isthmus was assessed *in vivo* by direct observation under the surgical microscope. For this purpose the oviducts and ovaries were exposed under ether anaesthesia through the same incision as used for the transfer of microspheres. During the 15-min observation period, care was taken to maintain the temperature and moisture of the organs exposed with the aid of a lamp and frequent irrigation with warm saline solution.

**Statistical analysis.** Differences in the number of eggs or microspheres between groups were analysed by the Wilcoxon two sample or Kruskal–Wallis test (Conover, 1980) as indicated in the ‘Results’. Differences in distribution along several segments were analysed by χ² tests. Probability values of <0.05 were considered statistically significant.

**Results**

Transport from the infundibulum of the ampullary–isthmic junction took (mean ± s.e.) 82 ± 6.8 and 367 ± 61.1 sec for cumuli and dextran blue microspheres, respectively. All cumuli moved continuously in the abovarian direction at a steady speed whereas some microspheres would stop and experience back and forth movements. Once they reached the ampullary–isthmic junction the cumulus masses and microspheres moved back and forth within the dilated loop. Since treatment with oestrogen reduced the oviducal transit of embryos from the normal 96 h to less than 24 h (Ortiz *et al.*, 1979) it became obvious that accelerated transport up to the ampullary–isthmic junction could not account for any significant part of this effect. Therefore transport through the ampullary segment under the action of exogenous oestrogen was not studied. Instead decreased retention time at the ampullary–isthmic junction or utero-tubal junction and/or accelerated passage through the isthmus were examined in the following experiments.

**Experiment 1**

This experiment was designed to investigate the effect of delayed transfer on the transport of microspheres and native eggs to the uterus.

Six starch microspheres were transferred at 10:00–12:00 h to each oviduct of 8 rats on Day 1, of 24 rats on Day 2 and of 24 rats on Day 3. Rats with microspheres transferred on Day 1 were killed on Day 5 and 6 rats of each of the other groups were autopsied on Day 5, 7, 9 or 11 to determine the number of microspheres and eggs in oviducts and uteri and implanted embryos when appropriate. As shown in Fig. 1, after synchronous transfer (on Day 1) all microspheres and eggs were recovered from the uterus on Day 5 whereas after delayed transfer (Day 2 or 3) most microspheres (90%) were in the oviduct and all eggs were in the uterus. At all times the mean number of embryos was >9. Nearly 50% of transferred microspheres were recovered on Day 5, 7 or 9, and recovery dropped even further on Day 11.
Fig. 1. Distribution of native eggs (solid bars) and microspheres (open bars) between oviduct and uterus on Days 5, 7, 9 or 11 of pregnancy. Six starch microspheres were transferred to the infundibulum of each oviduct on Day 1, 2 or 3 of pregnancy. Values are mean ± s.e.m.

Experiment 2

This experiment was designed to determine the oviducal distribution of microspheres after delayed transfer.

Six starch microspheres were transferred to each oviduct of 12 rats on Day 2. Groups of 4 rats were killed on Day 4, 5 or 7. To assess the segmental distribution of microspheres and eggs, the oviducts were divided in three segments: ampulla, distal and proximal isthmus. As shown in Fig. 2, most eggs were in the oviducal segment closest to the uterus on Day 4 and all of them were in the uterus on Day 5 or 7. At each time most of the microspheres were in the oviduct, distributed along its length, although 40% or more remained in the ampulla. Transfer on Day 3 (not shown) gave the same distribution as did transfers on Day 2.

Experiment 3

This experiment was designed to determine whether treatment with oestradiol accelerates the transport of microspheres.

Six starch microspheres were transferred to each oviduct of 24 rats on Day 1: 12 rats received a single s.c. injection of 1 µg oestradiol in the morning of Day 1 and the remaining rats served as controls. The number of microspheres and eggs in oviducts and uteri was determined 24 h after the injection of oestradiol. At 24 h after a single s.c. injection of oestradiol on Day 1, the mean numbers of eggs and microspheres in the oviducts were 13.4 ± 0.7 and 10.5 ± 0.5 in the control rats and 5.2 ± 1.3 and 7.2 ± 1 in the treated rats respectively. The differences between control and treated groups were statistically significant (Kruskal–Wallis test).

Experiment 4

This experiment was designed to determine whether treatment with oestradiol advances the passage of microspheres and eggs across the ampullary-isthmic junction.

Four dextran blue microspheres were transferred to each oviduct of 56 rats in the morning of Day 1. One-half of the animals received a single s.c. injection of 5 µg oestradiol on the same day at
Fig. 2. Distribution of native eggs (shaded bars) and microspheres (open bars) along the oviducal segments on Days 4, 5 or 7 of pregnancy. Six starch microspheres were transferred to the infundibulum of each oviduct on Day 2 of pregnancy. Values are based on a mean of >6 eggs per oviduct and >70% of the transferred microspheres. Values are mean ± s.e.m. of 8 oviducts.

10:00 h and the others served as controls. Seven treated and 7 control rats were killed at 18:00, 21:00, or 24:00 h on Day 1 or at 03:00 h on Day 2. To assess the distribution of microspheres and eggs across the ampullary–isthmic junction the oviducts were divided in two segments: ampulla and isthmus. As shown in Fig. 3 most microspheres and eggs in the control and treated group were in the ampulla until the autopsy done at 24:00 h on Day 1 and 50% or more had crossed the ampullary–isthmic junction at 03:00 h on Day 2. At this time there was a statistically significant loss of microspheres and eggs, in accordance with the loss of eggs that follows the onset of accelerated transport (Ortiz et al., 1979). Before this time there was no statistically significant difference in the distribution of microspheres and eggs between treated and control groups ($\chi^2$ test).

Experiment 5

This experiment was designed to assess the effect of oestradiol on oviducal transport of microspheres located in ampulla or isthmus.

Six starch microspheres were transferred to each oviduct of 16 rats on Day 1. In the morning of Day 3, when microspheres were in the isthmus, 8 animals received a single s.c. injection of 1 µg oestradiol; 8 animals did not receive oestradiol and served as controls.

Six starch microspheres were transferred to each oviduct of 12 rats on Day 3. In the morning of the same day, when microspheres were still in the ampulla, 6 animals received a single s.c. injection of 1 µg oestradiol; 6 animals did not receive oestradiol and served as controls. The numbers of microspheres in oviducts and uteri were determined 24 h after injecting oestradiol. Most microspheres transferred on Day 1 or 3 were in the oviduct in the control group (9·5 ± 0·6 and 10·3 ± 0·9, respectively). In the treated group most microspheres were in the uterus when the
transfer was done on Day 1 (6.1 ± 1.6) but all microspheres were in the oviduct when the transfer was done on Day 3 (10.7 ± 0.8). In oestrogen-treated rats, therefore, microspheres were accelerated only when they were located in the isthmus at the time of treatment. Most native eggs were in the oviduct in the control groups and all passed to the uterus in the treated groups (not shown).

Experiment 6

This experiment was designed to assess the effect of oestradiol on the movements of microspheres in the isthmus.

Four dextran blue microspheres were transferred to each oviduct of 127 rats in the morning of Day 1; 72 of these animals received a single s.c. injection of 5 µg oestradiol at different times on Day 1 or in the morning of Day 2, and 55 animals did not receive oestradiol and served as controls. On Day 2, between 2 and 24 h after the injection of oestradiol, the frequency of movements of the microspheres was determined. The same was done in the control group on Day 2. As shown in Fig. 4 the frequency of movements on Day 2 was 30–60% higher in rats treated 7–24 h earlier with oestradiol than in their controls tested at the same time of the day. The amplitude and velocity of back and forth movements appeared to be increased under the influence of oestrogen, but no attempt was made to verify this objectively.

Discussion

This study demonstrates that microspheres transferred to the rat oviduct with more than 24 h delay with respect to ovulation do not reproduce the normal time course of egg transport and that
oestradiol-induced acceleration of egg transport is restricted to the isthmus and is associated with increased frequency of the smooth muscle activity of this segment.

Transport of stained cumuli from the ostium abdominale to the ampullary–isthmic junction takes less than 2 min and seems to reflect only the ciliary activity, differing from the pattern of movement seen in the rabbit (Verdugo et al., 1975). Microspheres take a few more minutes than do cumuli but also respond to ciliary action and muscle activity. At any rate, this step of transport cannot contribute significantly to the overall effect of oestrogen on oviducal transport.

Microspheres transferred on Day 1 mimic quite well the transport of native eggs (Moore & Croxatto, 1988). In the present work, most microspheres transferred in the morning of Day 2 or 3 of pregnancy remained in the oviduct until Day 9 and nearly 50% of microspheres remained in the ampulla until Day 7 and the rest in the distal and proximal isthmus, when the transfer was done in the morning of Day 2. This suggests that at these times of transfer the passage of eggs and microspheres across the ampullary–isthmic junction was already restricted. In addition, microspheres transferred on Day 1 and native eggs began to pass to the isthmus between 21:00 and 24:00 h of the same day and, at 03:00 h on Day 2, more than half of the eggs or microspheres were in the isthmus. This means that in the rat the ampullary–isthmic junction becomes patent to the passage of particles nearly 18 h after ovulation and remains so for less than 12 h. This coincides with findings in the rabbit oviduct which established that the ampullary–isthmic junction becomes patent between 12 and 24 h after ovulation (Pauerstein et al., 1974) and remains open for about 16 h (Gómez & Croxatto, 1977).

The mean number of microspheres and eggs recovered from the oviduct 24 h after the injection of oestradiol on Day 1 was significantly diminished. This has been shown before to be the result of accelerated transport with expulsion of eggs from the uterus (Villalón et al., 1982a; Fuentealba et al., 1987). Thus, the residence of more than 50% of the eggs in the oviduct was shortened from 95 h to less than 24 h. Advanced passage of eggs or microspheres through the ampullary–isthmic junction did not contribute significantly to this effect. No difference was found in number and distribution of eggs and microspheres across the junction between control and oestradiol treated rats up to 24:00 h on Day 1. At 03:00 h on Day 2 more than 50% of eggs had crossed the ampullary–isthmic junction in the control group and at this time accelerated transport was evident for the first time as a significant decrease in egg recovery. Oestradiol therefore accelerated the transport of eggs and

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**Fig. 4.** Frequency of movements of dextran blue microspheres in the isthmus. Four microspheres were transferred to the infundibulum of each oviduct on Day 1 of pregnancy. Oestradiol was injected into 72 rats (open bars) and 55 served as controls (solid bar). Frequency of movements was determined at different times after oestradiol. Values are mean ± s.e.m. for the number of rats indicated.
microspheres without advancing their passage through the ampullary–isthmic junction, and accelerated transport was restricted to a step that follows temporally and anatomically the passage of eggs through the ampullary–isthmic junction. The fact that oestradiol administered on Day 3 advanced the passage to the uterus of the microspheres localized in the isthmus but not those localized in the ampulla gives further support to the concept that oestradiol accelerates transport of ova or microspheres only after they have negotiated the ampullary–isthmic junction. This suggests an independent response of oviducal segments to oestrogen in the rat.

Dextran blue microspheres were observed in vivo to move back and forth inside the isthmus. This pattern of movement had been observed before in the isthmus of the rabbit oviduct (Hodgson et al., 1977; Bourdage & Halbert, 1984). The frequency and amplitude of movements of microspheres in the isthmus of the rat oviduct was increased by oestradiol treatment. It is not possible to explain this effect without assuming an increased frequency of muscular contractions. Increased frequency of back and forth movement was first observed 7 h after oestradiol administration and a significant decrease in the number of oviducal eggs was seen 11 h after treatment (Ortiz et al., 1979). The time course of this smooth muscle response is therefore compatible with the possibility of being causal in the accelerated transport of eggs that follows a single dose of oestradiol.

The present investigation shows that it is possible to distinguish temporally related changes in the activity of specific segments of the rat oviduct during embryo transport. These changes are not reproduced necessarily each time that a new cohort of particles enters the oviduct. This means that in the rat the programme of oviducal transport is set in motion by endocrine signals accompanying ovulation and not by the entrance of eggs or microspheres. Exogenous oestradiol can accelerate the transport of microspheres and eggs, reprogramming the activity of the isthmus rather than the ampulla or ampullary–isthmic junction. This effect is probably mediated by increased frequency of muscular contractions which raise the frequency of back and forth movements of the luminal contents.

We thank Dr Lars-Eric Larsson of Pharmacia for starch and dextran blue microspheres and the Rockefeller Foundation for financial support.

References


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Received 17 November 1987